

Mutations in EMT-Related Genes in *ALK* Positive Crizotinib Resistant Non-Small Cell Lung Cancers

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Supplementary Table S1. Whole exome sequencing data in five patient samples.

Sample	ALK4			ALK6			ALK8		ALK14			ALK16			
	N	P	R	N	P	R	N	R	N	P	R	N	P	R	
Clean reads x10 ⁶	61	74	64	60	66	65	58	59	59	58	57	61	142	61	
Unique reads x10 ⁶	58	62	60	57	59	60	57	58	56	56	55	58	82	60	
Percentage of unique reads	95%	84%	94%	95%	89%	92%	97%	97%	96%	97%	96%	96%	58%	98%	
Unique aligned reads (x10 ⁶)	57	60	59	56	58	58	56	56	55	55	54	57	79	59	
Percentage of unique aligned reads	98%	97%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	96%	98%	
Average target coverage	68	70	66	65	71	68	65	65	68	65	65	65	63	66	
20X coverage	91%	90%	91%	91%	90%	91%	90%	65%	91%	89%	74%	91%	91%	73%	
Number of variants	0	82	169	0	44	61	0	31	0	168	180	0	29	38	
CADD Scores	<10	0	20	28	0	13	15	0	5	0	34	45	0	4	6
	[10,20)	0	34	89	0	17	23	0	16	0	77	77	0	14	14
	≥20	0	18	46	0	8	13	0	6	0	50	46	0	7	9
	NA	0	10	6	0	6	10	0	4	0	7	12	0	4	9

N: Normal sample; P: Primary tumour sample before the start of treatment with crizotinib; R: Resistant tumour sample taken when disease progression was observed by CT or PET/CT during crizotinib treatment.

Supplementary Table S2. Treatment-related variants in all tumor samples in ALK4, ALK6, ALK14, and ALK16.

Chr	Position (HG19)	REF	ALT	Gene symbol	Variant Type	Amino acid change ¹	CADD	Primary sample		Resistant sample		Involvement in EMT or metabolism pathways
								Altered reads	Total reads	Altered reads	Total reads	
ALK4												
1	1254808	G	C	CPSF3L	SNV	p.(Ile105Met)	20	0	99	9	44	Metabolism
1	2435753	C	G	PLCH2	SNV	p.(Pro1118Ala)	1	0	76	23	94	
1	110921947	C	A	SLC16A4	SNV	p.(Leu186Phe)	16	0	31	14	41	
1	149858085	C	T	HIST2H2BE	SNV	p.(Glu36Lys)	35	0	132	21	105	
1	161736226	G	C	ATF6	SNV	p.(Asp26His)	19	0	100	14	52	
1	173703378	G	C	KLHL20	SNV	p.(Glu184Gln)	29	0	137	43	178	EMT ²
1	175048588	G	A	TNN	SNV	p.(Gly177Ser)	11	0	134	15	73	
2	28762018	C	T	PLB1	SNV	p.(Ser224Phe)	15	1	39	19	39	Metabolism
2	28841228	G	C	PLB1	SNV	p.(Asp1093His)	19	0	53	11	39	Metabolism
2	97377492	C	T	LMAN2L	SNV	p.(Asp240Asn)	31	0	73	20	60	EMT
2	102413796	C	CTTT G	MAP4K4	Frameshift Insertion	p.(His26Phefs*3)	NA	0	33	8	35	
2	202208931	C	G	ALS2CR12	SNV	p.(Glu142Gln)	19	0	79	26	90	
2	202939778	G	A	AC079354.1	SNV	p.(Trp83*)	34	0	25	11	39	
2	211447349	G	T	CPS1	SNV	p.(Met185Ile)	10	0	131	32	111	
2	225360551	C	T	CUL3	SNV	p.(Glu614Lys)	15	2	76	18	66	Metabolism
2	234978605	G	A	SPP2	SNV	p.(Arg195Lys)	18	0	52	16	65	
3	15084402	G	C	NR2C2	SNV	p.(Glu579Gln)	30	0	78	18	70	
3	48541457	G	A	SHISA5	SNV	p.(Pro24Leu)	18	0	79	7	35	
3	50290516	G	A	GNAI2	SNV	p.(Asp122Asn)	23	0	151	16	79	
3	52397143	G	A	DNAH1	SNV	p.(Glu1743Lys)	34	0	17	11	41	EMT
3	133486927	G	T	TF	SNV	p.(Cys514Phe)	17	0	39	10	45	
3	182988049	G	C	B3GNT5	SNV	p.(Asp155His)	16	0	80	23	97	
3	190127781	C	T	CLDN16	SNV	p.(Arg292Cys)	15	0	53	15	43	
6	31655514	G	C	ABHD16A	SNV	p.(Ser484*)	38	0	81	23	104	
6	109768619	C	T	MICAL1	SNV	p.(Glu690Lys)	10	0	42	21	60	EMT
7	4153039	G	A	SDK1	SNV	p.(Ala1185Thr)	26	2	206	34	115	
7	99768020	G	A	GPC2	SNV	p.(Arg525Trp)	17	0	133	14	68	
8	11606590	G	A	GATA4	SNV	p.(Arg261Gln)	31	2	107	12	56	
8	23709762	T	C	STC1	SNV	p.(Asp85Gly)	23	0	52	10	49	
8	38034637	C	T	BAG4	SNV	p.(Arg84*)	37	1	141	21	91	

