

Table S1. ALK Inhibitors in Clinical Development

Generation	Agent	Manufacturer
1st Generation	Crizotinib ^a	Pfizer
2nd Generation	Ceritinib ^a	Novartis
	Alectinib ^a	Genentech/Roche
	Brigatinib	Ariad
	Entrectinib	Ignitya
	X-396	Xcovery
3rd Generation	Lorlatinib	Pfizer

^aAgents that are currently approved by the U.S. Food and Drug Administration

Table S2. Summary of Clinical Characteristics of ALK-Positive Patients Undergoing Biopsies at the Time of ALK Inhibitor Resistance

	Crizotinib-Resistant Biopsies	Ceritinib-Resistant Biopsies	Alectinib-Resistant Biopsies	Brigatinib-Resistant Biopsies
Patients (N)	51 ^a	23 ^b	17	6 ^b
Age (yrs)				
Median	54	47	54	48.5
Range	22-78	22-78	32-67	38-67
Gender – no. (%)				
Male	26 (51)	7 (30)	8 (47)	5 (83)
Female	25 (49)	16 (70)	9 (53)	1 (17)
Race – no. (%)				
Caucasian	42 (82)	20 (87)	17 (100)	4 (60)
Asian	6 (12)	3 (13)	0 (0)	1 (20)
Other	3 (6)	0 (0)	0 (0)	1 (20)
Prior ALK Inhibitors – no. (%)				
Crizotinib	51 (100)	21 (91)	17 (100)	5 (83)
Ceritinib	1 (2)	23 (100)	3 (18)	-
Alectinib	-	3 (13)	17 (100)	-
Brigatinib	-	-	-	6 (100)
Site of Biopsy – no. (%)				
Pleura/Pleural Effusion	17 (31)	4 (17)	3 (18)	0 (0)
Liver	12 (22)	7 (29)	7 (41)	1 (14)
Lymph Node	10 (18)	1 (4)	3 (17)	4 (57)
Lung	6 (11)	7 (29)	2 (12)	1 (14)
Brain	3 (5)	1 (4)	0 (0)	0 (0)
Other ^c	7 (13)	4 (17)	2 (12)	1 (14)
Sequencing for ALK Mutations– no. (%)				
Sanger Sequencing (cDNA)	44 (81)	9 (38)	3 (18)	1 (14)
Sanger Sequencing (gDNA)	2 (4)	1 (4)	0 (0)	1 (14)
Targeted Next-Generation Sequencing	8 (15) ^d	14 (58)	14 (82)	4 (57) ^d

^aFour patients underwent 2 separate biopsies. ^bOne patient underwent 2 separate biopsies. ^cNot available in 3 patients. ^dOne additional specimen underwent whole-exome sequencing. Percentages may not add up to 100% due to rounding.

Table S3. ALK-Positive Patients with Paired, Post-Crizotinib and Post Second-Generation ALK Inhibitor Biopsies

Post-Crizotinib Samples			Post 2 nd Generation ALK Inhibitor Samples				
ID	Biopsy Site	ALK Resistance Mutation	2 nd Generation ALK Inhibitor	Best Response by RECIST ^a	Duration of Therapy (months)	Biopsy Site	ALK Resistance Mutation
MGH011	Lung	S1206Y	Ceritinib	PR	15.4	Lung	G1202R
MGH015	Lung	WT	Ceritinib	N/A	8.2	Bone	WT
MGH023	Liver	WT	Ceritinib	PR	8.7	Liver	F1174C
MGH034	Liver	WT	Ceritinib	PR	9.8	Liver	WT
MGH040	Node	WT	Ceritinib	PR	34.1	Lung	G1202R
MGH049	Lung	WT	Ceritinib	PR	7.1	Pleural fluid	WT
MGH051	Liver	WT	Ceritinib	PR	6.5	Liver	G1202R
MGH061	Brain	WT	Ceritinib	SD	7.6	Brain	WT
MGH065	Node	WT	Ceritinib	PR	8.4	Adrenal	L1196M
MGH087	Node	WT	Alectinib	SD	14.7	Lung	V1180L
MGH090	Node	WT	Alectinib	SD	2.1	Peritoneum	WT
MGH098	Pleural fluid	WT	Alectinib	SD	3.2	Pleural fluid	WT
MGH032	Liver	WT	Brigatinib	N/A	N/A	CNS	G1202R
MGH086 ^b	Node	E1210K	Brigatinib	N/A	NR	Node	(1) E1210K + S1206C (2) E1210K + D1203N

