Genetically engineered Ba/F3 cell lines and *in vivo* models

WuXi AppTec Research Service Division, Oncology & Immunology Unit

2020.07

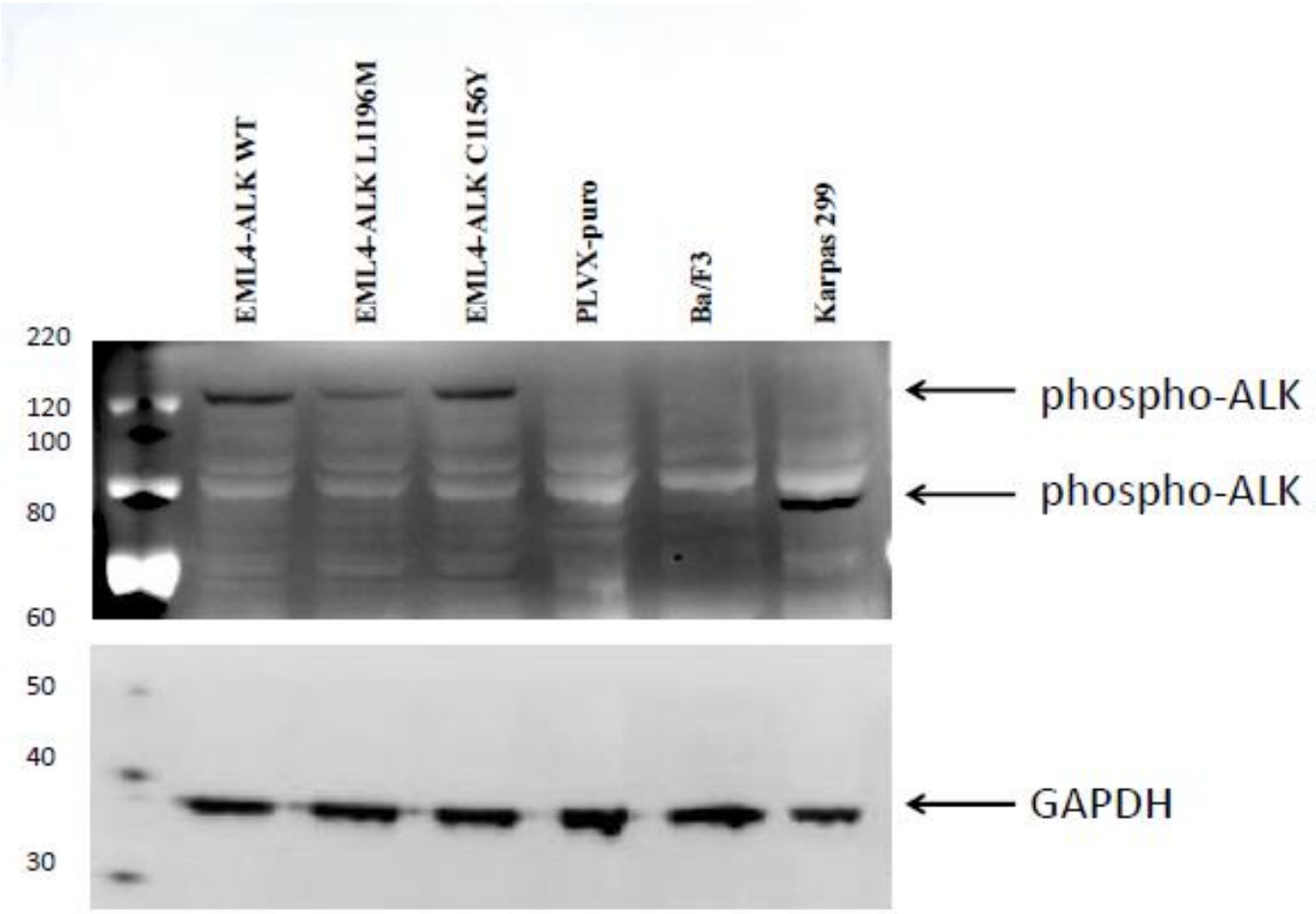
Outline

- ALK engineered Ba/F3 cell lines and *in-vitro/in-vivo* data
- EGFR engineered Ba/F3 cell lines and *in-vitro/in-vivo* data
- TRK engineered Ba/F3 cell lines and *in-vitro/in-vivo* data

Engineered Ba/F3 cell lines carrying ALK mutations

Cancer type	Cell line	Model growth curve
Pro-B cells	Ba/F3-EML4-ALK-WT	Yes
	Ba/F3-EML4-ALK-L1196M	Yes
	Ba/F3-EML4-ALK-C1156Y	Yes
	Ba/F3-EML4-ALK-G1202R/L1196M	Yes
	Ba/F3-EML4-ALK-G1202R	Yes

EML4-ALK expression in engineered Ba/F3 cells



- Loading protein: 80 ug
- Antibodies
  - Primary antibody:
    - –Phospho-ALK(Tyr1604) Antibody
    - –CST #3341
  - Secondary antibody:
    - – anti-rabbit

Activities of ALK inhibitors on Ba/F3-EML4-ALK cells

Cell line	Compound ID	IC50 (nM)	
		n=1	n=2
Ba/F3	Crizontinib	836	1733
	Brigatinib	2112	4523
Ba/F3(plvx-puro)	Crizontinib	368	1911
	Brigatinib	2444	4181
Ba/F3(ALK WT)	Crizontinib	63	123
	Brigatinib	8	10
Ba/F3(L1196M)	Crizontinib	204	278
	Brigatinib	7	19
Ba/F3(C1156Y)	Crizontinib	170	352
	Brigatinib	16	25

- Assay settings:
  - 3000 cells per well
  - 3 days' incubation
  - Ba/F3 and Ba/F3 (plvx-puro) as the negative control

- Results
  - S/N >50 folds
  - Z primes >0.7

- Both reference cpds were tested in 5 cell lines to see the IC50 shift between ALK WT and ALK two sites mutant (L1196M,C1156Y).

Activities of ALK inhibitors on Ba/F3-EML4-ALK-G1202R/L1196M and G1202R cells

Cell line	Reference compound IC50 (uM)					
	Crizotinib	Ceritinib	Alectinib	Entrectinib	Brigatinib	Lorlatinib
G1202R/L1196M	0.904±0.087	0.425±0.038	0.634±0.155	2.507±0.687	0.313±0.151	1.058±0.193
G1202R	0.368±0.025	0.174±0.035	0.284±0.038	2.589±0.392	0.128±0.005	0.052±0.002

