Clinical value of *EGFR* copy number gain determined by amplicon-based targeted next generation sequencing in *EGFR* mutated NSCLC patients

Jiacong Wei[1,4,+], Pei Meng[2,5,+], Miente Martijn Terpstra[1], Anke van Rijk[2], Menno Tamminga[3], Frank Scherpen[2], Arja ter Elst[2], Mohamed Z. Alimohamed [1,9], Lennart F. Johansson[1], Jos Stigt[6], Rolof P.G. Gijtenbeek[7], John van Putten[8], T. Jeroen N. Hiltermann[3], Harry J.M. Groen[3], Klaas Kok[1], Anthonie J. van der Wekken[3], Anke van den Berg[2]*

[1]Department of Genetics, University of Groningen, University Medical Center Groningen, Netherlands

[2]Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Netherlands

[3]Department of Pulmonary Diseases, University of Groningen, University Medical Center Groningen, Netherlands

[4]Department of Pathology, Cancer Hospital Chinese Academy of Medical Sciences, Beijing, China

[5]Department of Pathology, Collaborative and Creative Centre, Shantou University Medical College, Shantou, Guangdong, China

[6]Department of Pulmonary Diseases, Isala Clinic, Zwolle, Netherlands

[7]Department of Pulmonary Diseases, Medical Center Leeuwarden, Leeuwarden, Netherlands

[8]Department of Pulmonary Diseases, Martini Hospital, Groningen, Netherlands

[9]Department of Haematology and Blood Transfusion, Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, Tanzania

[+]Authors contributed equally

*Corresponding author

Address for correspondence:

