SUPPLEMENTARY INFORMATION CHAPTER 2

Supplementary Table 1. The *EGFR*ex20+ mutations (original data) from 36 studies and case reports to evaluate the distribution and relative frequency of different NSCLC *EGFR*ex20+ mutations by amino acid position. Some amino acid sequence changes within *EGFR* exon 20 were 'unknown' because (1) the exact variant was not reported, (2) the insertion mutations were identified just by PCR testing or (3) the exact variant could not be characterized by Sanger sequencing due to very low mutant peaks. The 'other' mutations within *EGFR* exon 20 are, for example, rare point mutations or coexisting mutations together with *EGFR* exons other than exon 20 and where therefore excluded for table 1. The point mutation p.S768I [a substitution at codon 768 of exon 20 (c.2303G>T, p.S768I)] was also excluded from table 1, since it is not a insertion/deletion.

Joan luis leal et al, 2021	#	Cardona et al, 2018	#	Beau Faller et al, 2014	#	Oxnard et al, 2013	#	Naidoo et al, 2015	#	Koogman et al, 2021	#	Wu et al, 2019	#	Yang et al, 2021	#	Arcila et al, 2013	#	Prelaj et al, 2021	#	Riess et al, 2018	#	Riely et al, 2022	#
V769_D770insASV	11	H773insH	19	A767 V769dupASV	5	V769_D770insASV	6	D770_N771insSVD	11	A763_Y764insFQEA	1	A763_Y764insFQEA	9	D770_N771insSVD	17	D770_N771insSVD	7	H773_V774insH	1	A767_V769dupASV	55	A763_Y764insFQEA	2
D770_N771insSVD	7	V769_D770insASV	16	D761_E762insEAFQ	1	H773insH	3	V769_D770insASV	10	A767_V769dup	16	A767_V769dupASV	30	V769_D770insASV	11	V769_D770insASV	4	H773_V774insAH	2	S768_D770dupSVD	52	A767_V769dup	5
N771_P772insPH	4	A763_Y764insFQEA	13	A763_Y764insFQEA	1	A763_Y764insFQEA	3	H773_v774insNPH	4	S768_D770dup	9	S768_D770dupSVD	17	A763_Y764insFQEA	5	A767_S768insTLA	1	D770_N771insSVD	4	N771_H773dupNPH	20	S768_D770dup	1
D770insG	3	H773_V774insPH	13	M766_V769insWPA	1	H773_V774PH	2	H773_V774insH	4	D770_H773dup	1	V769_D770insGSV	1	D770delinsGY	3	H773_V774insNPH	2	V769_D770insASV	1	A763_Y764insFQEA	15	V769dup	1
H773_V774insPHPH	3	H773_V774insNPH	9	A767_S768insSVR	3	H773_V774NPH	2	H773_V774insPH	2	D770_N771insG	3	D770delinsGY	3	D770_N771insG	3	V774_C775insHV	2	A767_V769dup	2	D770_N771insG	10	D770delinsGY	1
H773dup	3	H773_V774insSVD	5	S768_D770dupSVD	3	V774_C775insHV	2	H773_V774insAH	2	D770_N771insGF	1	D770_N771insG	4	H773_V774insNPH	2	H773_V774insPH	3	M766_A767insASV	4	P772_H773dupPH	9	D770_N771insH	1
D770delinsGY	1	V774_C775insHV	4	V769_D770insG	1	D770_N771insSVD	2	A763_Y764insFQEA	1	D770_N771insSVA	2	D770_N771insGTT	1	V769_D770insSV	2	H773_V774insAH	1	S768_D770dup	1	H773dupH	8	D770_N771insAGH	1
H773_V774dup	1	D770delinsGY	4	V769_D770insL	1	D770delinsGY	2	A767_S768insTLA	1	D770_N771insT	1	D770_N771insY	1	V769_D770insASE	1	D770_N771insGT	1	P772_H773insHV	1	D770_N771>GYN	6	D770_N771insNPG	1
H773_V774insNPH	1	N771_P772insV	2	D770_H773dupTTP	1	N771_P772insV	1	V769_D770insGE	1	D770_N771insY	1	N771delinsTH	1	D770_N771insGTD	1	D770_N771insGF	1	D770_N771insG	1	H773_V774dupHV	6	N771dup	1
M766_A767insASV	1	N771delinsGY	2	D770_N771insSVD	4	P772_H773insPNP	1	V774_C775insHV	1	D770_P772dup	1	N771delinsKH	1	D770delinsDGT	1	D770>GY	1	Other	4	V774_C775>AHVC	6	P772_H773dup	1
N771_delinsGF	1	H773_V774insAH	1	N771dupN	1	D770_N771insGL	1	N771_P772insH	1	D770delinsEQPP	1	N771_P772insS	1	N771delinsGY	1	D770_N771insY	1			N771_P772>GYP	5	P772_H773insNPH	2
N771_H773dup	1			N771delinsKPP	1	N771delinsGY	1	N771_P772insN	1	D770delinsGY	3	N771_H773dupNPH	6	N771delinsRD	1	H773_V774insH	3	Zweifenga et al, 2021	#	D770_N771>GSVDN	4	P772_H773insPNP	1
N771_P772insT	1	Yang et al, 2020	#	P772_C775dupPHVC	1	H773_V774insAH	1	P772_H773insNP	11	N771_H773dup	2	P772_H773insGHP	1	N771delinsRH	1	N771_P772insH	1	A763_Y764insFQEA	1	N771_P772insH	3	H773_V774dup	1
P772_H773dup	1	V769_D770insASV	38	P772_H773insLGNP	1			P772_H773insNPH	1	N771_P772insH	1	P772_H773dup	1	N771delinsKH	1	A763_Y764insFQEA	3	A767_V769dup	3	Other	64	H773delinsSNPY	1
P772_H773insHV	1	D770_N771insSVD	29	P772_H773insDNP	1	Bycon et al, 2019	#	D770_N771insGV	1	N771_P772insR	1	P772_H773insTNP	1	N771delinsGF	1	Other	1	S768_D770dup	2			H773_V774insNPH	2