9	33385784	C	G	AQP7	SNV	p.(Glu202Asp)	11	1	14	6	19	
9	33385786	C	G	AQP7	SNV	p.(Glu202Gln)	4	1	14	6	20	
9	75773645	C	T	ANXA1	SNV	p.(Ser34Leu)	12	1	57	13	56	
9	119249702	C	G	ASTN2	SNV	p.(Glu1145Gln)	11	0	56	12	34	
10	20357108	C	G	PLXDC2	SNV	p.(Leu161Val)	27	1	53	11	51	
10	65358936	G	C	REEP3	SNV	p.(Asp104His)	20	0	128	26	114	
10	94373137	G	A	KIF11	SNV	p.(Asp265Asn)	34	0	48	18	52	
10	114925655	C	G	TCF7L2	SNV	p.(Ser595Cys)	16	0	250	35	163	EMT
11	3060438	G	A	CARS	SNV	p.(Gln213*)	14	0	79	13	48	
11	6416928	G	A	APBB1	SNV	p.(Arg657Cys)	19	0	101	20	85	
11	47602214	G	A	NDUFS3	SNV	p.(Glu91Lys)	15	1	81	31	117	Metabolism
11	57191474	G	C	SLC43A3	SNV	p.(Ile127Met)	1	0	50	12	41	
11	72410500	C	G	ARAP1	SNV	p.(Glu800Asp)	12	0	50	18	61	
11	117117615	G	T	RNF214	SNV	p.(Glu304*)	34	0	121	73	119	
11	124857614	C	G	CCDC15	SNV	p.(Gln498Glu)	11	0	119	64	250	
11	129762618	G	C	NFRKB	SNV	p.(Leu56Val)	13	0	13	5	21	
12	10213832	G	A	CLEC9A	SNV	p.(Met93Ile)	4	0	40	17	60	
12	27849943	G	C	REP15	SNV	p.(Glu150Gln)	19	0	57	33	95	
12	43763100	C	T	ADAMTS20	SNV	p.(Cys1844Tyr)	17	0	32	6	22	
12	53445723	G	A	TENC1	SNV	p.(Asp64Asn)	33	0	49	34	90	
12	110206933	C	T	FAM222A	SNV	p.(Ser400Leu)	17	0	89	49	85	
14	21424243	G	C	RNASE2	SNV	p.(Val105Leu)	15	0	74	28	103	
14	64694303	G	A	ESR2	SNV	p.(Ser479Leu)	11	0	107	34	130	
14	102551674	C	G	HSP90AA1	SNV	p.(Lys330Asn)	17	0	14	3	15	EMT
15	51829933	C	A	DMXL2	SNV	p.(Glu457*)	38	0	92	16	54	
15	52404866	C	T	BCL2L10	SNV	p.(Glu20Lys)	12	0	67	10	44	
15	78927783	C	T	CHRN4	SNV	p.(Val68Met)	19	1	28	5	23	
15	85360163	G	A	ALPK3	SNV	p.(Arg29Lys)	12	1	103	14	67	
16	14758921	G	A	BFAR	SNV	p.(Glu385Lys)	28	0	16	6	28	
16	67858669	C	T	TSNAXIP1	SNV	p.(Ser222Leu)	29	0	41	21	87	
16	68894173	C	G	TANGO6	SNV	p.(Leu161Val)	18	0	81	26	80	
17	6014162	G	A	WSCD1	SNV	p.(Gly361Arg)	26	0	31	12	52	
17	6014163	G	A	WSCD1	SNV	p.(Gly361Glu)	25	0	33	12	52	
17	7577106	G	T	TP53	SNV	p.(Pro278Thr)	29	0	65	11	50	Metabolism; EMT
17	9515661	C	T	WDR16	SNV	p.(Thr307Ile)	17	0	42	11	55	
17	28706662	G	A	CPD	SNV	p.(Asp222Asn)	26	0	140	12	57	
17	37596699	C	G	MED1	SNV	p.(Gln113His)	11	0	66	17	66	
17	56389316	C	T	BZRAP1	SNV	p.(Glu956Lys)	28	0	16	8	27	

