Supplementary Material

Table S1: Additional Patient Characteristics

Demographics[a]	All patients (N=86)	Treatment-naïve patients (N=14)	Pretreated patients (N=72)
Age, years			
Range	25-88	50-88	25-86
< 65	38 (44)	3 (21)	35 (49)
⩾65	48 (56)	11 (79)	37 (51)
Race, n (%)			
Asian	1 (1)	0 (0)	1 (1)
Non-Asian	85 (99)	14 (100)	71 (99)
Stage at initial diagnosis, n (%)			
Stage I	2 (2)	1 (7)	1 (1)
Stage II	7 (8)	3 (21)	4 (6)
Stage III	11 (13)	2 (14)	9 (13)
Stage IIIa	5 (6)	1 (7)	4 (6)
Stage IIIb	4 (5)	1 (7)	3 (4)
Stage IIIc	2 (2)	0 (0)	2 (3)
Stage IV	64 (74)	8 (57)	56 (78)
Stage IVa	18 (21)	3 (21)	15 (21)
Stage IVb	48 (56)	5 (36)	43 (60)
Stage at mobocertinib initiation, n (%)			
Stage III	1 (1)	1 (7)	0
Stage IV	85 (99)	13 (93)	72
Location of metastasis at mobocertinib initiation, n (%)			
Bone	48 (56)	5 (36)	39 (54)

Lung	43 (50)	9 (64)	38 (53)
Pleura	29 (34)	1 (7)	25 (35)
Brain	25 (29)	4 (29)	23 (32)
Liver	20 (23)	2 (14)	19 (26)
Adrenal gland	11 (13)	1 (7)	10 (14)
Lymph nodes	14 (16)	1 (7)	13 (18)
Other	17 (20)	2 (14)	15 (21)
Site of metastasis, n (%)			
0- 1	29 (34)	8 (57)	21 (29)
2–3	40 (47)	4 (29)	36 (50)
>3	19 (22)	2 (14)	17 (24)
Brain metastasis at baseline, n (%)			
Asymptomatic	16 (19)	3 (21)	13 (18)
Symptomatic	7 (8)	1 (7)	6 (8)
Unknown	5 (6)	0 (0)	5 (7)
Previous regimens curative setting, n (%)			
Neoadjuvant	2 (2)	0 (0)	2 (3)
Adjuvant	11 (13)	3 (21)	8 (11)
Definitive Chemoradiotherapy with Immunotherapy	1 (1)	1 (7)	0 (0)
Radiotherapy, n (%)			
Prior to mobocertinib administration			
Patients without radiotherapy	59 (69)	10 (71)	49 (68)
Neoadjuvant or post-operative thoracic radiotherapy	4 (5)	0 (0)	4 (6)

Stereotactic radiotherapy of brain metastasis	10 (12)	3 (21)	7 (10)
Palliative radiotherapy of bone or soft-tissue metastasis	11 (13)	3 (21)	8 (11)
Stereotactic radiotherapy for oligo metastasis	2 (2)	0 (0)	2 (3)
Whole brain radiotherapy	3 (3)	0 (0)	3 (4)
Palliative thoracic radiotherapy with or without lymph nodes	3 (3)	1 (7)	2 (3)
During mobocertinib administration			
No radiotherapy	78 (91)	13 (93)	65 (90)
Stereotactic radiotherapy of brain metastasis	2 (2)	0 (0)	2 (3)
Palliative radiotherapy of bone or soft-tissue metastasis	4 (5)	1 (7)	3 (4)

Data cut-off date: 05-Apr-2023. [a]Percentage may not be 100 because of rounding.