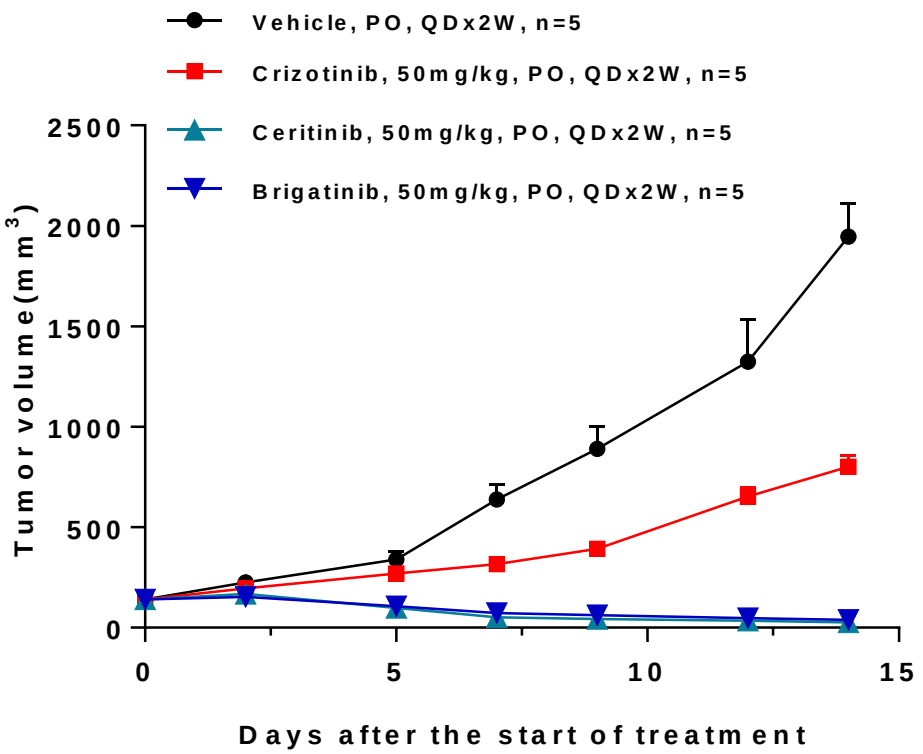
In vivo models derived from Ba/F3 genetically engineered cell lines for ALK

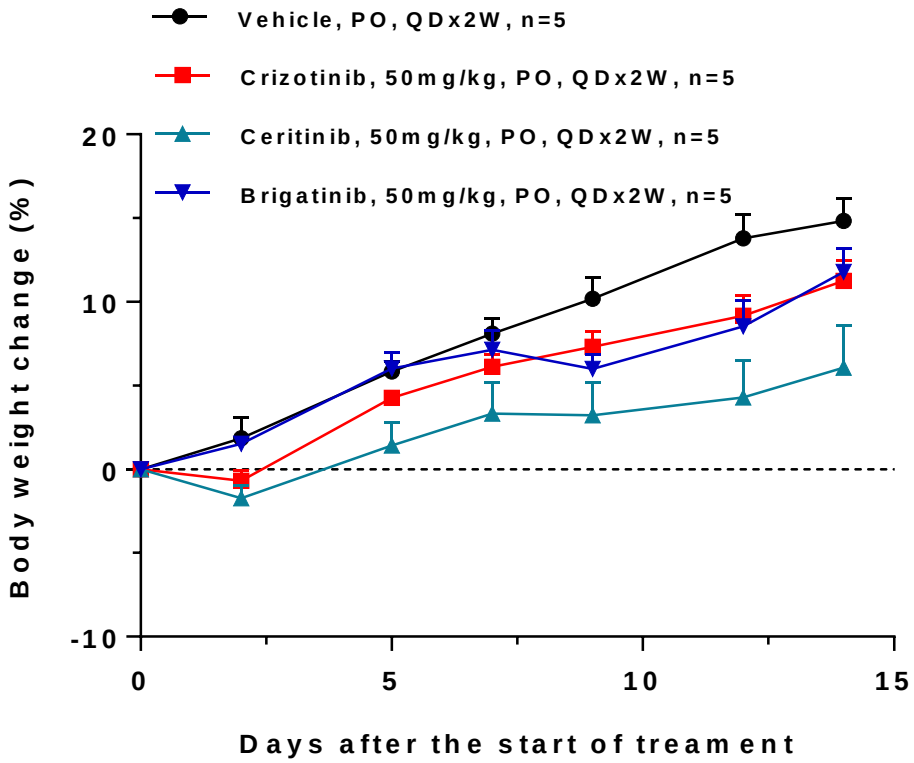
Cancer type	Model ID	Tumor growth curve	Drug tested and Dosage (mg/kg)	TGI (%)	Model genomics
Pro-B cells	Ba/F3-EML4-ALK-WT	Yes	Crizotinib (50) Ceritinib (50) Brigatinib (50)	63.4 106.3 105.6	EML4-ALK WT
	Ba/F3-EML4-ALK-L1196M	Yes	Crizotinib (50) Ceritinib (50, 100) Brigatinib (25)	4 70, 105 22	EML4-ALK L1196M
			Crizotinib (200) Alectinib (20) Ceritinib (50) Lorlatinib (20)	25 19 86 100	
	Ba/F3-EML4-ALK-C1156Y	Yes	Crizotinib (50, 100) Ceritinib (50, 100) Brigatinib (50)	0, 0 53, 90 116	EML4-ALK C1156Y
			Crizotinib (100) Ceritinib (25, 50) Lorlatinib (20) Alectinib (20, 60)	43.7 38.8, 67.9 114.7 117.2, 115.9	

In vivo models derived from ALK engineered Ba/F3 cell lines

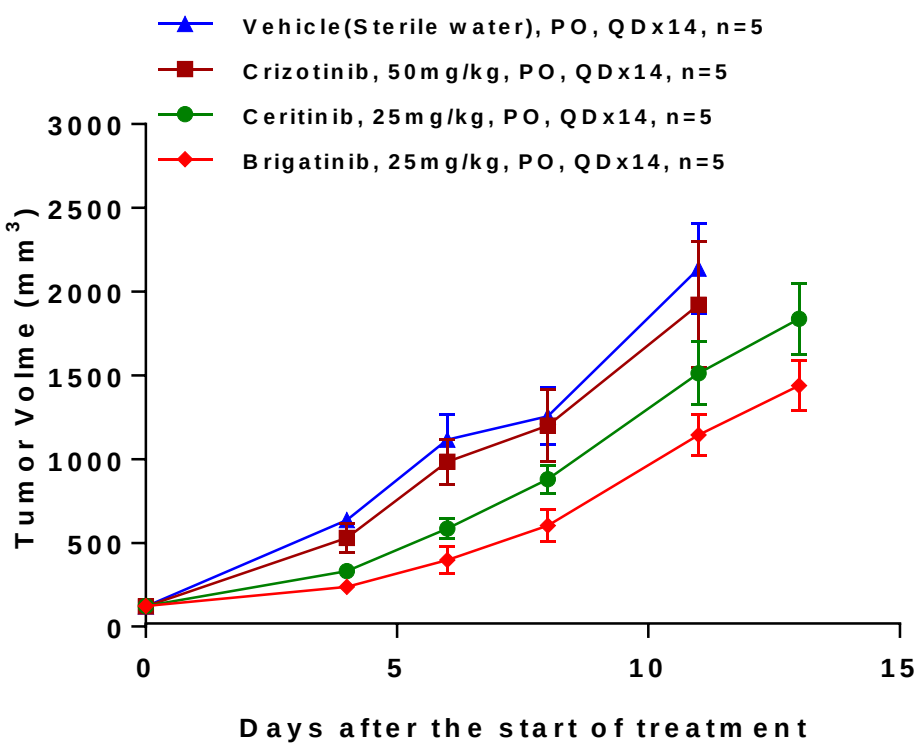
Cancer type	Model ID	Tumor growth curve	Drug tested and Dosage (mg/kg)	TGI (%)	Model genomics
Pro-B cells	Ba/F3-EML4-ALK G1202R	Yes	Ceritinib (50) Alectinib (60) Lorlatinib (20)	17 -0.05 111	EML4-ALK-G1202R
	Ba/F3-EML4-ALK G1202R/L1196M	Yes	Ceritinib (50) Alectinib (60) Lorlatinib (20)	-20 0.12 -18	EML4-ALK G1202R/L1196M