17	56833643	TGAG	T	PPM1E	Inframe Deletion	p.(Glu100del)	NA	1	14	3	13	
17	61623172	C	A	KCNH6	SNV	p.(Ser965Tyr)	22	0	74	26	123	
17	66042632	C	G	KPNA2	SNV	p.(Gln504Glu)	2	0	59	10	46	
17	77769081	C	T	CBX8	SNV	p.(Glu175Lys)	13	0	131	17	76	
18	3499195	C	A	DLGAP1	SNV	p.(Gln974His)	21	0	13	6	14	
18	3499203	C	A	DLGAP1	SNV	p.(Glu972*)	45	0	18	6	18	
18	60225942	G	C	ZCCHC2	SNV	p.(Lys477Asn)	19	0	14	4	16	
18	60999067	C	T	KDSR	SNV	p.(Arg316His)	36	0	43	29	84	Metabolism
19	1993141	C	G	BTBD2	SNV	p.(Glu188Gln)	28	0	200	24	95	
19	2252675	C	T	JSRP1	SNV	p.(Glu217Lys)	14	0	133	23	108	
19	6375401	C	A	PSPN	SNV	p.(Ala154Ser)	8	1	16	4	15	
19	17881285	G	A	FCHO1	SNV	p.(Val137Ile)	12	0	54	10	39	
19	50762440	C	T	MYH14	SNV	p.(Arg758Cys)	17	0	101	15	57	EMT
19	55697677	C	G	PTPRH	SNV	p.(Gln898His)	7	0	71	17	82	
19	55823305	C	T	BRSK1	SNV	p.(Pro752Ser)	14	0	83	7	33	
20	34091835	C	T	CEP250	SNV	p.(Arg1880Trp)	17	0	68	19	56	
20	54961418	G	C	AURKA	SNV	p.(Gln72Glu)	1	0	18	8	36	
22	32875149	G	A	FBXO7	SNV	p.(Glu102Lys)	20	0	75	35	146	
ALK6												
1	22161357	G	A	HSPG2	SNV	p.(Ala3512Val)	15	0	143	49	159	EMT
1	32221795	C	A	BAI2	SNV	p.(Ala215Ser)	10	1	33	22	58	
1	33960601	AG	A	ZSCAN20	Frameshift Deletion	p.(Ser887Alafs*176)	NA	0	62	25	79	
1	108686276	A	C	SLC25A24	SNV	p.(Asp329Glu)	19	0	94	35	133	
1	145457071	C	T	POLR3GL	SNV	p.(Glu164Lys)	19	0	26	13	26	Metabolism
2	99787087	C	A	MITD1	SNV	p.(Gly169Val)	6	0	144	26	93	
2	242681917	G	A	D2HGDH	SNV	p.(Val140Ile)	24	0	105	27	89	
3	11059618	A	AT	SLC6A1	Frameshift Insertion	p.(Gly111Argfs*96)	NA	0	26	9	41	
3	45943064	G	A	CCR9	SNV	p.(Val262Ile)	19	0	117	54	172	
3	56041309	G	C	ERC2	SNV	p.(Ala654Gly)	23	0	59	10	41	
4	82366740	TGAA ATA	T	RASGEF1B	Inframe Deletion	p.(Tyr288_Phe289del)	NA	0	114	24	105	
4	114279156	A	T	ANK2	SNV	p.(Ser3128Cys)	16	0	71	21	85	EMT
5	33947471	T	A	SLC45A2	SNV	p.(Lys389*)	32	9	46	26	58	
5	140798719	G	GT	PCDHGB7	Frameshift Insertion	p.(Leu432Phefs*13)	NA	0	232	56	167	