Anke van den Berg

Department of Pathology & Medical Biology, HPC: EA10, Room F0-15

University Medical Center Groningen

Hanzeplein 1

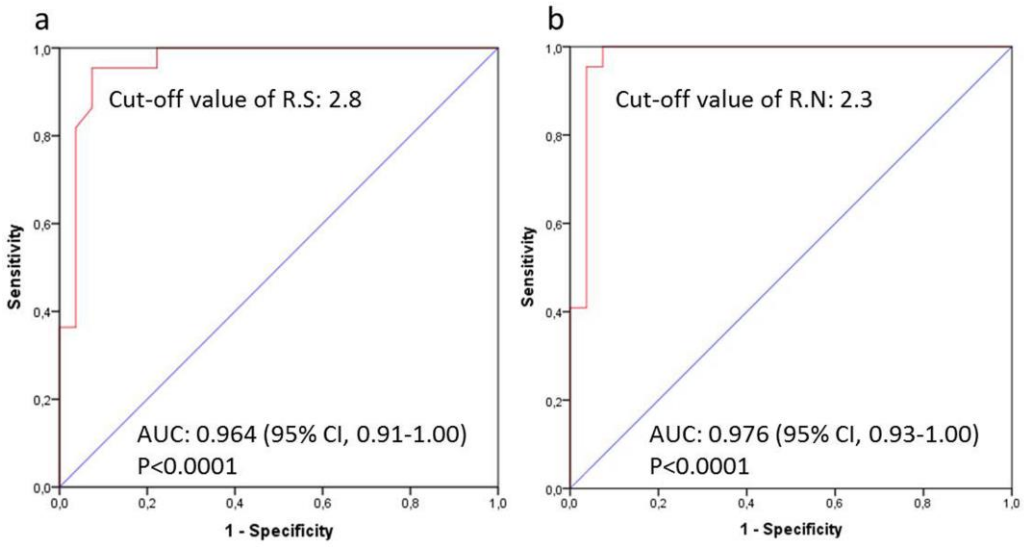
9713 GZ Groningen

Phone: +31-50-3611476

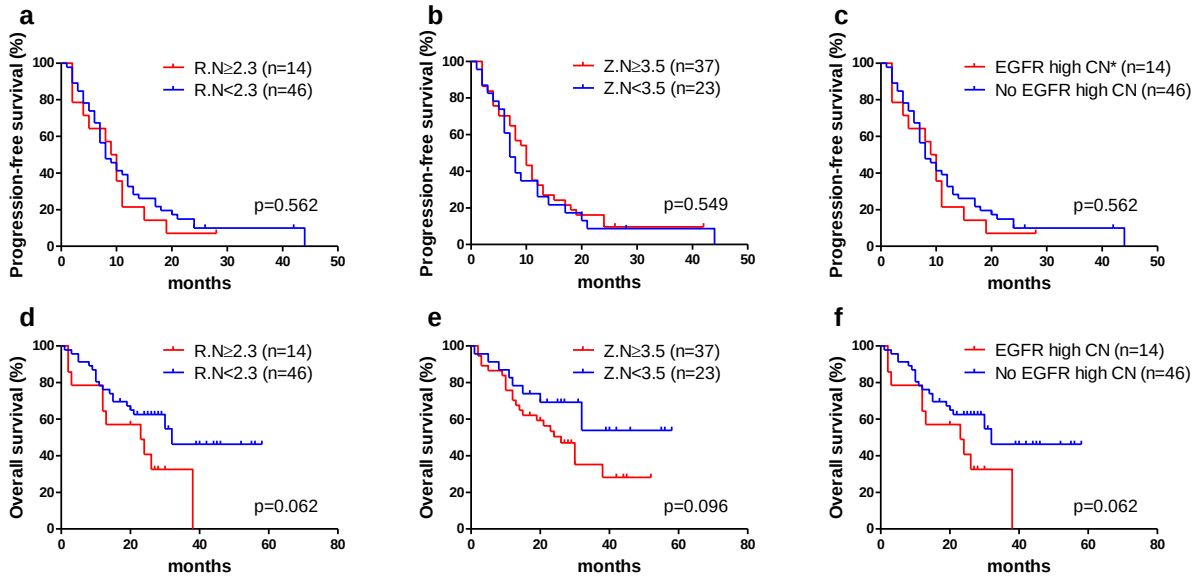
Email: a.van.den.berg01@umcg.nl

The authors declare no potential conflicts of interest.