P772_H773insPHPH	1	H773_V774insNPH	11	P772_H773insT	1	His-773_Val774insHis	7	D770_N771insGT	1	N771delinsGF	1	H773_V774dup	1	N771_P772insNPN	1	Geng et al, 2022	#	D770_N771insG	2	N771_H773dup	1	Prelaj et al, 2022	#
P772_H773insRCP	1	D770_N771insG	8	H773_V774dupH	3	Val774_Cys775insHisVal	3	H773_V774insY	1	N771delinsGY	1	H773_V774dup	1	N771_P772insNPN	1	Geng et al, 2022	#	D770_N771insG	2	N771_H773dup	1	Prelaj et al, 2022	#
S768_V769delinsIL	1	H773_V774insH	6	H773_V774insPH	2	His773_Val774insProHis	2	Unknown	2	N771delinsKG	1	N771dup	2	H773dup	1	S768_D770dup	1	D770_P772dup	1	D770_P772dup	2	D770_N771insSVD	1
V769_P772dup	1	A763_Y764insFQEA	5	Unknown	11	His773_Val774insAsnProHis	1			N771delinsTH	1	P772_C775dupPHVC	1	P772_H773insPHP	1	N771_H773dup	1	N771_P772insH	2	A767_S768insTLA	1		
S786I	27	V774_C775insHV	4	Other	7	Pro772_His773insThrThrPro	1	Fang et al, 2019	#	P772 P772_C775dup	1	H773_V774insGTNPH	1	H773_V774insTH	1	A767_V769dup	2	N771_P772insV	1	A767_V769dup	3	Fang et al, 2019	#
Unknown	47	D770delinsGY	3			Pro772_His773insGlyAsnPro	1	A767_V769dup	1	P772_H773dup	3			H773_V774insPH	1	A763_Y764insFQEA	1	N771_H773dup	3	S768_D770dup	1	A767_S768insSVD	1
		H773_V774insTH	3	Jenn-Yu Wu et al, 2008	#	Val769_Asn771insValGlyVal	1	S768_D770dup	2	P772_H773insANP	1	Takeda et al, 2018	#	H773delinsYNPY	1	H773dup	1	P772_H773dup	2	H773dup	1		
Piotrowska et al, 2020	#	P772_H773insGHP	2	A767_V769dupASV	3	Asp-770_Asn771insGly	1	N771_P772insL	1	H773_V774dup	2	M766_A767insASV	2	H773delinsYPNPY	1	S768_D770dup	1	P772_H773insR	1	N771delinsSVDS	1	Chan et al, 2018	#
A767_V769dupASV	5	H773delinsPNPY	2	S768_D770dupSVD	4	S768I	17	D770_N771insG	1	H773_V774insAH	2	A767_S768insSVD	3	V774_C775insHV	1	N771delinsGY	1	Other	2			A767_V769dup	1
D770_N771insG	2	N771delinsGY	2	D770_N771insD	1	Other	4	A763_Y764insFQEA	1	H773delinsYNPY	1	V769_D770insASV	1	C775_R776insPHVC	1	D770_N771insGF	1			Piotrowska et al, 2018	#		
N771_P772insH	1	V774_C775insCPHV	2	D770_N771insG	2	Unknown	29			V774delinsHC	1	D770_N771insGL	1	Unknown	1	H773insHA	1	Riess et al, 2021	#	A763_Y764insFQEA	1	Lin et al, 2020	#
P772_H773insF	1	Unknown	24	N771_H773dupNPH	1			Tsigelny et al, 2015	#	H773dup	5	P772_H773insGNP	1			Unknown	2	A767_V769dup	3	A767_V769dup	5	N771delinsKG	1
D770_N771insSVD	1	Other	26	Other	9	Robichauxet al, 2018	#	exon 20 (D770>GY)	1	S768I	5	S768I	2	Van Veggel et al, 2018	#			S768_D770dup	2	S768_D770dup	4		
N771_H773dup	1					V769_D770insGSV	1	D770_P772del_insKG	1	Unknown	4	Other	2	Ser768_Asp770dup	1	Yang et al, 2022	#	N771delinsGY	1	D770_P772dup	1	Zöchbauer-Müller et al, 2021	#
V769_D770insASV	1	Van Veggel et al, 2020	#	Yasuda et al, 2013	#	H773_V774insAH	1	Other	1	Other	1			Asn771_His773dup	1	A763_Y764insFQEA	8	H773dup	1	D770_N771insY	1	H773dup	1
H773_V774insNPH	1	H773delinsIM	2	A763_Y764insFQEA	3	H773_V774insPR	1							His773dup	1	D770delinsGY	4	H773_V774dup	1	D770_N771insGV	1		
H773_V774insPH	1	H773delinsYNPY	1	Y764_V765insHH	1	N771_P772insHH	1							Ala767_Val769dup	1	V769_D770insASV	7	H773_V774insGTNPH	1	D770_N771insNPY	1	Urbán et al, 2021	#
P772_H773insARG	1	N771delinsTH	1	M766_A767insASV	1	D770delinsGY	1									D770_N771insSVD	9	V774_C775insHNPHV	1	D770_N771insQVH	1	N771_H773dup	1
		H773_V774insAH	2	A767_V769dupASV	1	D770_N771insG	1									P772_H773insH	1			N771delinsFH	1		
		A767_V769dup	4	V769_D770insASV	1	A767_V769dupASV	1									P772_H773insGHP	1			N771delinsGF	1	Zhu et al, 2022	#
		N771_H773dup	2	D770_N771insGL	2	S768_D770dupSVD	1									H773delinsRY	1			N771_H773dup	1	N771delinsGF	1
		S768_D770dup	1	D770_N771insGT	1	S768I	1									D770_N771insGD	1			N771_P772insH	1		
		N771_P772insH	1	D770_N771insSVD	2	P772_H773insDNP	1									H773_V774insAH	1			P772_H773dup	4		
		N771delinsGY	1	D770delinsGY	2											N771_P772insH	1			H773dup	1		
		S768_V769delinsIL	1	P772_H773insYNP	1											P772_H773insH	1						
		V769_D770insGG	1	P772_H773dup	1																		
		N771delinsGH	1	H773_V774insH (H773dup)	2																		
		V769_D770insSFL	1	H773_V774insNPH	1																		

#; represents the count of each EGFRex20+ mutation present in the study.