5	178310282	A	G	<i>ZNF354B</i>	SNV	p.(Lys277Glu)	17	0	81	32	93	
7	12620815	A	G	<i>SCIN</i>	SNV	p.(Lys162Arg)	14	0	35	9	21	EMT
7	12620816	G	T	<i>SCIN</i>	SNV	p.(Lys162Asn)	11	0	36	9	22	EMT
8	131124478	C	A	<i>ASAP1</i>	SNV	p.(Gly755*)	44	0	175	30	110	
11	5632354	G	T	<i>TRIM6</i>	SNV	p.(Asp445Tyr)	15	0	79	28	92	
12	27915919	G	GT	<i>MANSC4</i>	Frameshift Insertion	p.(Leu259Thrfs*9)	NA	0	76	38	141	
12	55846835	G	A	<i>OR6C2</i>	SNV	p.(Ala280Thr)	16	0	103	35	124	
12	88383137	AAC	A	<i>C12orf50</i>	Frameshift Deletion	p.(Cys201Leufs*13)	NA	0	106	38	136	
12	108986065	G	A	<i>TMEM119</i>	SNV	p.(Ala32Val)	12	0	12	4	17	
12	125292384	C	T	<i>SCARB1</i>	SNV	p.(Gly311Glu)	25	0	66	16	62	
14	31619345	C	A	<i>HECTD1</i>	SNV	p.(Arg672Met)	32	0	120	25	93	
14	31619346	T	C	<i>HECTD1</i>	SNV	p.(Arg672Gly)	21	0	116	26	90	
15	23932268	G	A	<i>NDN</i>	SNV	p.(Pro33Ser)	8	3	23	11	32	
17	40489789	T	A	<i>STAT3</i>	SNV	p.(Met213Leu)	13	0	76	12	32	EMT
17	77758222	C	T	<i>CBX2</i>	SNV	p.(Pro327Leu)	2	0	25	17	63	
18	48591806	G	A	<i>SMAD4</i>	SNV	p.(Trp323*)	43	0	22	13	33	EMT
19	10600464	C	A	<i>KEAP1</i>	SNV	p.(Gly464Val)	27	0	75	11	35	
20	61528167	G	GA	<i>DIDO1</i>	Frameshift Insertion	p.(Phe590Leufs*9)	NA	0	85	30	92	
22	29977055	C	T	<i>NIPSNAP1</i>	SNV	p.(Cys6Tyr)	22	0	19	2	10	
ALK14												
1	16074055	G	C	<i>TMEM82</i>	SNV	p.(Gln320His)	16	16	88	54	141	
1	150795726	G	C	<i>ARNT</i>	SNV	p.(Phe446Leu)	32	0	76	13	57	EMT
1	172634924	T	A	<i>FASLG</i>	SNV	p.(Leu205Gln)	2	14	94	226	250	EMT
1	172634933	G	A	<i>FASLG</i>	SNV	p.(Ser208Asn)	10	13	87	225	250	EMT
1	172634968	C	G	<i>FASLG</i>	SNV	p.(Gln220Glu)	0	10	72	215	249	EMT
1	172634980	A	C	<i>FASLG</i>	SNV	p.(Met224Leu)	5	9	69	205	245	EMT
1	172634982	G	A	<i>FASLG</i>	SNV	p.(Met224Ile)	13	9	68	209	250	EMT
1	172634990	G	A	<i>FASLG</i>	SNV	p.(Gly227Glu)	11	9	71	211	249	EMT
1	172634996	T	G	<i>FASLG</i>	SNV	p.(Met229Arg)	3	7	73	200	240	EMT
1	172634998	A	T	<i>FASLG</i>	SNV	p.(Met230Leu)	7	6	72	200	242	EMT
1	172635002	G	A	<i>FASLG</i>	SNV	p.(Ser231Asn)	2	6	73	204	249	EMT
1	172635024	G	A	<i>FASLG</i>	SNV	p.(Met238Ile)	7	15	78	212	250	EMT
1	172635032	G	A	<i>FASLG</i>	SNV	p.(Arg241His)	6	16	85	209	249	EMT
1	180062254	G	A	<i>CEP350</i>	SNV	p.(Met2338Ile)	0	0	53	4	15	
1	180910336	G	A	<i>KIAA1614</i>	SNV	p.(Arg1025His)	24	0	41	14	54	