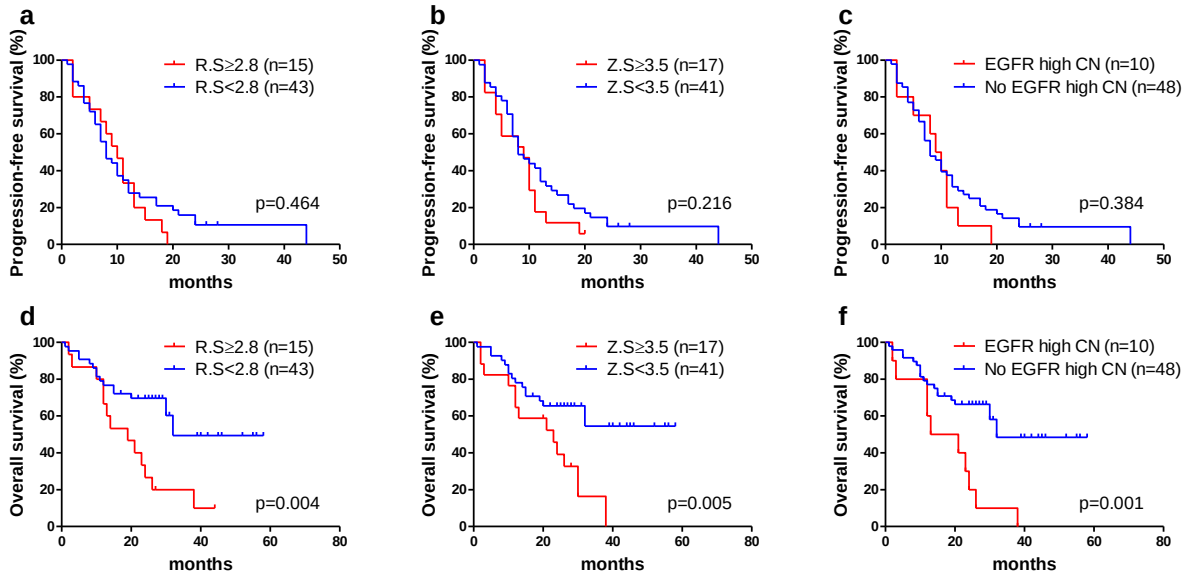
Supplementary Data



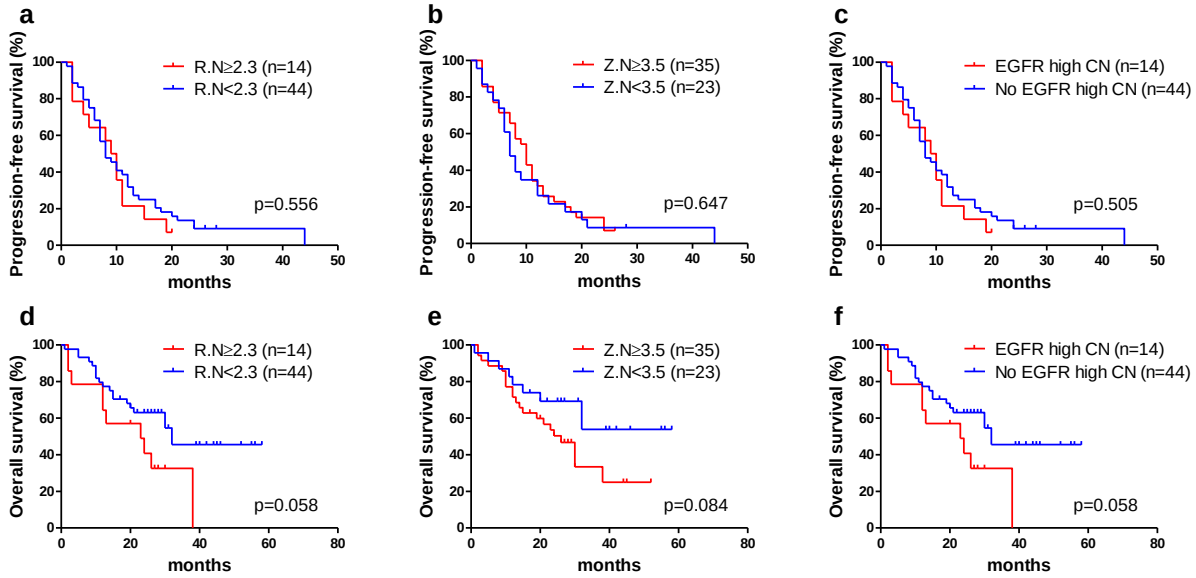
Supplementary Figure S1. ROC curve analysis to determine EGFR ratio cut-off by **a)** internal comparison approach and **b)** normal comparison approach.



Supplementary Figure S2. Kaplan Meier plots of progression free and overall survival for ratio, z score and the combined score (indicated as *EGFR* high CN or no *EGFR* high CN) of *EGFR* gain using the compared with normal samples approach. **a, b, c)** Progression-free survival time of *EGFR* mutated patients based on ratio ≥ 2.3, z score ≥ 3.5 and the combined criteria. **d, e, f)** Overall survival of *EGFR* mutated patients based on *EGFR* ratio ≥ 2.3, z score ≥ 3.5, and the combined criteria. No significant differences were observed. *CN: copy number

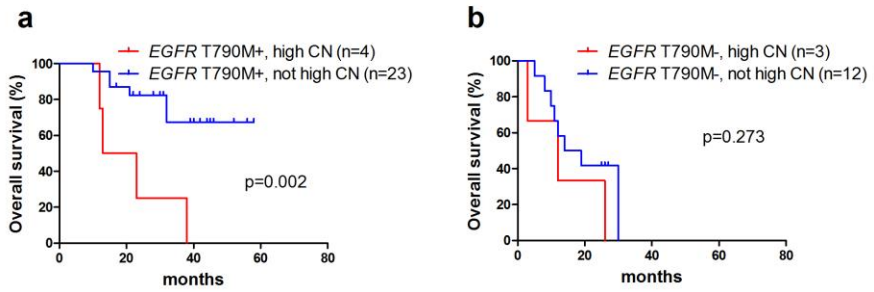


Supplementary Figure S3. Kaplan Meier plots of progression free and overall survival in 58 patients excluded two patients received osimertinib as first-line treatment using the internal comparison approach. **a, b, c)** Progression-free survival time of EGFR mutated patients based on ratio ≥ 2.8, z score ≥ 3.5 and the combined criteria. **d, e, f)** Overall survival of EGFR mutated patients based on EGFR ratio ≥ 2.8, z score ≥ 3.5, and the combined criteria.

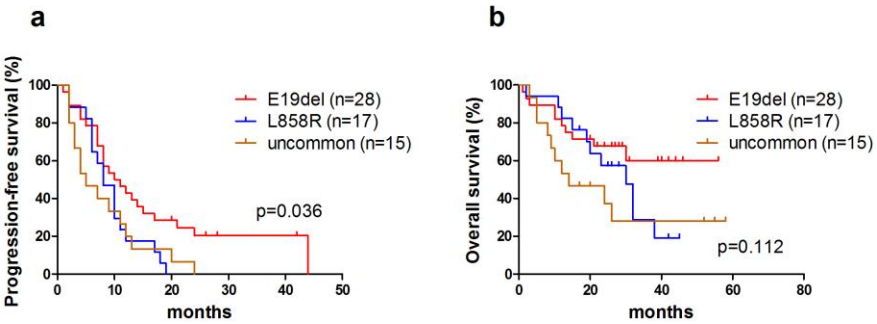


Supplementary Figure S4. Kaplan Meier plots of progression free and overall survival in 58 patients excluded two patients received osimertinib as first-line treatment using the normal

comparison approach. **a, b, c)** Progression-free survival time of EGFR mutated patients based on ratio ≥ 2.3, z score ≥ 3.5 and the combined criteria**. d, e, f)** Overall survival of EGFR mutated patients based on EGFR ratio ≥ 2.3, z score ≥ 3.5, and the combined criteria.