Supplementary Table 2. Overview of the original mutations (see supplementary table 1) and the annotation after converting according to the HGVS using the NP_005219.2 EGFR transcript. All variants were manually checked by adding the mutated amino acids into the *EGFR* exon 20 wild type amino acid position, as highlighted in yellow per variant. The *EGFR* exon 20 mutations variants M766_V769insWPA and D770_H773dupTTP (Beau Faller et al, 2014) were not recognized by the ncbi isoform and could not be manually reproduced, and are therefore excluded from the overview mentioned in supplementary Figure 1.

		Aminoacid position EGFR exon 20	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776			
			D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	R			
Input Status Normalized DNA	g. RNA Protein, mentioned in source article	EGFR mutation variant (protein abbreviation, after mutalyzer batch checker)																			
NP_005219.2:p.D761_E762insEAFQ	NP_005219.2:p.(Ala763_Tyr764insPheGlnGluAla)	A763_Y764insFQEA	D	E	A	F	Q	E	A	Y	V	M	A	S	V	D	N	P			
NP_005219.2:p.M766_A767insASV	NP_005219.2:p.(Ala767_Val769dup)	A767_V769dup	D	E	A	Y	V	M	A	S	V	A	S	V	D	N	P	H	V	C	R
NP_005219.2:p.M766_V769insWPA	N/A N/A N/A	EXCLUDE	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	R			
NP_005219.2:p.A767_S768insSVR	NP_005219.2:p.(Val769_Asp770insArgSerVal)	V769_D770insRSV	D	E	A	Y	V	M	A	S	V	R	S	V	D	N	P	H	V	C	R
NP_005219.2:p.A767_V769dupASV	NP_005219.2:p.(Ala767_Val769dup)	A767_V769dup	D	E	A	Y	V	M	A	S	V	A	S	V	D	N	P	H	V	C	R
NP_005219.2:p.S768_D770dupSVD	NP_005219.2:p.(Ser768_Asp770dup)	S768_D770dup	D	E	A	Y	V	M	A	S	V	D	S	V	D	N	P	H	V	C	R
NP_005219.2:p.A767_S768insSVD	NP_005219.2:p.(Ser768_Asp770dup)	S768_D770dup	D	E	A	Y	V	M	A	S	V	D	S	V	D	N	P	H	V	C	R
NP_005219.2:p.V769_D770insASV	NP_005219.2:p.(Ala767_Val769dup)	A767_V769dup	D	E	A	Y	V	M	A	S	V	A	S	V	D	N	P	H	V	C	R
NP_005219.2:p.D770_H773dupTTP	N/A N/A N/A	EXCLUDE	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	R			
NP_005219.2:p.D770_N771>GSVDN	N/A N/A N/A	D770_N771insGSVDN	D	E	A	Y	V	M	A	S	V	D	G	S	V	D	N	N	P	H	V	C	R
NP_005219.2:p.D770_N771>GYN	N/A N/A N/A	D770_N771insGYN	D	E	A	Y	V	M	A	S	V	D	G	Y	N	N	P	H	V	C	R
NP_005219.2:p.D770_N771insD	NP_005219.2:p.(Asp770dup)	D770dup	D	E	A	Y	V	M	A	S	V	D	D	N	P	H	V	C	R		
NP_005219.2:p.D770insG	NP_005219.2:p.(Asp770_Asn771insGly)	D770_N771insG	D	E	A	Y	V	M	A	S	V	D	G	N	P	H	V	C	R		
NP_005219.2:p.D770_N771insSVD	NP_005219.2:p.(Ser768_Asp770dup)	S768_D770dup	D	E	A	Y	V	M	A	S	V	D	S	V	D	N	P	H	V	C	R
NP_005219.2:p.D770>GY	N/A N/A N/A	D770_N771insGY	D	E	A	Y	V	M	A	S	V	D	G	Y	N	P	H	V	C	R	
NP_005219.2:p.N771_H773dupNPH	NP_005219.2:p.(Asn771_His773dup)	N771_H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	N	P	H	V	C	R
NP_005219.2:p.N771_P772>GYP	N/A N/A N/A	N771_P772insGYP	D	E	A	Y	V	M	A	S	V	D	N	G	Y	P	P	H	V	C	R
NP_005219.2:p.N771_P772insN	NP_005219.2:p.(Asn771dup)	N771dup	D	E	A	Y	V	M	A	S	V	D	N	N	P	H	V	C	R		
NP_005219.2:p.N771_P772insPH	NP_005219.2:p.(Pro772_His773dup)	P772_H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	P	H	V	C	R	
NP_005219.2: p.D770_P772del771NinsTH	NP_005219.2:p.(Asp770_Pro772delAsn771insThrHis)	N771delinsTH	D	E	A	Y	V	M	A	S	V	D	T	H	P	H	V	C	R		
NP_005219.2:p.N771dupN	NP_005219.2:p.(Asn771dup)	N771dup	D	E	A	Y	V	M	A	S	V	D	N	N	P	H	V	C	R		
NP_005219.2:p.P772_C775dupPHVC	NP_005219.2:p.(Pro772_Cys775dup)	P772_C775dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	P	H	V	C	R
NP_005219.2:p.P772_H773dupPH	NP_005219.2:p.(Pro772_His773dup)	P772_H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	P	H	V	C	R	
NP_005219.2:p.P772_H773insDNP	NP_005219.2:p.(Asp770_Pro772dup)	D770_P772dup	D	E	A	Y	V	M	A	S	V	D	N	P	D	N	P	H	V	C	R
NP_005219.2:p.P772_H773insNP	NP_005219.2:p.(Asn771_Pro772dup)	N771_P772dup	D	E	A	Y	V	M	A	S	V	D	N	P	N	P	H	V	C	R	
NP_005219.2:p.H773_V774dupHV	NP_005219.2:p.(His773_Val774dup)	H773_V774dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	H	V	C	R	
NP_005219.2:p.H773_V774insH	NP_005219.2:p.(His773dup)	H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	P	H	V	C	R	
NP_005219.2:p.H773_V774dupH	NP_005219.2:p.(His773dup)	H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	P	H	V	C	R	
NP_005219.2:p.H773_V774insPH	NP_005219.2:p.(Pro772_His773dup)	P772_H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	P	H	V	C	R	
NP_005219.2:p.H773dupH	NP_005219.2:p.(His773dup)	H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	H	V	C	R		
NP_005219.2:p.H773insH	N/A N/A N/A	H773dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	H	V	C	R		
NP_005219.2:p.V774_C775>AHVC	N/A N/A N/A	V774_C775insAHVC	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	A	H	V	C	C	R
NP_005219.2:p.V774_C775insCPHV	NP_005219.2:p.(Pro772_Cys775dup)	P772_C775dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	P	H	V	C	R
NP_005219.2:p.V774_C775insHV	NP_005219.2:p.(His773_Val774dup)	H773_V774dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	H	V	C	R		
NP_005219.2:p.C775_R776insPHVC	NP_005219.2:p.(Pro772_Cys775dup)	P772_C775dup	D	E	A	Y	V	M	A	S	V	D	N	P	H	V	C	P	H	V	C	R