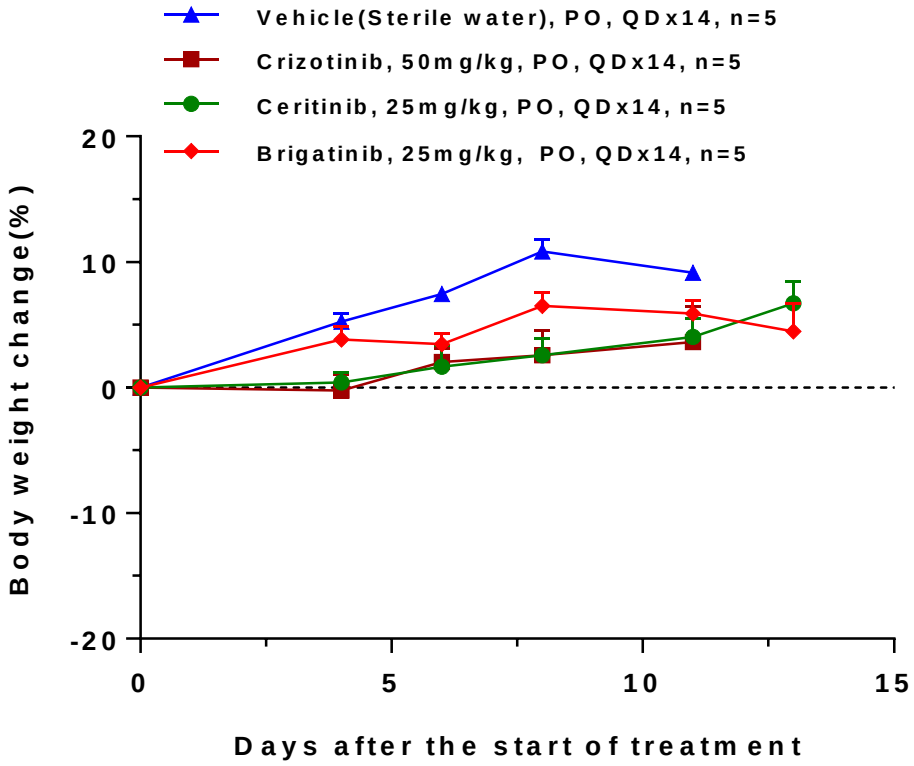
ALK inhibitors in Ba/F3-EML4-ALK-WT model



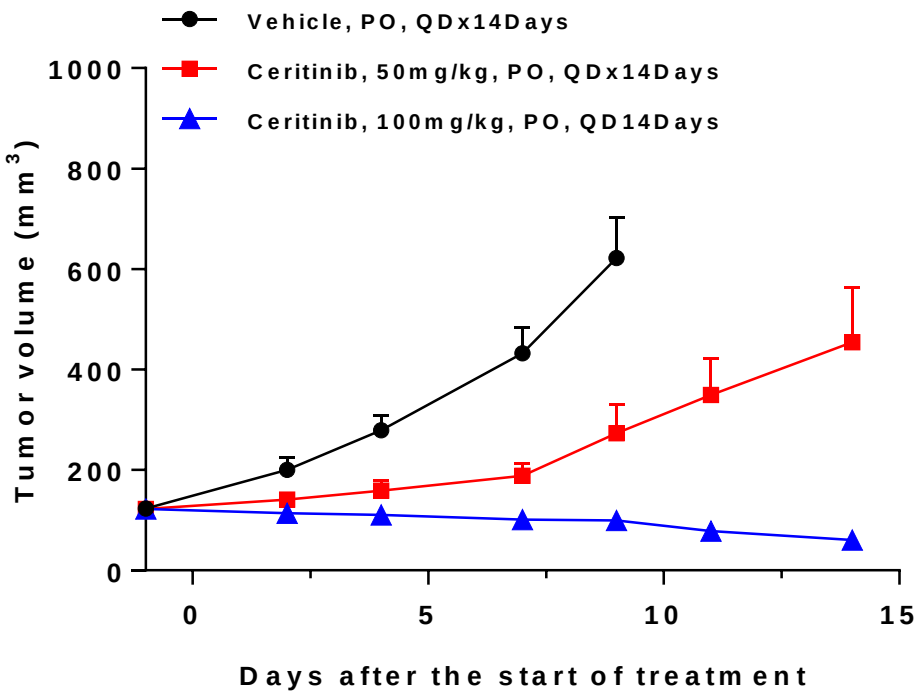


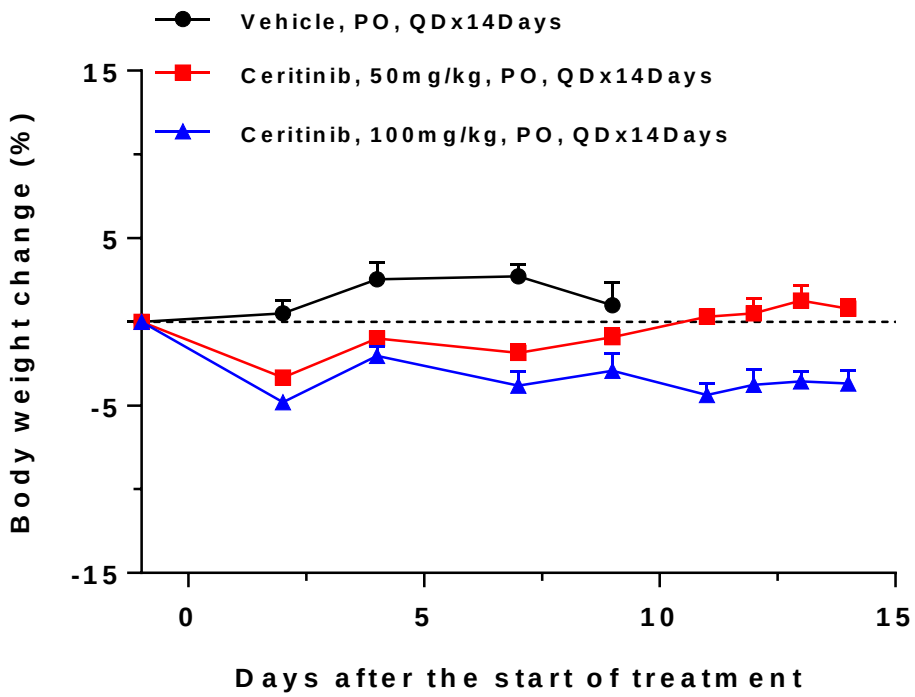
ALK inhibitors in Ba/F3-EML4-ALK-L1196M model