Supplementary Figure S5. Kaplan Meier analysis of copy number using the Internal comparison approach on OS for patients with or without an *EGFR* T790M at relapse. **a)** OS of 27 patients with T790M. T790M+ patients with *EGFR* high copy numbers had a shorter survival compared to those without high copy numbers. **b)** OS of 15 patients without T790M. All these T790M- patients had a short survival compared to *EGFR* T790M+, not *EGFR* high copy number patients.



Supplementary Figure S6. Kaplan Meier plots of survival analysis among different *EGFR* activating mutation types in baseline biopsies. **a)** Patients with *EGFR* E19DEL in baseline biopsy had a longer PFS time compared with patients with L858R mutation or uncommon *EGFR* activating mutation. **b)** There was no difference for OS among patients with different mutation types. Uncommon: other *EGFR* TKI-sensitive mutations.

Supplementary Table S1. Overview of the hotspot regions included in the two designs used in the routine diagnostics.

Amplicon ID	Panel Design	Library Pool	Gene	Exon	Chr	Start	Stop	Coefficient of Variation	Reference Amplicon
NRAS_E2	Design 1	NA	*NRAS*	E2	1	115,252,142	115,252,336	0.47	YES
NRAS_E3	Design 1	NA	*NRAS*	E3	1	115,256,404	115,256,624	0.51	NO
NRAS_E4	Design 1	NA	*NRAS*	E4	1	115,258,640	115,258,864	0.42	YES
ALK_E23	Design 1	NA	*ALK*	E23	2	29,432,582	29,432,798	0.50	NO
ALK_E25	Design 1	NA	*ALK*	E25	2	29,443,544	29,443,753	0.44	YES
PIK3CA_E10	Design 1	NA	*PIK3CA*	E10	3	178,936,053	178,936,181	0.80	NO
PIK3CA_E21	Design 1	NA	*PIK3CA*	E21	3	178,951,972	178,952,197	0.49	YES
PDGFRA_E12	Design 1	NA	*PDGFRA*	E12	4	55,140,935	55,141,138	0.75	NO
PDGFRA_E14	Design 1	NA	*PDGFRA*	E14	4	55,144,042	55,144,265	0.35	YES
PDGFRA_E18	Design 1	NA	*PDGFRA*	E18	4	55,151,981	55,152,185	0.36	YES
KIT_E8	Design 1	NA	*KIT*	E8	4	55,589,673	55,589,886	0.50	NO
KIT_E9	Design 1	NA	*KIT*	E9	4	55,592,034	55,592,251	0.47	YES
KIT_E11	Design 1	NA	*KIT*	E11	4	55,593,531	55,593,755	0.53	NO
KIT_E13	Design 1	NA	*KIT*	E13	4	55,594,129	55,594,354	0.53	NO
KIT_E14	Design 1	NA	*KIT*	E14	4	55,595,452	55,595,680	0.56	NO
KIT_E17	Design 1	NA	*KIT*	E17	4	55,599,231	55,599,444	0.47	YES
EGFR_E18	Design 1	NA	*EGFR*	E18	7	55,241,597	55,241,800	0.68	NO
EGFR_E19	Design 1	NA	*EGFR*	E19	7	55,242,353	55,242,556	0.62	NO
EGFR_E20	Design 1	NA	*EGFR*	E20	7	55,248,917	55,249,145	0.89	NO
EGFR_E21	Design 1	NA	*EGFR*	E21	7	55,259,368	55,259,594	0.67	NO
BRAF_E11	Design 1	NA	*BRAF*	E11	7	140,453,030	140,453,241	0.92	NO
BRAF_E15	Design 1	NA	*BRAF*	E15	7	140,481,298	140,481,518	0.84	NO
GNAQ_E5	Design 1	NA	*GNAQ*	E5	9	80,409,462	80,409,591	0.71	NO
KRAS_E2	Design 1	NA	*KRAS*	E2	12	25,378,432	25,378,651	0.79	NO
KRAS_E3	Design 1	NA	*KRAS*	E3	12	25,380,180	25,380,336	0.50	NO
KRAS_E4	Design 1	NA	*KRAS*	E4	12	25,398,184	25,398,387	0.55	NO
ERBB2_E23	Design 1	NA	*ERBB2*	E23	17	37,880,117	37,880,341	0.67	NO
ERBB2_E24	Design 1	NA	*ERBB2*	E24	17	37,880,910	37,881,090	0.66	NO