Table S2: Exploratory analysis of covariates for progression-free survival

Variable	Availability of Data	Hazard Ratio (95% CI)	P-value for Cox regression	Proportional hazards assumption
TP53: mutation vs. wildtype	N = 55 / 86 (64%)	0.959 (0.526 – 1.747)	0.891	True
Mobo given in firstline vs. later line	N = 86/86 (100%)	1.346 (0.685 -2.642)	0.389	Not true
Baseline brain metastases vs. no brain metastases	**N = 86/86 (100%)**	**0.358 (0.209 – 0.615)**	**<0.001**	**True**
ECOG 0 vs. ECOG 1-3	N = 86/86 (100%)	0.585 (0.358 – 0.956)	0.32	True
Female vs. male	N = 86/86 (100%)	0.977 (0.571 – 1.672)	0.934	True
Age ≤ 65 years vs. > 65 years	N = 86/86 (100%)	1.112 (0.685 – 1.805)	0.668	Not true
Discontinuation due to AE vs. no discontinuation	**N = 86/86 (100%)**	**0.426 (0.231 – 0.787)**	**0.006**	**True**
Near-loop vs. far-loop mutation	N = 65/86 (76%)	0.961 (0.404 – 2.285)	0.928	Not true

Hazard ratios and p-values were calculated using standard Cox regression without adjustment for multiple testing. The proportional hazards assumption was assessed visually on Kaplan-Meier plots. Significant results are labelled blue. Mobo = Mobocertinib. AE = Adverse event.

Table S3: In vitro sensitivity of EGFR exon 20 mutation with or without EGFR p.G721S to EGFR inhibitors

Mutation	Erlotinib	Gefitinib	Osimertinib	Afatinib	Poziotinib	Mobo-certinib
p.L858R	8,7	6,0	9,3	0,6	0,2	2,6
p.L858R + p.T790M	N.A.	N.A	34,2	98,4	26,6	32,8
p.D770_772dup	>3000	>3000	312,6	82,8	2,4	45,4
p.S768_D770dup	**>3000**	**>3000**	**262,9**	**57,1**	**0,6**	**21,2**
p.S768_D770dup + p.G721S	**>3000**	**>3000**	**252,3**	**53,1**	**0,7**	**22,2**

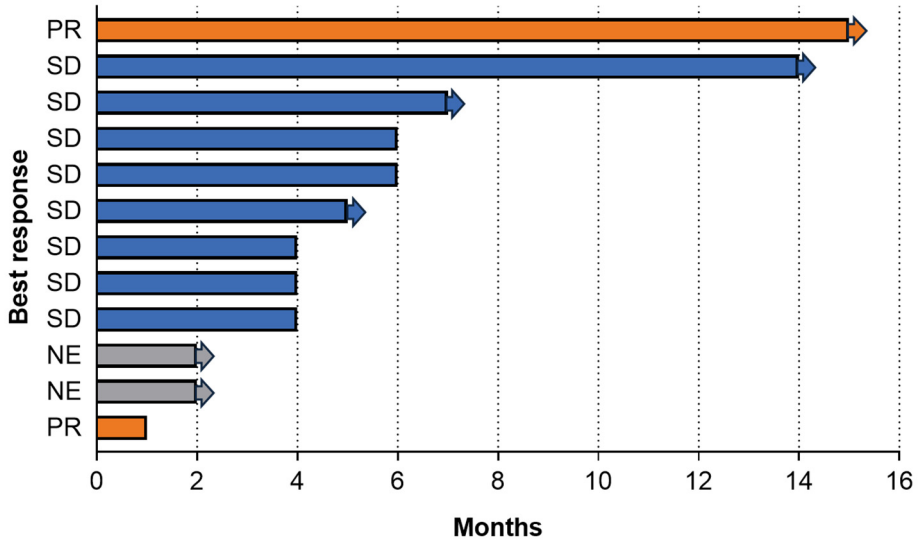
Ba/F3 cells retrovirally transduced with overexpression vectors encoding the EGFR exon 20 insertion p.S768_D770dup alone or in combination with the acquired EGFR p.G721S variant, and controls were deprived of IL-3 and treated with different concentrations of the indicated EGFR inhibitors. Half maximal inhibitory concentration (IC50) is provided in nM.