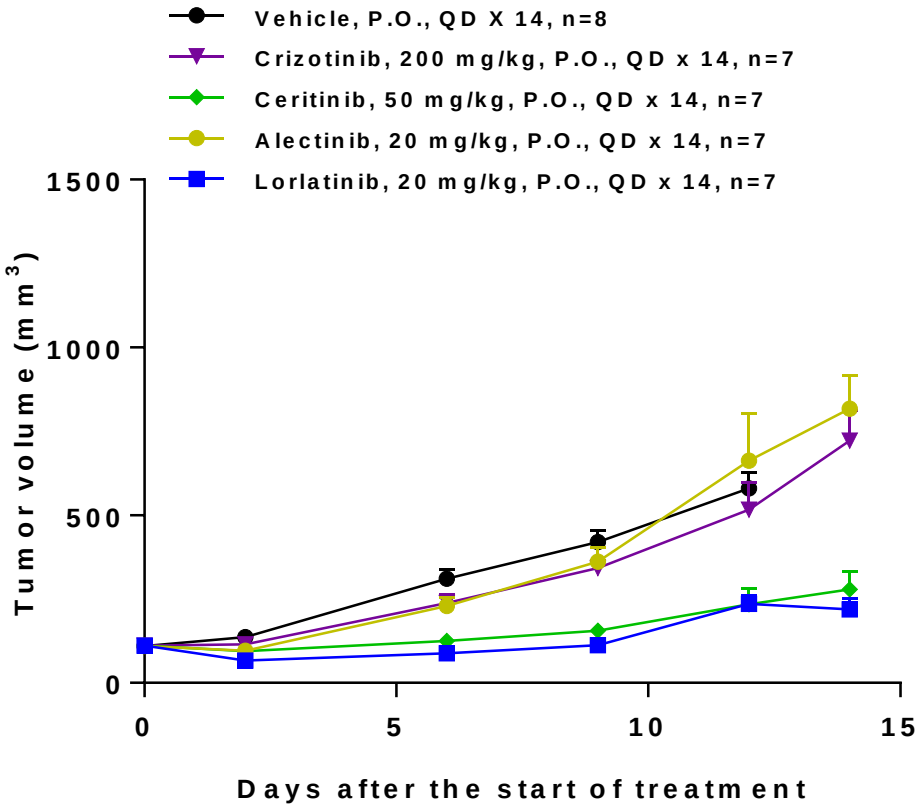


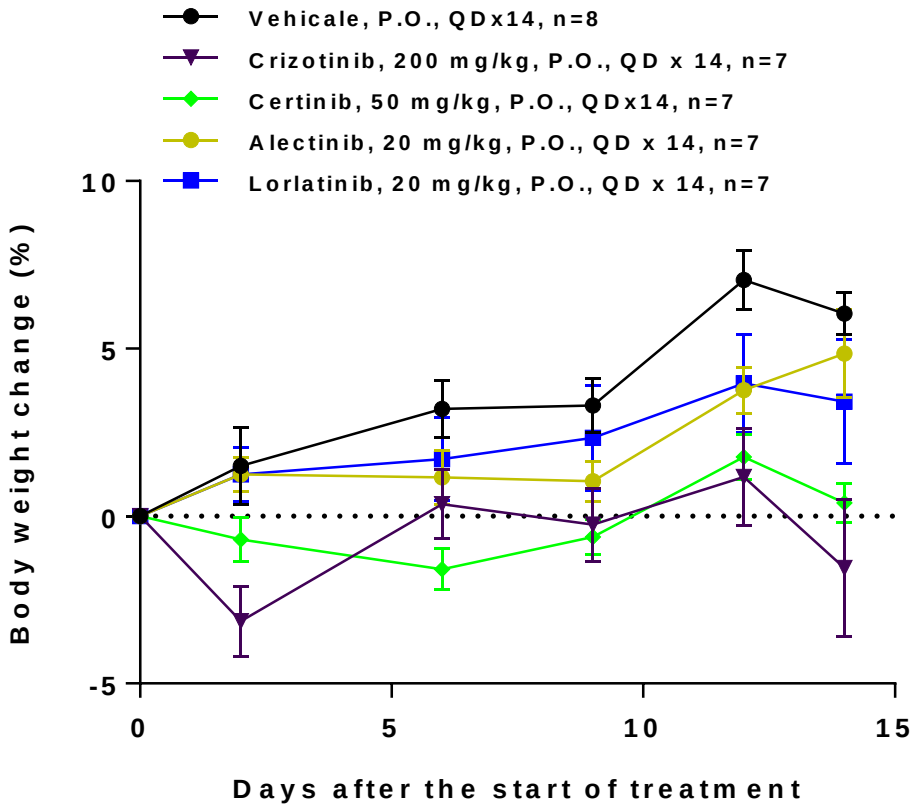
ALK inhibitors in Ba/F3-EML4-ALK-L1196M model