Amplicon ID	Panel Design	Library Pool	Gene	Exon	Chr	Start	Stop	Coefficient of Variation	Reference Amplicon
ERBB2_E25	Design 1	NA	*ERBB2*	E25	17	37,881,284	37,881,499	0.68	NO
GNA11_E5	Design 1	NA	*GNA11*	E5	19	3,118,790	3,119,018	0.77	NO
NRAS_E4	Design 2	1	*NRAS*	E4	1	115,252,186	115,252,314	1.00	YES
NRAS_E3	Design 2	2	*NRAS*	E3	1	115,256,462	115,256,578	0.48	NO
NRAS_E2	Design 2	1	*NRAS*	E2	1	115,258,686	115,258,805	0.28	YES
H3F3A_E2	Design 2	2	*H3F3A*	E2	1	226,252,098	226,252,197	0.67	NO
ALK_E25	Design 2	1	*ALK*	E25	2	29,432,581	29,432,710	1.45	NO
ALK_E23.2	Design 2	2	*ALK*	E23.2	2	29,443,568	29,443,695	0.34	YES
ALK_E23.1	Design 2	1	*ALK*	E23.1	2	29,443,686	29,443,766	0.57	NO
ALK_E22	Design 2	2	*ALK*	E22	2	29,445,209	29,445,319	0.35	YES
IDH1_E4	Design 2	1	*IDH1*	E4	2	209,113,081	209,113,196	0.27	YES
PIK3CA_E10.1	Design 2	1	*PIK3CA*	E10.1	3	178,936,052	178,936,169	0.63	NO
PIK3CA_E21.2	Design 2	2	*PIK3CA*	E21.2	3	178,952,018	178,952,135	0.61	NO
PDGFRA_E12.1	Design 2	1	*PDGFRA*	E12.1	4	55,140,936	55,141,058	0.33	YES
PDGFRA_E12.2	Design 2	2	*PDGFRA*	E12.2	4	55,141,047	55,141,154	0.26	YES
PDGFRA_E14.2	Design 2	1	*PDGFRA*	E14.2	4	55,144,027	55,144,127	0.40	NO
PDGFRA_E14.1	Design 2	2	*PDGFRA*	E14.1	4	55,144,116	55,144,191	0.55	NO
PDGFRA_E18.2	Design 2	1	*PDGFRA*	E18.2	4	55,151,969	55,152,087	0.31	YES
PDGFRA_E18.1	Design 2	2	*PDGFRA*	E18.1	4	55,152,076	55,152,162	0.38	NO
KIT_E8.2	Design 2	1	*KIT*	E8.2	4	55,589,635	55,589,765	0.42	NO
KIT_E8.1	Design 2	2	*KIT*	E8.1	4	55,589,754	55,589,875	0.58	NO
KIT_E9.1	Design 2	1	*KIT*	E9.1	4	55,592,014	55,592,139	0.34	NO
KIT_E9.2	Design 2	2	*KIT*	E9.2	4	55,592,130	55,592,244	0.83	NO
KIT_E11.1	Design 2	1	*KIT*	E11.1	4	55,593,518	55,593,634	0.50	NO
KIT_E11.2	Design 2	2	*KIT*	E11.2	4	55,593,623	55,593,725	0.38	NO
KIT_E13.2	Design 2	1	*KIT*	E13.2	4	55,594,096	55,594,221	0.34	NO
KIT_E13.1	Design 2	2	*KIT*	E13.1	4	55,594,210	55,594,296	0.33	YES
KIT_E14.2	Design 2	1	*KIT*	E14.2	4	55,595,494	55,595,605	0.70	NO
KIT_E14.1	Design 2	2	*KIT*	E14.1	4	55,595,594	55,595,661	0.62	NO
KIT_E17.1	Design 2	1	*KIT*	E17.1	4	55,599,228	55,599,303	0.78	NO
KIT_E17.2	Design 2	2	*KIT*	E17.2	4	55,599,292	55,599,376	0.33	YES