Supplementary Table 3. Overview of the Ba/F3 cell line models used for the *in vitro* TKI sensitivity studies.

Reference	Tested *EGFR*ex20+ mutational variants	TKI	Cell viability instrument	Reported value	Year published	DOIs
Hasako et al, 2018	Wild type EGFR A763_Y764insFQEA A767_V769dup S768_D770dup D770_N771insG H773_V774insNPH H773_V774insPH	Erlotinib Afatinib TAS6417	CellTiter-Glo luminescent cell viability assay (Promega)	IC_{50}	2018	10.1158/1535-7163.MCT-17-1206
Lee et al, 2019	Wild type EGFR A763_Y764insFQEA A767_V769dup S768_D770dup D770_N771insNPG P772insPR H773insH H773insNPH	Erlotinib Gefitinib Afatinib Dacomitinib Rociletinib Olmutinib Nazartinib	CellTiter-Glo luminescent cell viability assay (Promega)	IC_{50}	2019	10.1016/j.jtho.2019.05.006
Hirano et al, 2015	Wild type EGFR A763_Y764insFQEA A767_V769dup D770_N771insNPG Y764_V765insHH	Erlotinib Afatinib Osimertinib Rociletinib	CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega)	IC_{50}	2015	10.18632/oncotarget.5887
Udagawa et al, 2019	Wild type EGFR A763_Y764insFQEA A767_V769dup S768_D770dup D770_N771insG H773_V774insNPH H773_V774insPH	Erlotinib Afatinib Osimertinib TAS6417 Poziotinib	CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega)	IC_{50}	2019	10.1158/1541-7786.MCR-19-0419
Hirano et al, 2018	Wild type EGFR A763_Y764insFQEA Y764_V765insHH A767_V769dup D770_N771insNPG	Erlotinib Afatinib Osimertinib Naquotinib	CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega)	IC_{50}	2018	10.1158/1535-7163.MCT-17-1033
Jia et al, 2016	Wild type EGFR A767_V769dup S768_D770dup	EGF816	Bright-Glo Luciferase Assay System (Promega)	EC_{50}	2016	10.1158/0008-5472

	H773_V774insNPH					
Fan et al, 2020	Wild type EGFR A763_Y764insFQEA A767_V769dup S768I S768_D770dup N771_H773dup P772_H773dup	Osimertinib BEBT-109	CellTiter-Glo luminescent cell viability assay (Promega)	IC_{50}	2020	10.1016/j.tranon.2020.100961
Yun et al, 2020	Wild type EGFR Y764_V765insHH A767_V769dup D770delinsGY S768_D770dup H773dup	Poziotinib	CellTiter-Glo 2.0 Assay Kit (Promega)	IC_{50}	2020	10.1158/2159-8290.CD-20-0116
Vasconcelos et al, 2021	Wild type EGFR A763_Y764insFQEA A767_V769dupA S768_D770dup H773dup	Osimertinib Pozitionib Mobocertinib	CellTiter 96 AQueous One Solution proliferation kit (Promega) and/or Cell Counting Kit-8 (Dojindo Molecular Technologies)	IC_{50}	2021	10.1016/j.jtocrr.2020.100105
Jorge et al, 2018	Wild type EGFR A763_Y764insFQEA Y764_V765insHH M766_A767insAI A767_V769dup S768_D770dup D770_N771insNPG H773dup	Erlotinib Afatinib Rociletinib Luminespib	CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega)	IC_{50}	2018	10.1158/1078-0432.CCR-18-1541
Robichaux et al, 2021	A763_Y764insFQEA A767_V769dup S768I D770_ N771insNPG H773_V774insNPH	Erlotinib Afatinib Gefitinib Osimertinib AZD3759 Sapatinib Dacomitinib Neratinib Poziotinib Tarlox-TKI	CellTiter-Glo luminescent cell viability assay (Promega)	Mutant to WT ratios (dividing the IC50 values of mutant cell lines by the average IC50 value of Ba/F3 cells expressing WT EGFR)	2021	10.1038/s41586-021-03898-1

		CLN-081 AZ5104 Mobocertinib Nazartinib Olmutinib Rociletinib Naquotinib Lazertinib				

Supplementary Table 4. IC_{50} values (recalculated to nmol/L) and mutant to wild type ratios for the published models mentioned in Supplementary Table 3.