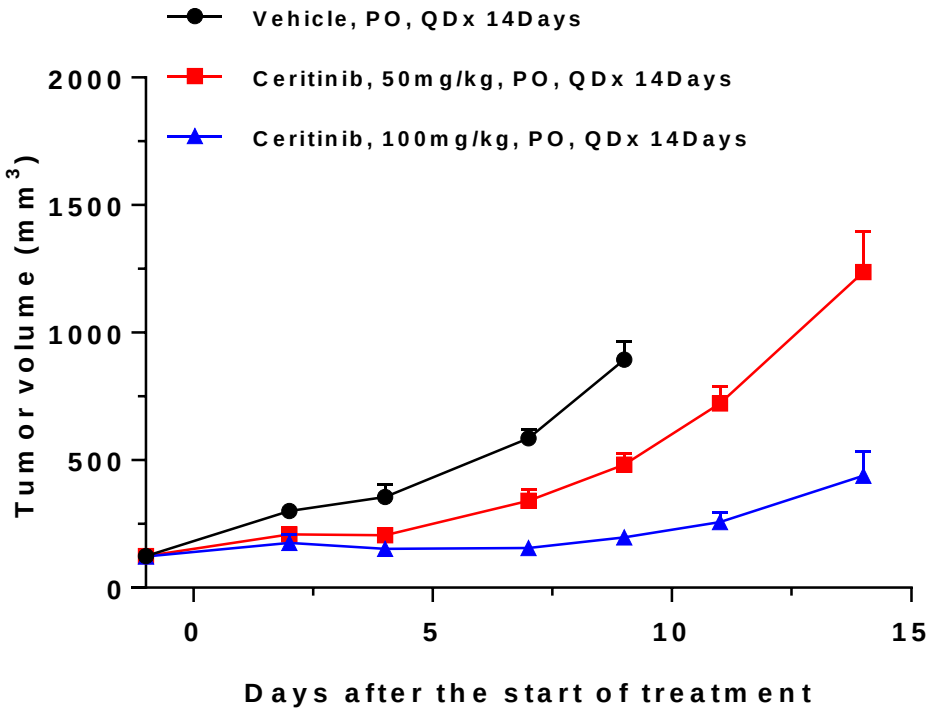


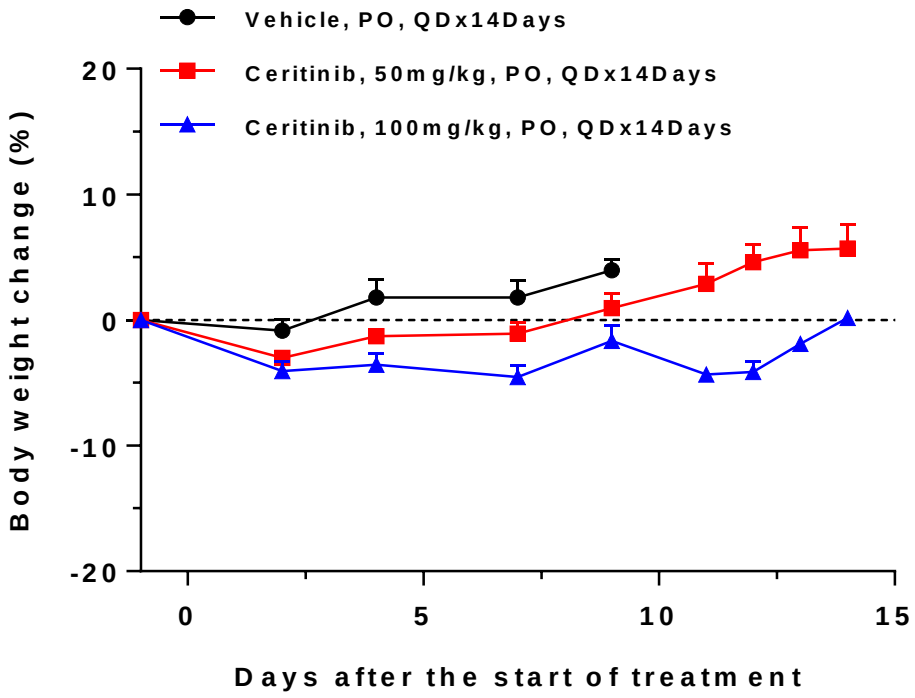
ALK inhibitors in Ba/F3-EML4-ALK-L1196M model



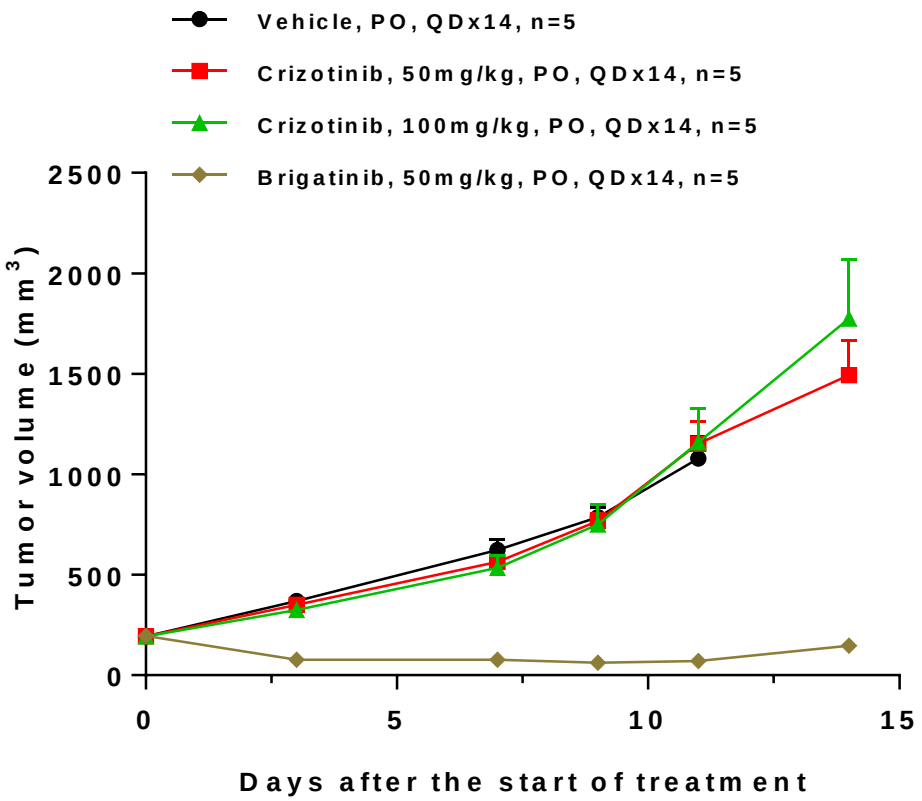


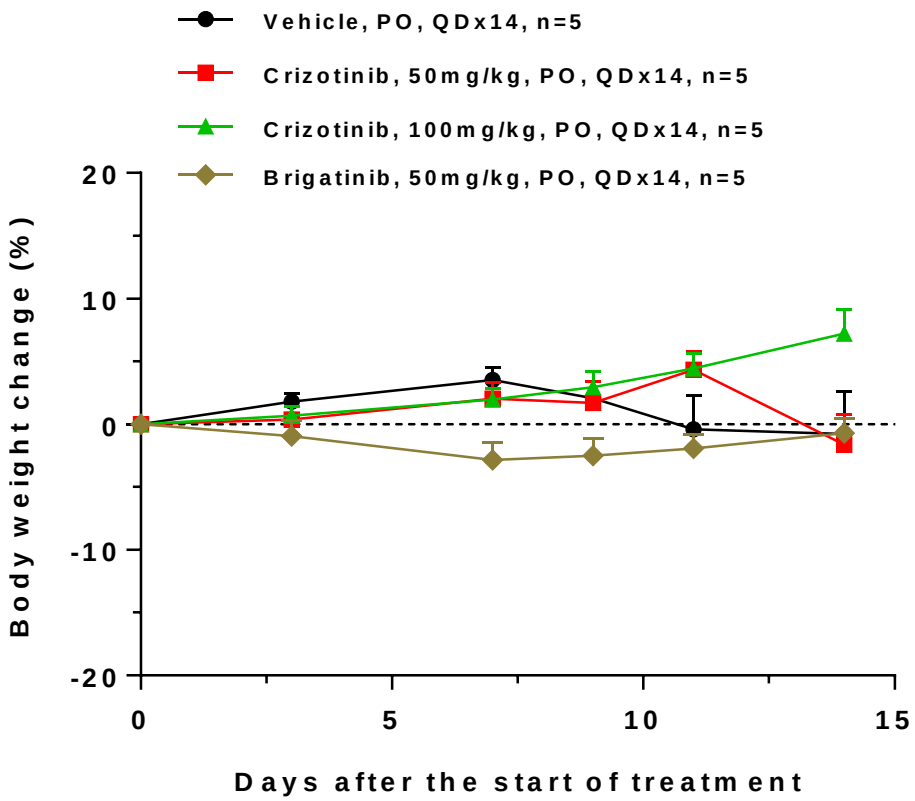
ALK inhibitors in Ba/F3-EML4-ALK-C1156Y model