Amplicon ID	Panel Design	Library Pool	Gene	Exon	Chr	Start	Stop	Coefficient of Variation	Reference Amplicon
KIT_E18.1	Design 2	1	*KIT*	E18.1	4	55,602,574	55,602,691	0.44	NO
KIT_E18.2	Design 2	2	*KIT*	E18.2	4	55,602,680	55,602,790	0.39	NO
ROS1_E41	Design 2	1	*ROS1*	E41	6	117,630,009	117,630,131	0.52	NO
ROS1_E38	Design 2	2	*ROS1*	E38	6	117,638,290	117,638,405	0.42	NO
ESR1_E9.1	Design 2	1	*ESR1*	E9.1	6	152,415,476	152,415,600	0.41	NO
ESR1_E9.2	Design 2	2	*ESR1*	E9.2	6	152,415,600	152,415,719	0.40	NO
ESR1_E10.1	Design 2	1	*ESR1*	E10.1	6	152,419,789	152,419,918	0.31	YES
ESR1_E10.2	Design 2	2	*ESR1*	E10.2	6	152,419,907	152,420,028	0.55	NO
EGFR_E12	Design 2	1	*EGFR*	E12	7	55,227,949	55,228,060	0.91	NO
EGFR_E18.2	Design 2	2	*EGFR*	E18.2	7	55,241,600	55,241,688	0.73	NO
EGFR_E18.1	Design 2	1	*EGFR*	E18.1	7	55,241,677	55,241,801	0.85	NO
EGFR_E19.1	Design 2	2	*EGFR*	E19.1	7	55,242,350	55,242,454	0.66	NO
EGFR_E19.2	Design 2	1	*EGFR*	E19.2	7	55,242,443	55,242,563	0.69	NO
EGFR_E20.1	Design 2	2	*EGFR*	E20.1	7	55,248,956	55,249,064	0.83	NO
EGFR_E20.2	Design 2	1	*EGFR*	E20.2	7	55,249,004	55,249,131	1.39	NO
EGFR_E20.3	Design 2	2	*EGFR*	E20.3	7	55,249,120	55,249,200	1.25	NO
EGFR_E21.2	Design 2	1	*EGFR*	E21.2	7	55,259,364	55,259,492	0.68	NO
EGFR_E21.1	Design 2	2	*EGFR*	E21.1	7	55,259,481	55,259,578	0.64	NO
MET_E14.1	Design 2	1	*MET*	E14.1	7	116,411,819	116,411,941	0.37	NO
MET_E14.2	Design 2	2	*MET*	E14.2	7	116,411,949	116,412,070	0.51	NO
BRAF_E15	Design 2	1	*BRAF*	E15	7	140,453,097	140,453,215	0.44	NO
BRAF_E11.2	Design 2	2	*BRAF*	E11.2	7	140,481,361	140,481,448	0.35	YES
BRAF_E11.1	Design 2	1	*BRAF*	E11.1	7	140,481,391	140,481,511	0.38	NO
JAK2_E13	Design 2	1	*JAK2*	E13	9	5,073,679	5,073,801	0.76	NO
GNAQ_E5	Design 2	2	*GNAQ*	E5	9	80,409,381	80,409,502	0.47	NO
GNAQ_E4	Design 2	1	*GNAQ*	E4	9	80,412,424	80,412,546	0.30	YES
HRAS_E4	Design 2	1	*HRAS*	E4	11	533,482	533,581	0.56	NO
HRAS_E3	Design 2	2	*HRAS*	E3	11	533,781	533,882	0.34	YES
HRAS_E2	Design 2	1	*HRAS*	E2	11	534,219	534,306	0.81	NO
KRAS_E4.1	Design 2	1	*KRAS*	E4.1	12	25,378,526	25,378,605	0.72	NO
KRAS_E4.2	Design 2	2	*KRAS*	E4.2	12	25,378,587	25,378,663	0.43	NO