Table S4: EGFR mutation subtypes

Region	Mutation	Number of patients
αC helix	p.A763_Y764insFQEA	2
αC helix (but resistant to EGFRi)	p.Y764_V765insHH	1
Near-loop mutations	p.A767_S768insTLA	1
	p.A767_V769dup	15
	p.S768_D770dup	8
	p.V769_D770insQ	1
	p.V769_D770insTSV	1
	p.D770_N771insGF	1
	p.D770_N771insGL	1
	p.D770_N771insSVQ	1
	p.D770delinsGY	1
	p.D770_P772dup	2
	p.D770_N771insG	6
	p.N771dup	1
	p.N771_H773delinsLM	1
	p.N771_P772insT	1
	p.N771delinsGF	1
	p.N771delinsKH	2
	p.N771_H773dup	6
	p.P772_H773dup	1
	p.P772_H773insPNP	1
Far-loop mutations	p.H773dup	6
	p.H773_C775delinsPTP	1
	p.H773_V774delinsLM	1
	p.H773_V774dup	1
	p.H773_V774insPHPH	1

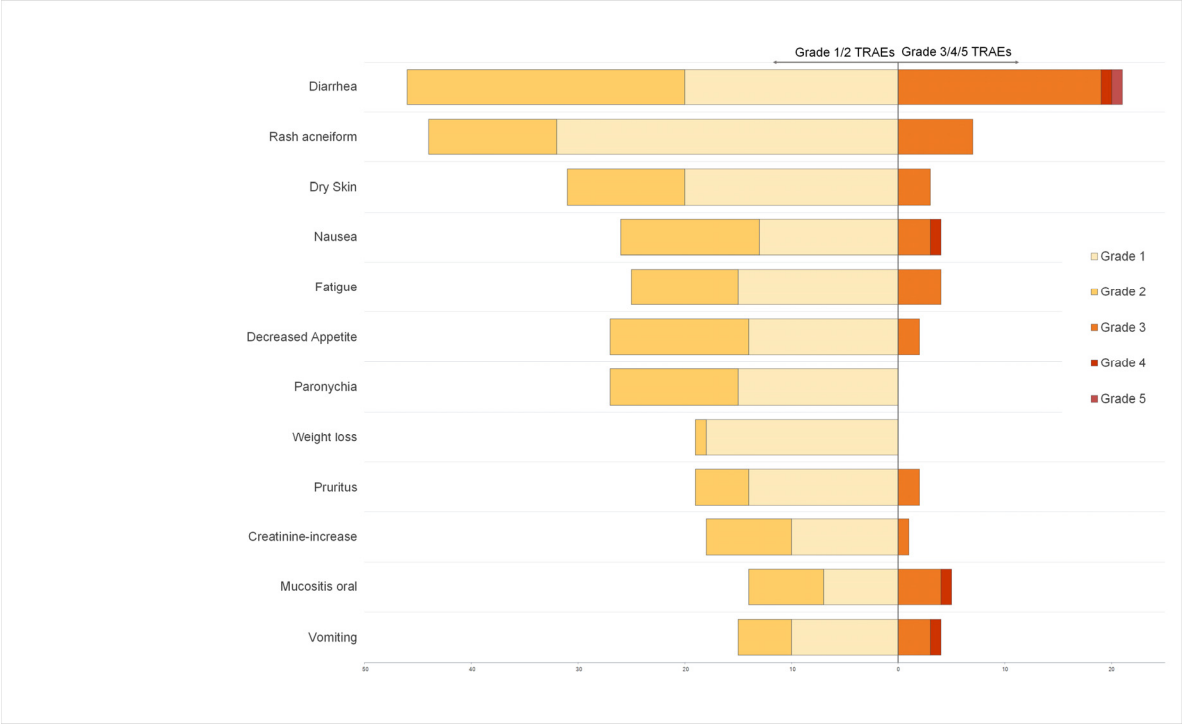
Amino acid changes as predicted from DNA variants based on transcript NM_005228.5 / ENST00000275493.7. Near-lopp/Far-loop classification is based on Robichaux et al..[9] Sequencing was performed by massive parallel sequencing panel analysis (NGS) and region-specific PCR assays in 87% and 13% of cases, respectively. Exact mutation name as depicted here was available for 65 of 86 patients.