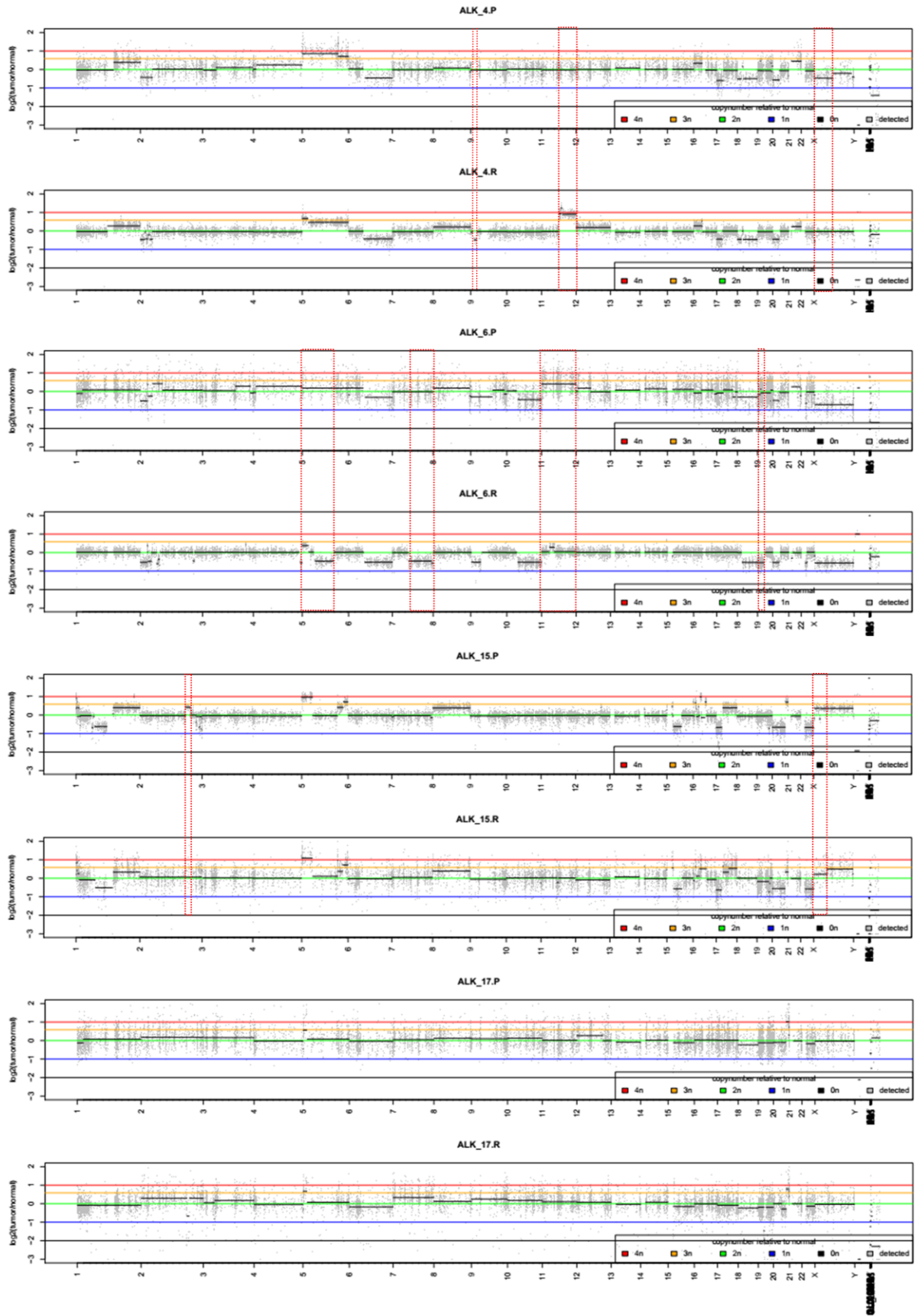
1	202724554	C	G	KDM5B	SNV	p.(Leu497Phe)	19	0	80	7	19	
2	64779826	G	T	AFTPH	SNV	p.(Leu406Phe)	13	0	95	2	10	
2	159077181	C	A	CCDC148	SNV	p.(Met441Ile)	9	0	25	2	10	
2	174055794	C	G	MLTK	SNV	p.(Phe157Leu)	21	0	109	2	10	EMT
2	174055799	AC	A	MLTK	Frameshift Deletion	p.(His160Ilefs*41)	NA	0	109	2	10	EMT
2	241938334	C	G	SNED1	SNV	p.(Ala27Gly)	16	0	12	3	14	
3	38061829	C	T	PLCD1	SNV	p.(Glu38Lys)	9	0	95	35	146	Metabolism
3	99514816	G	A	COL8A1	SNV	p.(Glu691Lys)	18	0	71	21	68	
6	34026857	G	C	GRM4	SNV	p.(Phe307Leu)	0	0	46	15	60	
6	43748488	G	A	VEGFA	SNV	p.(Gly328Arg)	22	0	18	15	39	EMT
6	72892467	G	C	RIMS1	SNV	p.(Glu431Asp)	11	0	39	16	61	
6	129571258	TCC	T	LAMA2	Frameshift Deletion	p.(Pro596Serfs*13)	NA	0	82	2	10	EMT
10	26575335	A	T	GAD2	SNV	p.(Tyr433Phe)	25	0	66	2	10	Metabolism
10	26575337	G	T	GAD2	SNV	p.(Asp434Tyr)	24	0	65	2	10	Metabolism
10	67829157	C	T	CTNNA3	SNV	p.(Glu690Lys)	33	0	186	5	24	EMT
12	27809577	TAGA	T	PPFIBP1	Inframe Deletion	p.(Glu275del)	NA	0	43	2	10	
12	75816737	GCA	G	GLIPR1L2	Frameshift Deletion	p.(Arg214Thrfs*2)	NA	0	27	2	10	
12	112888210	G	A	PTPN11	SNV	p.(Glu76Lys)	36	0	45	7	17	EMT
15	59934411	CAG	C	GTF2A2	Frameshift Deletion	p.(Leu76Glufs*2)	NA	0	69	2	10	
			CTTC AGGG CCGG TCAG CTCA TTGA TCTC GGTA CAGT TT	EMP2	frameshift Insertion	p.(Leu61Phefs*73)	NA	1	26	28	64	
17	19246713	C	T	B9D1	SNV	p.(Met178Ile)	19	0	22	17	42	
17	56388384	G	A	BZRAP1	SNV	p.(Ser1091Leu)	9	4	30	12	43	
19	8922711	T	TAAG	ZNF558	Inframe Insertion	p.(Asn152_Asn152 delinsThrTyr)	NA	0	48	2	10	