^aCeritinib response assessments were performed using RECIST version 1.0; alectinib assessments were performed using RECIST version 1.1.

^bPatient MGH086 underwent two separate post-brigatinib biopsies.

Abbreviations: anaplastic lymphoma kinase, ALK; central nervous system, CNS; not available, N/A; not reached, NR; RECIST, Response Evaluation Criteria in Solid Tumors

Table S4. Treatment Course of ALK-Positive Patients Undergoing Post-Ceritinib Biopsies

ID	Crizotinib – Duration of Therapy (Months)	Intervening Therapy Between Crizotinib and Ceritinib	Ceritinib – Duration of Therapy (Months)	Site of Ceritinib-Resistant Biopsy	ALK Resistance Mutation	Immediate Post-Ceritinib Therapy
MGH011	35.1	-	15.4	Lung	G1202R	Chemotherapy
MGH015	20.6	(1) Chemotherapy (2) Clinical Trial #1 (3) Clinical Trial #2	8.2	Bone	WT	None
MGH023	17.0	(1) Chemotherapy (2) Clinical Trial	8.7	Liver	F1174C	None
MGH034	8.4	-	9.8	Liver	WT	Clinical Trial
MGH040	9.8	-	34.1	Lung	G1202R	Clinical Trial
MGH036/49	13.7	-	7.1	Pleural fluid	WT	Clinical Trial
MGH051	2.8	-	6.5	Liver	G1202R	Chemotherapy
MGH057	8.1	-	5.8	Liver	WT	None
MGH061	8.3	-	7.6	Brain	WT	None
MGH065	5.6	-	8.4	Adrenal	L1196M	Chemotherapy
MGH067	6.5	(1) Chemotherapy (2) Alectinib (3) Chemotherapy	2.3	Subcutaneous mass	L1196M	None
MGH068	10.5	(1) Chemotherapy	14.7	Liver	F1174C	None
MGH075 ^a	N/A	N/A	7.1	Lung	WT	Chemotherapy
MGH084	18.2	(1) Alectinib	8.2	Liver	I1171N, C1156Y	Clinical Trial
MGH089	5.8	-	1.1	Pleural fluid	WT	Alectinib
MGH092	10.3	-	12.0	Lung	G1202del	Chemotherapy
MGH901	28.4	(1) Alectinib (2) Chemotherapy	9.2	N/A	G1202R	N/A
MGH902	4.8	-	3.0	Lung	WT	Chemotherapy
MGH905	8.2	-	27.1	Liver	F1174C, D1203N	Clinical Trial
MGH908	7.6	-	7.8	Pleural fluid	WT	Clinical Trial
MGH915 ^a	N/A	N/A	32.7	Lung	WT	Clinical Trial

MGH932	7.1	-	N/A	Node	C1156Y, G1202del, V1180L	N/A
MGH970	7.2	-	5.5	Lung	F1174L, G1202R	Chemotherapy

^aPatients MGH075 and MGH915 were treated with first-line ceritinib.