Figure S1: Amivantamab following progression to mobocertinib



Swimmer plot for time on treatment. Arrows indicating ongoing treatment. Colors correspond to best objective response to amivantamab. PR: partial response (orange). SD: stable disease (blue). NE: not estimated (grey).

Figure S2: Treatment related adverse events



Data cut-off date: 05-Apr-2023. Treatment-related adverse events (TRAEs) that occurred at any grade in at least 20% of patients. The X-axis shows the number of patients who experienced the TRAE. The analysis included all patients who received at least one dose of mobocertinib. Relatedness of any adverse event to the treatment was assessed by the treating physician. TRAEs were graded as per Common Terminology Criteria for Adverse Events (CTCAE, version 5.0). Grading was not available for one case of diarrhea and fatigue and two cases of weight loss. Percentage may not equal to 100 because of rounding.

Methods S1: Targets of custom EGFR panels

	Gene	Transcript ID	Exons	Gene	Transcript ID	Exons
nNGM Panel Version 2.0	ALK	ENST00000389048.3	Exon 22-25	BRAF	ENST00000288602.6	Exon 11,15
	CTNNB1	ENST00000349496	Exon 3	EGFR	ENST00000275493.2	Exon 18-21
	ERBB2	ENST00000269571.5	Exon 8,19,20	FGFR1	ENST00000447712.2	Exon 4-7,10,12,13-15
	FGFR2	ENST00000358487.9	Exon 6-15,18	FGFR2	ENST00000457416	Exon 8, 9, 12, 18
	FGFR3	ENST00000440486.2	Exon 3,6,7,9,10,12,14,16,18	FGFR4	ENST00000292408.4	Exon 3,6,9,12,13,15,16
	HRAS	ENST00000311189.8	Exon 2-4	IDH1	ENST00000345146.2	Exon 4
	IDH2	ENST00000330062.3	Exon 4	KEAP1	ENST00000171111.10	Exon 2-6
	KRAS	ENST00000311936.7	Exon 2-4	MAP2K1	ENST00000307102.5	Exon 2,3
	MET	ENST00000397752.3	Intron 13,14 + Exon 14,16-19	NRAS	ENST00000369535.4	Exon 2-4
	NTRK1	ENST00000524377.5	Exon 13-17	NTRK2	ENST00000277120.7	Exon 14-19
	NTRK3	ENST00000360948.6	Exon 15-20	PIK3CA	ENST00000263967.3	Exon 8, 10,21
	PTEN	ENST00000371953.3	Exon 1-8	RET	ENST00000355710.8	Exon 10-18
	ROS1	ENST00000326873.11	Exon 34-41	STK11	ENST00000326873.11	Exon 1-9
	TP53	ENST00000269305.4	Exon 4-8			
Mini-nNGM Panel Version 2.0	ALK	ENST00000389048.3	Exon 22-25	BRAF	ENST00000288602.6	Exon 11,15
	EGFR	ENST00000275493.2	Exon 18-21	KRAS	ENST00000311936.7	Exon 2-4
	MET	ENST00000397752.3	Intron 13,14 + Exon 14,16-19	NRAS	ENST00000369535.4	Exon 2-4
	PIK3CA	ENST00000263967.3	Exon 8, 10,21			
RNA Scan CFHS-10224Z-571 Panel Whole Genes only	ALK	BAG4	BRAF	CCDC6	CD74	CUX1
	DCBLD1	EGFR	EGFR-AS1	EML4	ETV6	EZR
	FGFR1	FGFR2	FGFR3	GOPC	HIP1	KIF5B
	KLC1	LOC107984219	LOC107985874	LRIG3	MET	MPRIP
	NRG1	NTRK1	NTRK2	NTRK3	NTRK3-AS1	RAD51
	RET	ROS1	SDC4	SLC34A2	STRN	TACC3
	TFG	TPM3	TPR	TRIM33		