19	14184014	C	T	<i>MISP3</i>	SNV	p.(Pro107Ser)	12	0	49	17	56	
19	56538437	C	T	<i>NLRP5</i>	SNV	p.(Arg280Cys)	10	0	164	75	250	
20	57042661	G	T	<i>APCDD1L</i>	SNV	p.(Pro92His)	14	0	59	17	84	
X	57358218	G	T	<i>FAAH2</i>	SNV	p.(Gln200His)	7	4	25	7	13	
X	153069641	G	A	<i>PDZD4</i>	SNV	p.(Arg493Trp)	14	0	22	10	37	
ALK16												
1	85656064	T	A	<i>SYDE2</i>	SNV	p.(Asn373Tyr)	23	0	92	9	38	
1	104118133	C	A	<i>AMY2B</i>	SNV	p.(Arg358Ser)	6	0	24	2	10	Metabolism
2	31399464	G	A	<i>CAPN14</i>	SNV	p.(Gln673*)	38	2	29	19	83	
3	152132776	ATT	A	<i>MBNL1</i>	Frameshift Deletion	p.(Leu75Lysfs*11)	NA	0	190	2	10	
3	152132779	T	A	<i>MBNL1</i>	SNV	p.(Leu75*)	45	0	198	2	10	
5	58571190	CT	AGA	<i>PDE4D</i>	Frameshift Insertion	p.(Ser14Phefs*11)	NA	0	75	3	11	Metabolism
7	72984907	C	A	<i>TBL2</i>	SNV	p.(Arg425Leu)	22	2	47	18	69	
7	150498790	G	T	<i>TMEM176A</i>	SNV	p.(Ser51Ile)	14	1	28	16	57	
11	61508817	G	A	<i>DAGLA</i>	SNV	p.(Val723Ile)	13	1	11	8	21	
12	6840167	C	A	<i>COPS7A</i>	SNV	p.(Leu264Ile)	20	1	26	9	31	
14	24772980	G	A	<i>NOP9</i>	SNV	p.(Val443Met)	18	7	77	15	67	
14	81251682	TATG G	T	<i>CEP128</i>	Frameshift Deletion	p.(His589Aspfs*6)	NA	0	50	2	10	
19	40885573	A	G	<i>HIPK4</i>	SNV	p.(Leu591Pro)	14	2	10	6	15	