		Reported IC_{50} values (unit: nmol/L) per inhibitory compound														
Reference	**Tested EGFRex20+ mutational variants**	**Erlotinib**	**Afatinib**	**Gefitinib**	**Osimertinib**	**Olmutinib**	**Nazartinib**	**Rociletinib**	**BEBT-109**	**Naquotinib**	**TAS6417**	**Luminespib**	**EGF816**	**Dacomitinib**	**Poziotinib**	**Mobocertinib**
Hasako et al, 2018	Wild type EGFR	919	34								676					
	A763_Y764insFQEA	100	2								5.05					
	A767_V769dup	1591	93								103					
	S768_D770dup	2063	121								38					
	D770_N771insG	1012	41								38					
	H773_V774insNPH	1459	230								145					
	H773_V774insPH	2675	325								150					
Lee et al, 2019	Wild type EGFR	1333	7	1127	259	236	82	263						39		
	A763_Y764insFQEA	33	0	22	132	352	352	1022						0		
	A767_V769dup	5051	54	4870	41	262	108	1325						55		
	S768_D770dup	5180	64	3479	28	288	129	732						86		
	D770_N771insNPG	3240	17	2422	15	30	36	97						8		
	P772insPR	5391	200	3611	22	314	148	3035						162		
	H773insNPH	1391	20	949	11	97	50	288						11		
	H773insH	2859	353	1980	66	498	510	2309						320		
Hirano et al, 2015	Wild type EGFR	1020	31		938			2052								
	A763_Y764insFQEA	154	3		44			673								
	A767_V769dup	1679	158		333			5290								
	D770_N771insNPG	1146	43		42			262								
	Y764_V765insHH	>10000	134		237			1730								
Udagawa et al, 2019	Wild type EGFR	980	48		688						702				6	
	A763_Y764insFQEA	3145	279		10						4				22	
	A767_V769dup	91	69		77						26				41	
	S768_D770dup	7	0		4						1				0	
	D770_N771insG	2970	270		10						3				30	
	H773_V774insNPH	138	3		45						5				1	
	H773_V774insPH	1511	100		244						121				5	
Hirano et al, 2018	Wild type EGFR	667	28		704					830						
	A763_Y764insFQEA	84	0.6		16					19						
	Y764_V765insHH	>10000	734		701					681						
	A767_V769dup	2021	48		230					148						
	D770_N771insNPG	1835	92		38					56						
Jia et al, 2016	Wild type EGFR												166			
	A767_V769dup												11			
	S768_D770dup												7			
	H773_V774insNPH												190			
Fan et al, 2020	Wild type EGFR				1112				3415							
	A763_Y764insFQEA				45				11							
	A767_V769dup				101				21							
	S768I				198				52							
	S768_D770dup				110				32							
	N771_H773dup				89				21							
	P772_H773dup				59				15							
Yun et al, 2020	Wild type EGFR														0.8	
	Y764_V765insHH														6.0	
	A767_V769dup														1.3	
	D770delinsGY														0.8	
	S768_D770dup														1.5	
	H773dup														10.4	
Vasconcelos et al, 2021	Wild type EGFR				65.83										28	763
	A763_Y764insFQEA				30										2	109
	A767_V769dup				179										2	63
	S768_D770dup				133										2	203
	H773dup				100										18	180
Jorge et al, 2018	Wild type EGFR	1535	15					119				3				
	A763_Y764insFQEA	111	1					1053				3				
	Y764_V765insHH	3400	99					1035				2				
	M766_A767insAI	3276	36					1762				8				
	A767_V769dup	2628	34					1210				3				
	S768_D770dup	3187	25					1237				4				
	D770_N771insNPG	2138	31					341				8				
	H773dup	3884	161					1194				2				

Reported mutant to wild type ratios per inhibitory compound

Reference	Tested EGFRex20+ mutational variants	Erlotinib	Afatinib	Gefitinib	Osimertinib	Olmutinib	Rociletinib	Lazertinib	Naquotinib	AZD3759	Sapatinib	Dacomitinib	Neratinib	Poziotinib	Tarlox-TKI	CLN-081	AZ5104	Mobocertinib	Nazartinib
Robichaux et al, 2021	A763_Y764insFQEA	0,42	0,07	0,17	0,12	0,03	0,18	0,04	0,19	0,02	0,31	0,04	0,07	0,07	0,05	0,00	0,40	0,47	0,06
	A767_V769dup	60,28	4,07	30,47	2,39	0,68	1,86	10,93	1,61	4,15	2,23	3,06	3,52	0,32	0,99	0,14	4,66	1,55	0,64
	S768I	1,57	42,75	3,30	0,37	0,01	0,85	1,26	1,09	0,14	0,19	17,66	10,70	4,86	0,39	0,08	1,36	0,78	0,53
	D770_ N771insNPG	16,70	6,26	26,32	1,33	0,31	0,69	4,93	1,30	3,84	5,02	5,40	27,86	0,29	5,02	0,02	1,98	1,21	0,53
	H773_V774insNPH	5,55	2,05	28,96	0,93	0,43	0,87	5,10	1,08	1,56	1,26	1,65	7,72	0,39	11,56	0,18	4,00	0,30	0,42

Supplementary Table 5. Published *EGFR*ex20+ patient derived cell lines with IC_{50} values (calculated to nmol/L) of several inhibitory compounds and the IC_{50} values of the control matching mutational cell lines (if applicable). All experiments were performed in triplo.

*; When experiments were performed twice instead of in triplo, both values were mentioned. #; Ba/F3 cell lines were used as a control. $; A431 cell line was used as a control.