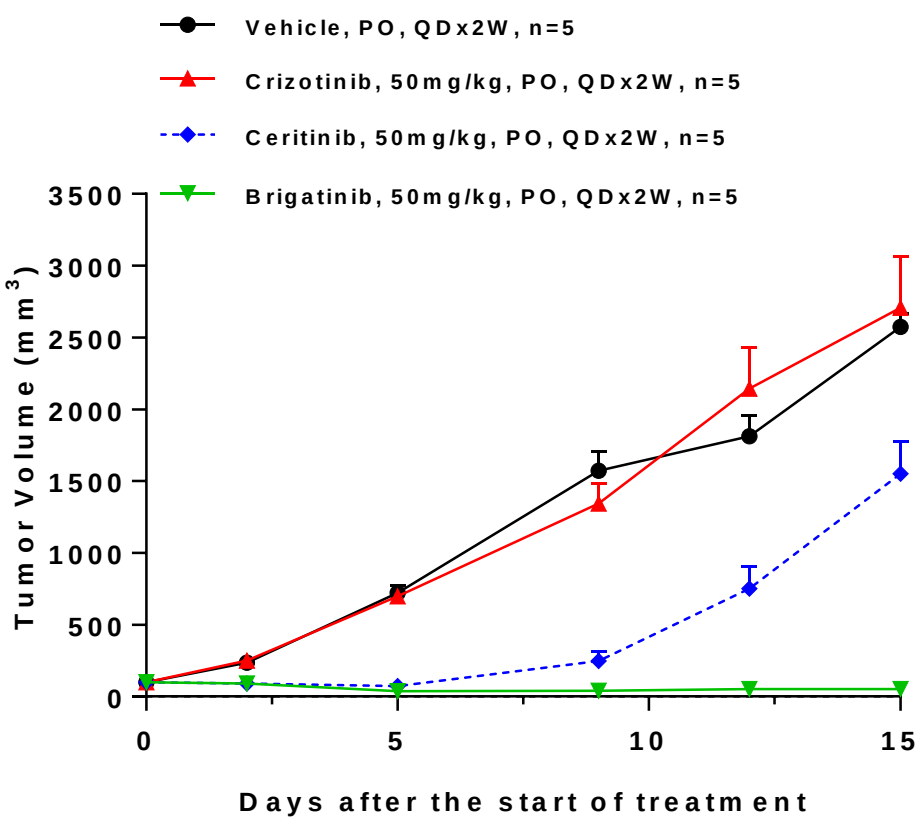


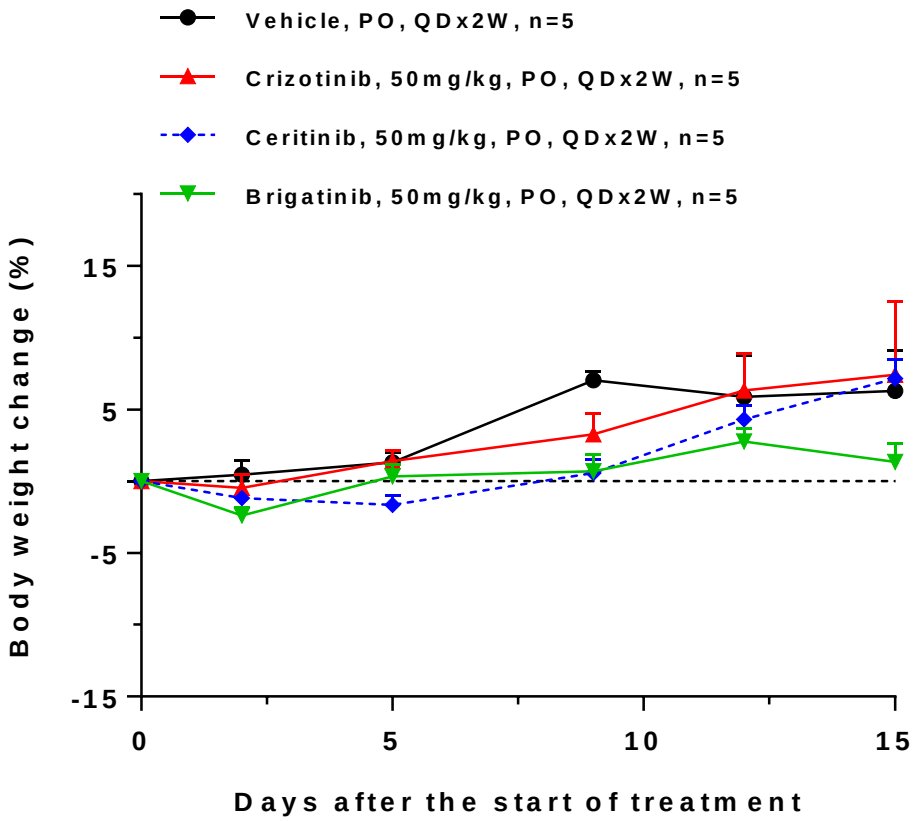
ALK inhibitors in Ba/F3-EML4-ALK-C1156Y model