¹: Annotation of mutations according to ALAMUT and mutalyzer <https://mutalyzer.nl/>; ²: EMT, epithelial-mesenchymal transition.

Supplementary Table S3. Significantly enriched pathways harboring treatment-related variants in patients ALK6, ALK14 and ALK16.

Pathway name	Patient	Genes mutated	Enrichment score	Enrichment p-value
Proteoglycans in cancer	ALK6, ALK14	<i>ANK2, FASLG, HSPG2, PTPN11, STAT3, VEGFA</i>	8.0	0.00
Pathways in cancer	ALK6, ALK14	<i>ARNT, CTNNA3, FASLG, LAMA2, SMAD4, STAT3, VEGFA</i>	6.5	0.00
AGE-RAGE signaling pathway in diabetic complications	ALK6, ALK14	<i>PLCD1, SMAD4, STAT3, VEGFA</i>	6.4	0.00
Renal cell carcinoma	ALK14	<i>ARNT, PTPN11, VEGFA</i>	5.4	0.00
Hepatitis B	ALK6, ALK14	<i>FASLG, HSPG2, SMAD4, STAT3</i>	5.3	0.00
Pancreatic cancer	ALK6, ALK14	<i>SMAD4, STAT3, VEGFA</i>	5.0	0.01
HIF-1 signaling pathway	ALK6, ALK14	<i>ARNT, STAT3, VEGFA</i>	4.3	0.01
Type I diabetes mellitus	ALK14	<i>FASLG, GAD2</i>	4.2	0.01
FoxO signaling pathway	ALK6, ALK14	<i>FASLG, SMAD4, STAT3</i>	3.7	0.03
GABAergic synapse	ALK6, ALK14	<i>GAD2, SLC6A1</i>	3.4	0.03
Arrhythmogenic right ventricular cardiomyopathy	ALK14	<i>CTNNA3, LAMA2</i>	3.3	0.04
Taurine and hypotaurine metabolism	ALK14	<i>GAD2</i>	3.3	0.04
ECM-receptor interaction	ALK6, ALK14	<i>HSPG2, LAMA2</i>	3.3	0.04
Aldosterone synthesis and secretion	ALK6, ALK16	<i>DAGLA, SCARB1</i>	3.1	0.05
Adipocytokine signaling pathway	ALK6, ALK14	<i>PTPN11, STAT3</i>	3.1	0.05



Supplementary Figure S1. Copy number variant plots across four patients with ALK rearranged advanced NSCLC with matched primary and resistant tumor samples. The chromosomal segments with conspicuous copy number differences between primary and resistant samples in each patient are marked by the red boxes.