Reported IC_{50} values (calculated to nmol/L) per inhibitory compound

Reference	Patient-derived cell line	Mutational status	Erlotinib	Afatinib	Gefitinib	Osimertinib	Rocelitenib	Poziotinib	Naquotinib	Luminespib	TAS6417	Mobocertinib	Cetuximab	Dacometinib	Nazartinib	Olmutinib	Tarloxotinib	Tarlox-E
Yesuda et al, 2013	BID007	A763_Y764insFQEA	82	5	565													
	#	A763_Y764insFQEA	48	4	174													
Hirano et al, 2015	BID007	A763_Y764insFQEA	45	8		40	1278											
	#	A763_Y764insFQEA	154	3		44	673											
	#	Wild type EGFR	1020	31		938	2052											
Hirano et al, 2018	BID007	A763_Y764insFQEA	233	13		108			52									
	#	A763_Y764insFQEA	84	0.6		16			19									
	#	Wild type EGFR	667	28		704			830									
Udagawa et al, 2019	BID007	A763_Y764insFQEA	53	3		87		1			3							
	BID019	N771_P772insH		221				1			21							
	#	A763_Y764insFQEA	138	3		45		1			5							
	#	Wild type EGFR	980	48		688		6			702							
Jorge et al, 2018	BID007	A763_Y764insFQEA								7								
	#	A763_Y764insFQEA								3								
	#	Wild type EGFR								3								
Vasconcelos et al, 2021	BID007	A763_Y764insFQEA										60.36						
	BID019	N771_P772insH										109.70						
	#	A763_Y764insFQEA										109						
	#	Wild type EGFR										763,4						
Yang et al, 2016*	LU0387	H773_V774insNPH	11621 >10000	10823 >10000	>10000 >10000	3298 5721	2636 3442						Insensitive Insensitive					
	LU3075	P772_H773insDNP	96665 >100000	1230 1816	4369 6790	1591 2570	2095 2501						Insensitive Insensitive					
Gonzalez et al, 2021	LU0387	H773_V774insNPH	2793	20	364	195						21						
	CUTO14	A767_V769dup	2679	66	1021	575						33						
	#	H773_V774insNPH	2764	103	4054	469						18						
	#	A767_V769dup	1340	46	369	173						11						
	$	Wild type EGFR	71	3.9	56	-						35						
Lee et al, 2019	SNU-3173	H773_V774insAH	4535	16.7	1050	62.7	1202.5							13.7	139.7	627		
	#	Wild type EGFR	1333	7	1127	259								39	82	236		
Estrada et al, 2021	CUTO14	A767_V769dup		110.9	374	303											4645	72.2
	CUTO17	N771_H773dup		219.7	4197	426											3090	48.1
	CUTO18	S768_D770dup		841.3	>10000	647											>10000	158.4

Supplementary Table 6. Articles reporting clinical treatment results (ORR and PFS if applicable) of in total 672 patients per *EGFR*ex20+ mutation or insertion location dividing them between C-helix, near loop or far loop insertions. *Platinum based chemotherapy regime. Abbreviations: ICI = immune checkpoint inhibitor (ICI)-based treatment.

Reference	Type of study	Treatment	Year published	DOIs
Wu, Jenn-Yu et al. "Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response." Clinical cancer research : an official journal of the American Association for Cancer Research vol. 14,15 (2008): 4877-82.	Cohort	Gefitinib	2008	10.1158/1078-0432.CCR-07-5123
Yasuda, Hiroyuki et al. "Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer." Science translational medicine vol. 5,216 (2013): 216ra177.	Cohort	Erlotinib Geftinib	2013	10.1126/scitranslmed.3007205
Beau-Faller, M et al. "Rare EGFR exon 18 and exon 20 mutations in non-small-cell lung cancer on 10 117 patients: a multicentre observational study by the French ERMETIC-IFCT network." Annals of oncology : official journal of the European Society for Medical Oncology vol. 25,1 (2014): 126-31.	Cohort	Erlotinib Gefitinib	2014	10.1093/annonc/mdt418
Naidoo, J et al. "Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: Clinical outcomes and response to erlotinib." Cancer vol. 121,18 (2015): 3212-3220.	Cohort	Erlotinib	2015	10.1002/cncr.29493