Abbreviations: anaplastic lymphoma kinase, ALK; not available, N/A; wild-type, WT

Table S5. Treatment Course of ALK-Positive Patients Undergoing Post-Alectinib Biopsies

ID	Crizotinib – Duration of Therapy (Months)	Intervening Therapy Between Crizotinib and Alectinib	Alectinib – Duration of Therapy (Months)	Site of Alectinib-Resistant Biopsy	ALK Resistance Mutation	Immediate Post-Alectinib Therapy
MGH056	14.0	-	4.2	Liver	I1171T	Chemotherapy
MGH063	27.4	-	7.4	Pleural fluid	WT	Ceritinib
MGH074	26.1	-	21.1	Lymph node	WT	Clinical trial
MGH087	11.5	-	14.7	Lung	V1180L	Ceritinib
MGH090	19.5	-	2.1	Peritoneum	WT	None
MGH098	5.8	-	3.2	Pleural fluid	WT	Chemotherapy
MGH099	7.7	-	6.7	Liver	WT	Clinical Trial
MGH907	20.0	Ceritinib	5.1	Lung	WT	None
MGH910	8.1	(1) Chemotherapy (2) Chemotherapy (3) Ceritinib	1.3	Liver	G1202R	Lost to follow-up
MGH913	8.8	-	15.1	Adrenal	I1171S	Chemotherapy
MGH917	14.4	-	33.0	Lymph node	G1202R	Lost to follow-up
MGH919	1.7	-	1.8	Liver	G1202R	Chemotherapy
MGH921	0.6	-	15.2	Lymph node	WT	Clinical trial
MGH935	41.6	-	12.2	Liver	G1202R	Clinical trial
MGH936	9.6	-	16.0	Liver	G1202R	Clinical trial
MGH964	6.7	Ceritinib	7.6	Pleural fluid	WT	Clinical trial
MGH988	12.1	Chemotherapy	26.1	Liver	L1196M	Lost to follow-up

Abbreviations: anaplastic lymphoma kinase, ALK; wild-type, WT

Table S6. Treatment Course of ALK-Positive Patients Undergoing Post-Brigatinib Biopsies

ID	Crizotinib – Duration of Therapy (Months)	Intervening Therapy Between Crizotinib and Brigatinib	Brigatinib – Duration of Therapy (Months)	Site of Post-Brigatinib Biopsy	ALK Resistance Mutation	Immediate Post-Brigatinib Therapy
MGH032	13.4	(1) Chemotherapy (2) Clinical Trial (3) Clinical Trial	N/A ^a	Central nervous system	G1202R	Lost to follow-up
MGH086	21.0	-	44.4 ^b	Lymph node	(1): E1210K + S1206C (2): E1210K + D1203N	None ^b
MGH093	14.5	-	20.2	Lung	G1202R	Clinical trial
MGH900	1.6 ^c	-	18.1	Lymph node	WT	Clinical trial
MGH9003-1	20.7	-	12.1	Liver	G1202R	Chemotherapy
MGH952	N/A	-	28.1 ^b	Lymph node	WT	None ^b

^aSpecific dates surrounding therapy were not available.

^bRemains on therapy to date.

^cDiscontinued due to hepatotoxicity.

Abbreviations: anaplastic lymphoma kinase, ALK; wild-type, WT

Table S7. Second-Generation ALK Inhibitor-Resistant Specimens with ≥ 2 ALK Resistance Mutations

Specimen	ALK Inhibitors (Prior To Biopsy)	ALK Resistance Mutations
MGH084-1	Crizotinib, Alectinib, Ceritinib	I1171N, C1156Y
MGH905-1	Crizotinib, Ceritinib	D1203N, F1174C
MGH932-1	Crizotinib, Ceritinib	C1156Y, G1202del, V1180L
MGH970-1	Crizotinib, Ceritinib	F1174L, G1202R
MGH086-0	Crizotinib, Brigatinib	E1210K, S1206C
MGH086-1	Crizotinib, Brigatinib	E1210K, D1203N