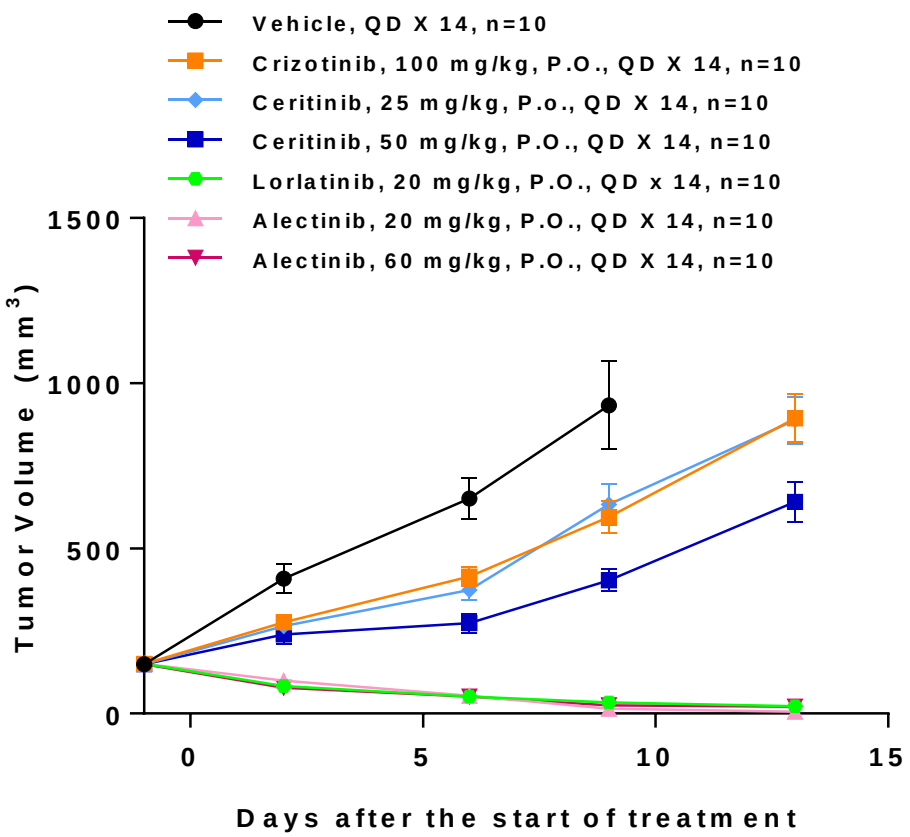


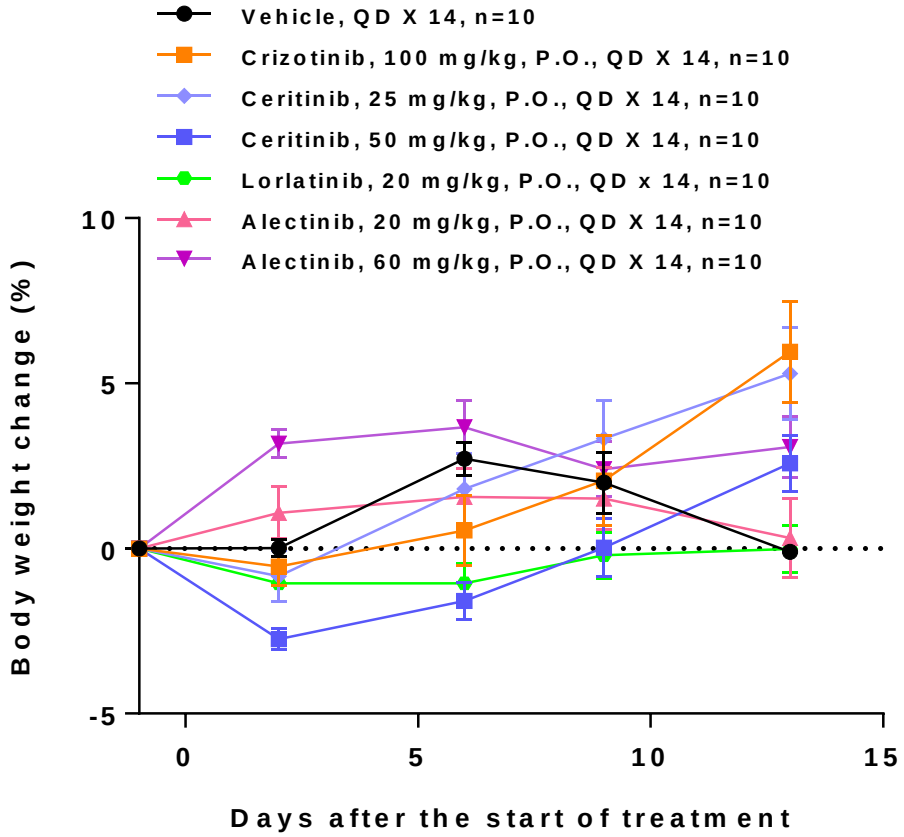
ALK inhibitors in Ba/F3-EML4-ALK-C1156Y model



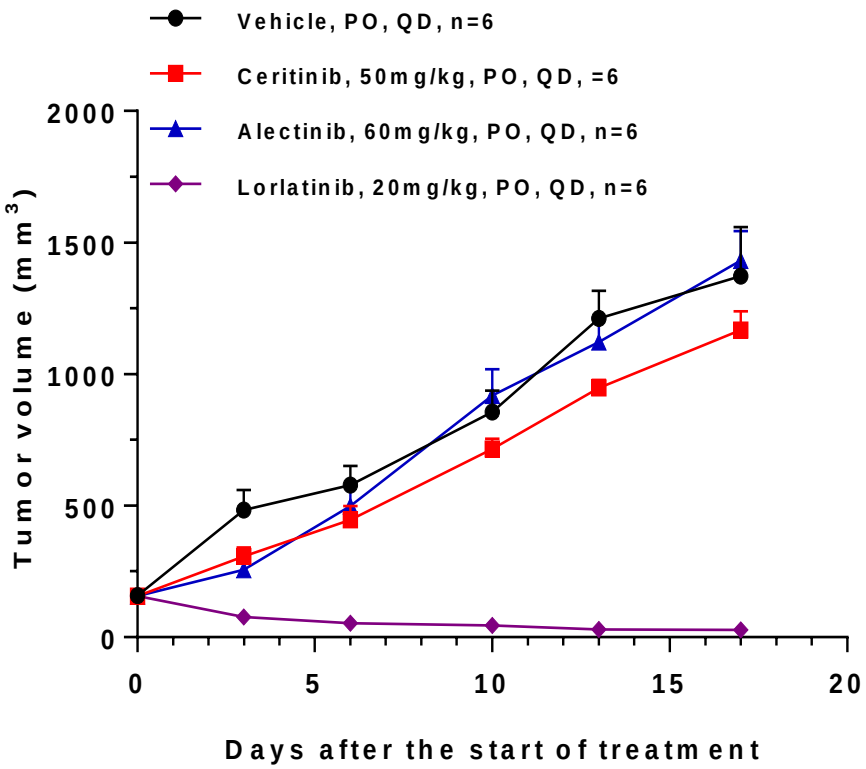


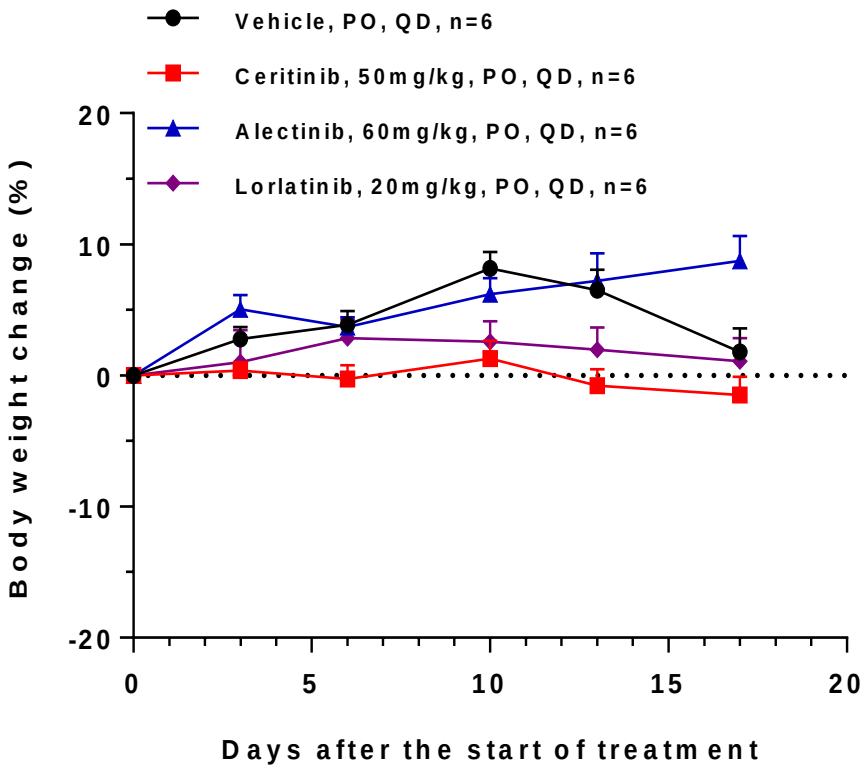
ALK inhibitors in Ba/F3-EML4-ALK-C1156Y model