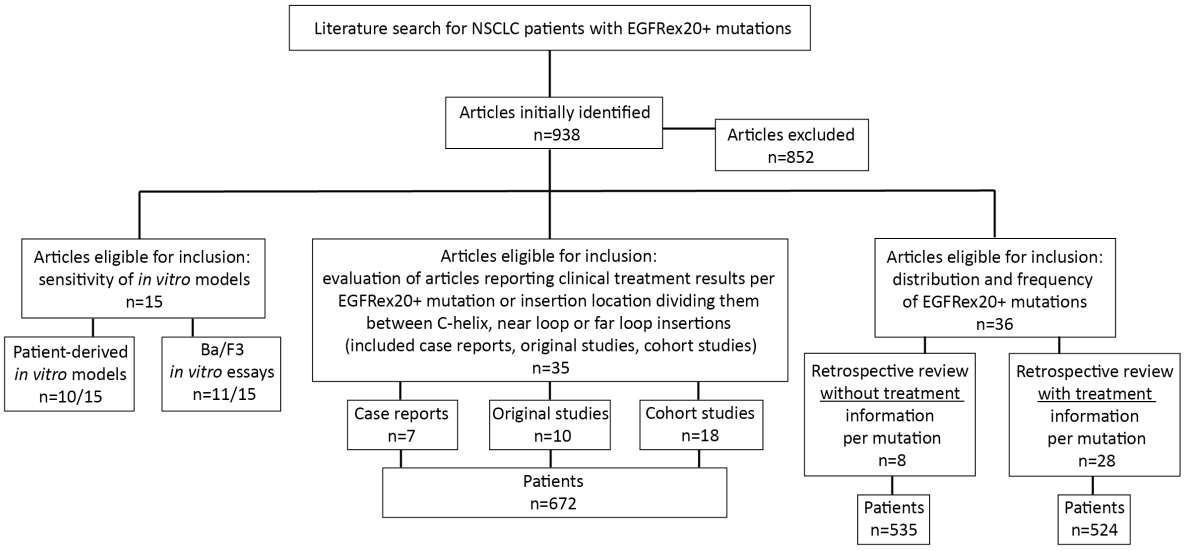
Tsigelny, Igor F et al. "Molecular determinants of drug-specific sensitivity for epidermal growth factor receptor (EGFR) exon 19 and 20 mutants in non-small cell lung cancer." Oncotarget vol. 6,8 (2015): 6029-39.	Cohort	Erlotinib/ cetuximab	2015	10.18632/oncotarget.3472
Chan, Raymond Tsz-Tong. "Afatinib for an EGFR exon 20 insertion mutation: A case report of progressive stage IV metastatic lung adenocarcinoma with 54 months' survival." Asia-Pacific journal of clinical oncology vol. 14 Suppl 1 (2018): 7-9.	Case report	Afatinib	2018	10.1111/ajco.12853
van Veggel, Bianca et al. "Afatinib and Cetuximab in Four Patients With EGFR Exon 20 Insertion-Positive Advanced NSCLC." Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer vol. 13,8 (2018): 1222-1226.	Cohort	Afatinib/ cetuximab	2018	10.1016/j.jtho.2018.04.012
Takeda, Masayuki et al. "Clinical characteristics of non-small cell lung cancer harboring mutations in exon 20 of EGFR or HER2." Oncotarget vol. 9,30 21132-21140. 20 Apr. 2018.	Cohort	ICI	2018	10.18632/oncotarget.24958
Piotrowska, Z et al. "Activity of the Hsp90 inhibitor luminespib among non-small-cell lung cancers harboring EGFR exon 20 insertions." Annals of oncology : official journal of the European Society for Medical Oncology vol. 29,10 (2018): 2092-2097.	Original study	Luminespib	2018	10.1093/annonc/mdy336
Robichaux, Jacqulyne P et al. "Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer." Nature medicine vol. 24,5 (2018): 638-646.	Original study	Poziotinib	2018	10.1038/s41591-018-0007-9
Fang, Wenfeng et al. "A Patient with EGFR Exon 20 Insertion-Mutant Non-Small Cell Lung Cancer Responded to Osimertinib plus Cetuximab Combination Therapy." Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer vol. 14,9 (2019): e201-e202.	Case report	Afatinib/ cetuximab	2019	10.1016/j.jtho.2019.04.013
Wu, Jenn-Yu et al. "Effectiveness of Treatments for Advanced Non-Small-Cell Lung Cancer With Exon 20 Insertion Epidermal Growth Factor Receptor Mutations." Clinical lung cancer vol. 20,6 (2019): e620-e630.	Cohort	Chemotherapy* Gefitinib Erlotinib Afatinib	2019	10.1016/j.cllc.2019.06.018
Byeon, Seonggyu et al. "Clinical Outcomes of EGFR Exon 20 Insertion Mutations in Advanced Non-small Cell Lung Cancer in Korea." Cancer research and treatment vol. 51,2 (2019): 623-631.	Cohort	Chemotherapy*	2019	10.4143/crt.2018.151
Fang, Wenfeng et al. "EGFR exon 20 insertion mutations and response to osimertinib in non-small-cell lung cancer." BMC cancer vol. 19,1 595. 17 Jun. 2019.	Cohort	Osimertinib 80mg	2019	10.1186/s12885-019-5820-0
Lin, Ling et al. "Response to Afatinib in a Patient with NSCLC Harboring Novel EGFR Exon 20 Insertion Mutations." OncoTargets and therapy vol. 13 9753-9757. 30 Sep. 2020.	Case report	Afatinib	2020	10.2147/OTT.S268694
Piotrowska, Zofia et al. "ECOG-ACRIN 5162: A phase II study of osimertinib 160 mg in NSCLC with EGFR exon 20 insertions.," Journal of Clinical Oncology, vol. 38, no. 15_suppl, p. 9513, May 2020.	Cohort	Osimertinib 160mg	2020	10.1200/JCO.2020.38.15_suppl.9513
Yang, Guang-Jian et al. "Osimertinib for Chinese advanced non-small cell lung cancer patients harboring diverse EGFR exon 20 insertion mutations." Lung cancer (Amsterdam, Netherlands) vol. 152 (2021): 39-48.	Cohort	Osimertinib 80mg and 160mg	2020	10.1016/j.lungcan.2020.11.027
van Veggel, B et al. "Osimertinib treatment for patients with EGFR exon 20 mutation positive non-small cell lung cancer." Lung cancer (Amsterdam, Netherlands) vol. 141 (2020): 9-13.	Cohort	Osimertinib 80mg and 160mg	2020	10.1016/j.lungcan.2019.12.013
Le, Ziuning et al. "Poziotinib shows activity and durability of responses in subgroups of previously treated EGFR exon 20 NSCLC patients." Journal of Clinical Oncology, vol. 38, no. 15_suppl, p. 9514, May 2020.	Original study	Poziotinib	2020	10.1200/JCO.2020.38.15_suppl.9514
Zöchbauer-Müller, Sabine et al. "Case Report: Afatinib Treatment in a Patient With NSCLC Harboring a Rare EGFR Exon 20 Mutation." Frontiers in oncology vol. 10 593852. 26 Jan. 2021.	Case report	Afatinib	2021	10.3389/fonc.2020.593852
Urbán, László et al. "Major Clinical Response to Afatinib Monotherapy in Lung Adenocarcinoma Harboring EGFR Exon 20 Insertion Mutation." Clinical lung cancer vol. 22,1 (2021): e112-e115.	Case report	Afatinib	2021	10.1016/j.cllc.2020.09.005
Park, Keunchil et al. "Amivantamab in EGFR Exon 20 Insertion-Mutated Non-Small-Cell Lung Cancer Progressing on Platinum Chemotherapy: Initial Results From the CHRYSALIS Phase I	Original study	Amivantamab	2021	10.1200/JCO.21.00662