Table S8: Distribution of Mutations in Ceritinib-Resistant Biopsies

MGH ID	Sequencing Platform	ALK Resistance Mutation	Gene	Alteration
MGH040-2	Snapshot-NGS-V1	G1202R	MET	T992I
MGH065-2	Snapshot-NGS-V1	L1196M	TP53	S314fs
MGH068-2	Snapshot-NGS-V1	F1174C	-	-
MGH092-1	Snapshot-NGS-V1	G1202del	-	-
MGH902-1	Snapshot-NGS-V1	WT	-	-
MGH905-1	Snapshot-NGS-V1	F1174C D1203N	FGFR2	F645L
MGH908-1	Snapshot-NGS-V1	WT	BRAF TP53	G615V L321fs
MGH915-0	Snapshot-NGS-V1	WT	-	-
MGH015-2	Foundation One™	WT	NKX2-1	Amplification
MGH084-1	Foundation One™	C1156Y I1171N	-	-
MGH901-1	Foundation One™	G1202R	-	-
MGH932-1	Foundation One™	C1156Y V1180L G1202del	CDKN2A/B NOTCH2 NOTCH2 ARID2	Loss S2395* S2437* Loss (exons 1-3)
MGH970-1	Foundation One™	F1174L G1202R	ACVR1B APC	Y151* I1307K

Table S9: Distribution of Mutations in Alectinib-Resistant Biopsies

MGH ID	Sequencing Platform	ALK Resistance Mutation	Gene	Alteration
MGH074-2	Snapshot-NGS-V1	WT	PIK3CA TP53	G106V E224D
MGH087-2	Snapshot-NGS-V1	V1180L	DDR2 TP53	L610F I195T
MGH098-2	Snapshot-NGS-V1	WT	-	-
MGH099-1	Snapshot-NGS-V1	WT	TP53	Splice acceptor variant
MGH907-1	Snapshot-NGS-V1	WT	-	-
MGH910-1	Snapshot-NGS-V1	G1202R	TP53	C176Y
MGH913-1	Snapshot-NGS-V1	I1171S	TP53	R248W
MGH917-1	Snapshot-NGS-V1	G1202R	TP53	V172F
MGH919-4	Snapshot-NGS-V1	G1202R	-	-
MGH921-1	Snapshot-NGS-V1	WT	TP53	V1173G
MGH936-1	Snapshot-NGS-V1	G1202R	-	-
MGH964-1	Snapshot-NGS-V1	WT	BRAF NRAS	Splice acceptor variant A155T

Table S10: Distribution of Mutations in Brigatinib-Resistant Biopsies by Snapshot

MGH ID	Sequencing Platform	ALK Resistance Mutation	Gene	Alteration
MGH9003-1	Snapshot-NGS-V1	G1202R	-	-
MGH952-1	Snapshot-NGS-V1	WT	-	-

Table S11. Single-Nucleotide Variants Identified in Patient-Derived ALK-Positive Cell Lines by NGS

Cell line	Control	2° ALK mutation	Gene	cDNA change	AA change	Allelic Fraction
MGH021-5A	HapMap	G1202R	AKT1	655A>C	T219P	0.088
			ALK	3604G>A	G1202R	0.430
			ALPK3	4698G>C	E1566D	0.489
			ARID1A	3778T>C	Y1260H	0.059
			CADM1	1064T>G	V355G	0.044
			CADM1	1070T>G	V357G	0.039
			CAMK2B	1918A>C	T640P	0.049
			CBLB	1754A>C	D585A	0.031
			CBLC	397G>A	A133T	0.536
			CDK4	409G>A	V137I	0.471
			COL1A1	1259C>G	P420R	0.520
			CREB3L2	1406C>A	P469Q	0.457
			DGKZ	3316C>A	Q1106K	0.048
			DYRK1B	212C>G	A71G	0.075
			EPHA1	1679C>G	A560G	0.490
			EPHA1	529A>C	T177P	0.075
			FGFR3	797T>G	V266G	0.051
			IL21R	1445T>C	L482P	0.113
			IP6K1	1033C>G	R345G	0.039
			IRS2	275T>G	V92G	0.037
			MAP3K6	2246G>T	S749I	0.034
			MEF2B	130G>C	A44P	0.032
			MITF	283A>C	T95P	0.046