Amplicon ID	Panel Design	Library Pool	Gene	Exon	Chr	Start	Stop	Coefficient of Variation	Reference Amplicon
KRAS_E3.2	Design 2	1	*KRAS*	E3.2	12	25,380,169	25,380,270	4.48	NO
KRAS_E3.1	Design 2	2	*KRAS*	E3.1	12	25,380,259	25,380,337	1.08	NO
KRAS_E2	Design 2	1	*KRAS*	E2	12	25,398,188	25,398,310	0.55	NO
POLE_E13	Design 2	2	*POLE*	E13	12	133,250,220	133,250,310	0.28	YES
POLE_E9	Design 2	1	*POLE*	E9	12	133,253,118	133,253,245	0.35	NO
AKT1_E3	Design 2	2	*AKT1*	E3	14	105,246,472	105,246,583	0.34	YES
MAP2K1_E2	Design 2	1	*MAP2K1*	E2	15	66,727,413	66,727,523	0.31	YES
MAP2K1_E3	Design 2	2	*MAP2K1*	E3	15	66,729,091	66,729,218	0.42	NO
MAP2K1_E6	Design 2	1	*MAP2K1*	E6	15	66,774,070	66,774,199	0.27	YES
MAP2K1_E7.1	Design 2	2	*MAP2K1*	E7.1	15	66,777,302	66,777,413	0.27	YES
MAP2K1_E7.2	Design 2	1	*MAP2K1*	E7.2	15	66,777,402	66,777,522	0.42	NO
MAP2K1_E11	Design 2	2	*MAP2K1*	E11	15	66,782,826	66,782,955	0.45	NO
IDH2_E4	Design 2	1	*IDH2*	E4	15	90,631,757	90,631,886	0.53	NO
ERBB2_E23.2	Design 2	2	*ERBB2*	E23.2	17	37,880,146	37,880,238	0.69	NO
ERBB2_E23.1	Design 2	1	*ERBB2*	E23.1	17	37,880,227	37,880,352	1.48	NO
ERBB2_E24	Design 2	2	*ERBB2*	E24	17	37,880,960	37,881,060	0.62	NO
ERBB2_E25	Design 2	1	*ERBB2*	E25	17	37,881,324	37,881,453	0.72	YES
H3F3B_E3	Design 2	2	*H3F3B*	E3	17	73,775,027	73,775,156	0.51	NO
H3F3B_E2	Design 2	1	*H3F3B*	E2	17	73,775,144	73,775,227	0.91	NO
GNA11_E4	Design 2	2	*GNA11*	E4	19	3,114,959	3,115,051	0.61	NO
GNA11_E5	Design 2	1	*GNA11*	E5	19	3,118,875	3,118,993	0.52	NO
GNAS_E8	Design 2	2	*GNAS*	E8	20	57,484,359	57,484,474	0.58	NO
GNAS_E9	Design 2	1	*GNAS*	E9	20	57,484,534	57,484,659	0.30	YES
AMELY_E3*	Design 2	2	*AMELY*	E3	Y	6,738,019	6,738,142	Na	NO

* AMELY is a housekeeping gene that is in chromosome Y, thus not applicable for coefficient of variation calculation

Supplementary Table S2. Type of *EGFR* mutations reported in the diagnostic setting in NSCLC patients (2014-2017).

***EGFR* mutation**	**No. Samples / Patients**	**Frequency in cohort**
E19 DEL	85 / 66	4.2%
L858R	71 / 51	3.3%
G719A/C/S	28 / 17	1.1%
E20 INDEL[b]	23 / 17	1.1%
S768I	9 / 6	0.4%
C797S[b]	4 / 2	0.1%
E709A/K	6 / 3	0.2%
L861Q	6 / 4	0.3%
A840T[b]	3 / 3	0.2%
V689L	2 / 2	0.1%
L747P[b]	2 / 2	0.1%
D761N	2 / 1	0.1%
R776G[a]	2 / 1	0.1%
T488N[a]	1 / 1	0.1%
K714N[a]	1 / 1	0.1%
T751_S752del[a]	1 / 1	0.1%
A755V[a]	1 / 1	0.1%
Y764Y[a]	1 / 1	0.1%
V769*[b]	1 / 1	0.1%
C775Y[a]	1 / 1	0.1%
R776H	1 / 1	0.1%
R776L	1 / 1	0.1%
T790S[a]	1 / 1	0.1%
L792H	1 / 1	0.1%
G810V[a]	1 / 1	0.1%
D830Y	1 / 1	0.1%
V834L	1 / 1	0.1%
R836L[a]	1 / 1	0.1%
P848L[b]	1 / 1	0.1%
L861R	1 / 1	0.1%
H870R[a]	1 / 1	0.1%

[a] Response of this variant to EGFR-TKI is unknown
[b] Variants reported to be unresponsive or resistant to EGFR-TKI