Study." Journal of clinical oncology : official journal of the American Society of Clinical Oncology vol. 39,30 (2021): 3391-3402.				
Riely, Gregory J et al. "Activity and Safety of Mobocertinib (TAK-788) in Previously Treated Non-Small Cell Lung Cancer with EGFR Exon 20 Insertion Mutations from a Phase I/II Trial." Cancer discovery vol. 11,7 (2021): 1688-1699.	Original study	Mobocertinib	2021	10.1158/2159-8290.CD-20-1598
Zhou, Caicun et al. "Treatment Outcomes and Safety of Mobocertinib in Platinum-Pretreated Patients With EGFR Exon 20 Insertion-Positive Metastatic Non-Small Cell Lung Cancer: A Phase 1/2 Open-label Nonrandomized Clinical Trial." JAMA oncology vol. 7,12 (2021): e214761.	Original study	Mobocertinib	2021	10.1001/jamaoncol.2021.4761
Riess, Jonathan W et al. "Erlotinib and Onalespib Lactate Focused on EGFR Exon 20 Insertion Non-Small Cell Lung Cancer (NSCLC): A California Cancer Consortium Phase I/II Trial (NCI 9878)." Clinical lung cancer vol. 22,6 (2021): 541-548.	Original study	Onalespib Erlotinib	2021	10.1016/j.cllc.2021.05.001
Yasuda, Hiroyuki et al. "A phase I/II study of osimertinib in EGFR exon 20 insertion mutation-positive non-small cell lung cancer." Lung cancer (Amsterdam, Netherlands) vol. 162 (2021): 140-146.	Original study	Osimertinib 80mg	2021	10.1016/j.lungcan.2021.10.006
Prelaj, Arsela et al. "Poziotinib for EGFR and HER2 exon 20 insertion mutation in advanced NSCLC: Results from the expanded access program." European journal of cancer (Oxford, England : 1990) vol. 149 (2021): 235-248.	Cohort	Poziotinib	2021	10.1016/j.ejca.2021.02.038
Nikanjam, Mina et al. "Cetuximab in Patients with Non-Small Cell Lung Cancer and EGFR Exon 20 Insertion Alterations." Clinical oncology, case reports vol. 5,1 (2022): 210.	Cohort	Afatinib/ cetuximab	2022	
Geng, D et al. "Clinical and molecular characteristics of epidermal growth factor receptor exon 20 insertion mutations in non-small-cell lung cancer." Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico vol. 24,2 (2022): 379-387.	Cohort	ICI	2022	10.1007/s12094-021-02701-x
Zhu, Lingling et al. "Case Report: Partial Response Following Nivolumab Plus Docetaxel in a Patient With EGFR Exon 20 Deletion/Insertion (p.N771delinsGF) Mutant Lung Adenocarcinoma Transdifferentiated From Squamous Cell Carcinoma." Frontiers in cell and developmental biology vol. 9 755135. 10 Jan. 2022.	Case report	ICI/chemotherapy*	2022	10.3389/fcell.2021.755135
Shi, Chao et al. "Real-world clinical treatment outcomes in Chinese non-small cell lung cancer with EGFR exon 20 insertion mutations." Frontiers in oncology vol. 12 949304. 2 Sep. 2022.	Cohort	ICI/chemotherapy*	2022	10.3389/fonc.2022.949304
Zwierenga, Fenneke et al. "High dose osimertinib in patients with advanced stage EGFR exon 20 mutation-positive NSCLC: Results from the phase 2 multicenter POSITION20 trial." Lung cancer (Amsterdam, Netherlands) vol. 170 (2022): 133-140.	Original study	Osimertinib 160mg	2022	10.1016/j.lungcan.2022.06.012
Yang, Guangjian et al. "*EGFR* exon 20 insertion variants A763_Y764insFQEA and D770delinsGY confer favorable sensitivity to currently approved EGFR-specific tyrosine kinase inhibitors." *Frontiers in pharmacology* vol. 13 984503. 8 Nov. 2022.	Cohort	Osimertinib 80mg Gefitinib Erlotinib Afatinib	2022	10.3389/fphar.2022.984503
Prelaj, Arsela et al. "Case Report: Exceptional Response to Poziotinib in Patient with Metastatic Non-Small Cell Lung Cancer With EGFR Exon 20 Insertion Mutation." Frontiers in oncology vol. 12 902967. 8 Jun. 2022.	Case report	Poziotinib	2022	10.3389/fonc.2022.902967
Elamin, Yasir Y et al. "Poziotinib for EGFR exon 20-mutant NSCLC: Clinical efficacy, resistance mechanisms, and impact of insertion location on drug sensitivity." Cancer cell vol. 40,7 (2022): 754-767.e6.	Original study	Poziotinib	2022	10.1016/j.ccell.2022.06.006

Supplementary Figure 1. Flowchart on number of articles selected from literature search with results on in vitro experiments and clinical treatment and how patient selection was performed. Fifteen articles

were found to be eligible for reporting sensitivity of in vitro models. Six articles reported data both on patient derived in vitro models and Ba/F3 in vitro essays. Thirty-five articles were found to report an evaluation on clinical treatment per *EGFR*ex20+ mutation location, including articles reporting results only dividing the location between C-helix, near loop or far loop insertions. For the distribution and frequency of *EGFR*ex20+ mutations, 36 articles were retrospectively analyzed. The 28 articles founded which contained treatment information, are also included within the 35 articles for the evaluation of clinical treatment per *EGFR*ex20+ mutation.

Abbreviations: NSCLC = non-small cell lung cancer; *EGFR*ex20+ = in-frame deletion and/or insertion mutations clustering within the *EGFR* exon 20 region.



Supplementary Figure 2. A cross-tabulation comparing observed clinical benefit with chemotherapy, ICI and chemotherapy/ICI combination therapy based on tumor responses in NSCLC patients across different *EGFR*ex20+ mutations (Sup. Table 6). The size of the bar representing the responses reflects the relative frequency of all responses mentioned for the corresponding mutation. Used chemotherapy was platinum-based and the used anti PD1 are camrelizumab, sintilimab, pembrolizumab and nivolumab. The mPFS is the median progressive free survival of all reported mPFS in the different studies, if applicable. Abbreviations: ICI = immune checkpoint inhibitor (ICI)-based treatment, PD1 = programmed death protein 1.