			MYCL1	518C>G	A173G	0.458
			MYLK	5395A>G	R1799G	0.024
			OBSCN	3269C>G	A1090G	0.032
			OBSCN	5201C>G	A1734G	0.028
			PIK3C2B	485G>A	R162Q	0.492
			PIP4K2B	38T>G	V13G	0.149
			RIPK2	100T>C	S34P	0.037
			ROCK1	3649C>G	Q1217E	0.078
			ROR2	433A>C	T145P	0.051
			SPEG	9038T>G	V3013G	0.026
			STK38L	415A>G	R139G	0.422
			TAOK2	2116A>C	T706P	0.043
			TRPM6	218C>T	A73V	0.471
			ULK2	280G>A	A94T	0.477
			WIF1	722G>A	C241Y	0.423
MGH051-2	Blood	G1202R	ALK	c.3604G>A	p.G1202R	0.288
			KDM6A	c.1525C>T	p.Q509*	0.952
			KIT	c.2866C>T	p.R956W	0.107
			MAP3K6	c.274C>T	p.R92C	0.657
			PRKCB	c.841G>A	p.E281K	0.343
			TRRAP	c.1892T>G	p.L631W	0.279
MGH075-2E	Blood	WT	AR	c.170T>A	p.L57Q	0.459
			CSMD3	c.1186G>A	p.V396M	0.321
			GRK6	c.1109T>A	p.V370D	0.060
			ROCK2	c.3779G>T	p.G1260V	0.305
			TP53	c.839G>C	p.R280T	1.000
MGH084-1D	Blood	I1171N, C1156Y	EPS15	c.1497G>A	p.M499I	0.504

			AKT3	c.1034C>A	p.P345H	0.192
			ALK	c.3512T>A	p.I1171N	0.168
			ALK	c.3467G>A	p.C1156Y	0.161
			MYO3B	c.583C>T	p.P195S	0.658
			MLTK	c.1672G>A	p.D558N	0.293
			STK16	c.14T>A	p.L5Q	0.339
			EPHA6	c.1697C>T	p.A566V	0.290
			TFG	c.1075C>G	p.P359A	0.232
			DGKB	c.1337A>T	p.K446I	0.186
			PRKD1	c.1349G>A	p.C450Y	0.211
			TSC2	c.3725A>G	p.K1242R	0.220
			PIM3	c.239G>A	p.G80D	0.382
			ATRX	c.2719C>T	p.R907*	0.561

Table S12: Assessments of Epithelial Mesenchymal Transition in Ceritinib-Resistant Biopsies

ID	Baseline, Initial Diagnostic Specimen		Ceritinib-Resistant Specimen	
	Vimentin	E-Cadherin	Vimentin	E-Cadherin
MGH023	Negative	Positive	Positive	Negative
MGH034	Negative	Positive	Positive	Negative
MGH065	Positive	Positive	Positive	Negative
MGH067	Negative	Positive	Positive	Negative
MGH902	Negative	Positive	Positive	Negative ^a

^aPartial loss

Table S13. Cells Seeded for Survival Assays

Cell line	Cell seeding	Assay time (d)	Proliferation index
MGH034-2A	4000	7	5.1
MGH049-1A	2000	5	4.2
MGH075-2E	7500	3	4.2
MGH021-5A	4000	7	3.9
MGH051-2C	5000	5	4
MGH084-1D	8000	14	3.9

Table S14. Cells Seeded for Growth Assays

Cell line	Cell seeding
MGH034-2A	4000
MGH049-1A	1500
MGH075-2E	2000
MGH021-5A	5000
MGH051-2C	4000
MGH084-1D	8000