Supplementary Table S3: Univariate analysis for progression-free survival

Variables		n	Univariate analysis		
			HR	95% confidence interval	p
Age	<60	17			
	≥60	43	1.02	0.56-1.86	0.939
Gender	Female	40			
	Male	20	1.31	0.74-2.31	0.353
Smoking	Non-smoker	24			
	Smoker (former and current)	36	1.09	0.63-1.88	0.764
VAF	<50%	28			
	≥50%	32	1.29	0.75-2.23	0.354
Activating mutation	E19DEL	28			
	L858R	17	1.89	0.98-3.62	0.058
	Uncommon	15	2.1	1.08-4.08	0.028
First-line EGFR-TKI	Afatinib	14			
	Gefitinib	28	1.05	0.54-2.07	0.884
	Erlotinib	16	1.19	0.57-2.52	0.641
	Osimertinib[#]	2	-	-	-
High copy number (internal comparison)	Low	49			
	High	11	1.51	0.77-2.96	0.235
High copy number (normal comparison)	Low	46			
	High	14	1.26	0.67-2.37	0.479

[#] Limited two patients that did not include for univariate analysis

Supplementary Table S4: Univariate and multivariate analysis for overall survival information

Variables		n	Univariate analysis			multivariate analysis			multivariate analysis*		
			HR	95% confidence interval	p	HR	95% confidence interval	p	HR	95% confidence interval	p
Age	<60	17									
	≥60	43	1.44	0.62-3.35	0.396						
Gender	Female	40									
	Male	20	1.54	0.75-3.15	0.237						
Smoking	Non-smoker	24									
	Smoker (former and current)	36	1.17	0.56-2.44	0.682						
VAF	<50%	28									
	≥50%	32	1.61	0.78-3.33	0.195						
Activating mutation	E19DEL	28									
	L858R	17	1.74	0.74-4.11	0.204	1.52	0.64-3.61	0.349	1.81	0.77-4.27	0.349
	Uncommon	15	2.46	1.02-5.93	0.046	1.89	0.774.68	0.17	2.4	0.99-5.78	0.052
First-line EGFR-TKI	Afatinib	14									
	Gefitinib	28	0.55	0.22-1.36	0.195						
	Erlotinib	16	0.81	0.30-2.13	0.663						
	Osimertinib[#]	2	-	-	-						
EGFR High copy number (internal comparison)	Low	49									
	High	11	3.62	1.71-7.64	0.001	3.14	1.46-6.78	0.003			
EGFR High copy number (normal comparison)	Low	46									
	High	14	2.02	0.94-4.33	0.07				2.01	0.94-4.32	0.074

* Because EGFR high copy number determined by internal and normal comparisom approaches are not independent covariates, we did multivariate analysis for 2 separate times. [#]Limited two patients that did not include for univariate analysis

Supplementary Table S5. Second-line treatment of the 60 NSCLC patients

Second-line treatment	n=60	
1st and 2nd generation EGFR-TKIs	12	
afatinib		4
gefitinib		4
gefitinib/crizotinib		1
erlotinib		3
3rd generation EGFR-TKIs	26	
osimertinib*		25
rociletinib		1
Chemotherapy	7	
Pemetrexed/Carboplatin	6	
pemetrexed/cisplatin	1	
trastuzumab/pertuzumab	1	
Stereotactic body radiation therapy	1	
no further treatment	12	
unknown	1	

*Two patients received third-line and one patient received fourth-line osimertinib treatment.

Supplementary Table S6: Univariate and multivariate analysis for overall survival regarding second-line or subsequent lines treatment

Variables			Univariate analysis			multivariate analysis			multivariate analysis*		
			HR	95% confidence interval	p	HR	95% confidence interval	p	HR	95% confidence interval	p
EGFR High copy number (internal comparison)	Low	49									
	High	11	3.62	1.71-7.64	0.001	3.8	1.78-8.1	0.001	2.79	1.29-6.02	0.009
presence of T790M at progressive disease	no	15									
	yes	27	0.24	0.1-0.58	0.002	0.24	0.1-0.59	0.002			
	missing	18	0.59	0.25-1.41	0.233	0.67	0.28-1.62	0.38			
3rd generation EGFR-TKI as second-line or more lines treatmenr	No	26									
	Yes	29	0.38	0.18-0.81	0.012				0.43	0.20-0.91	0.028
	Others#	5									

* Because presence of T790M mutation after resistance to firsr-line EGFR-TKI and osimertinib treatment are not independent covariates, we did multivariate analysis for 2 separate times. # Others include one patient for whom no second-line treatment information could be retrieved, the two patients received radiotherapy or EGFR antibody treatment as second-line treatment, and the two patients that had osimertinib in the first-line treatment.