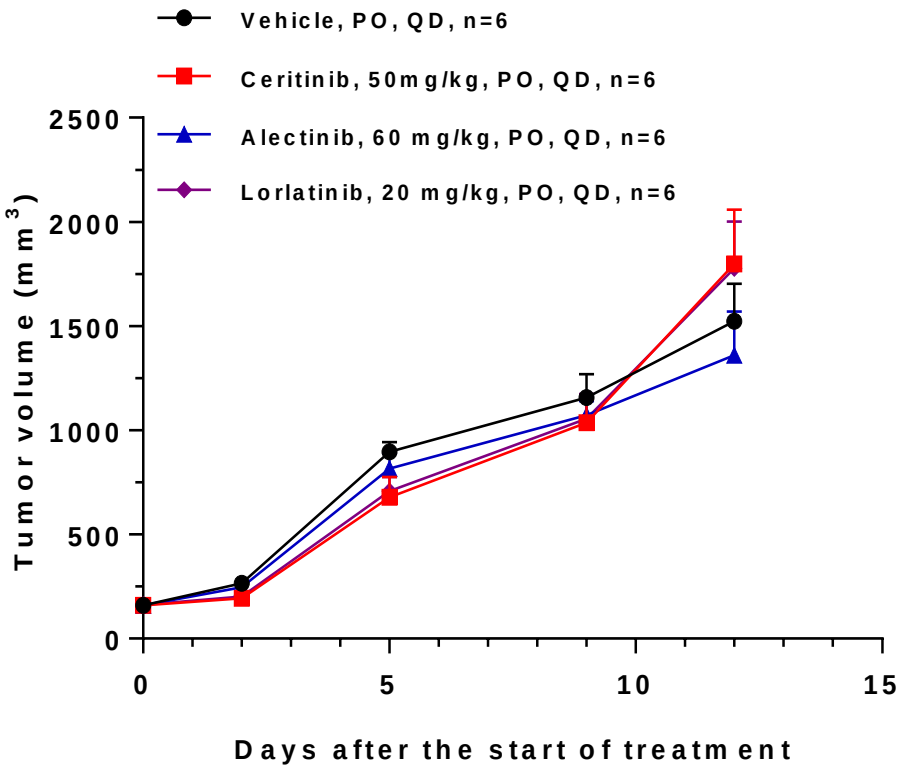


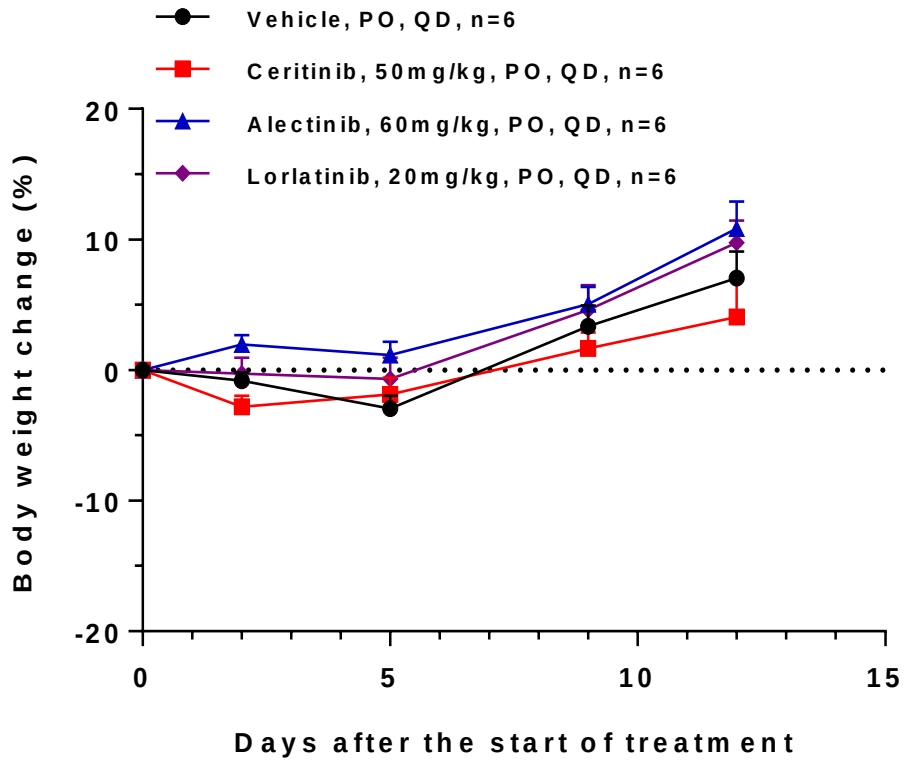
ALK inhibitors in Ba/F3-EML4-ALK G1202R model





ALK inhibitors in Ba/F3-EML4-ALK G1202R/L1196M model

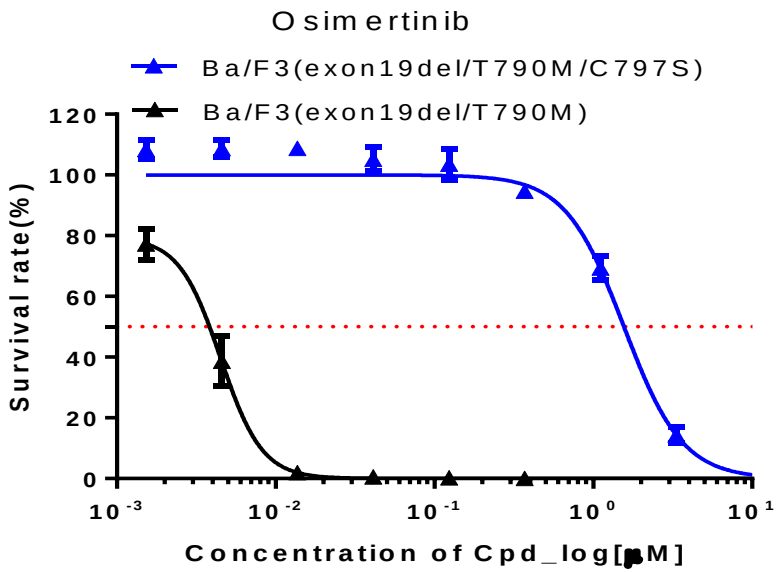


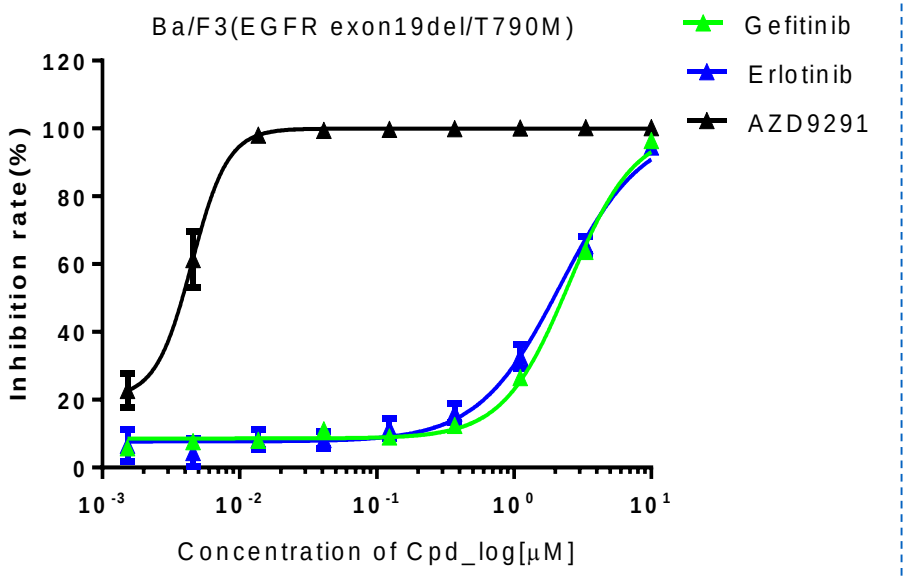


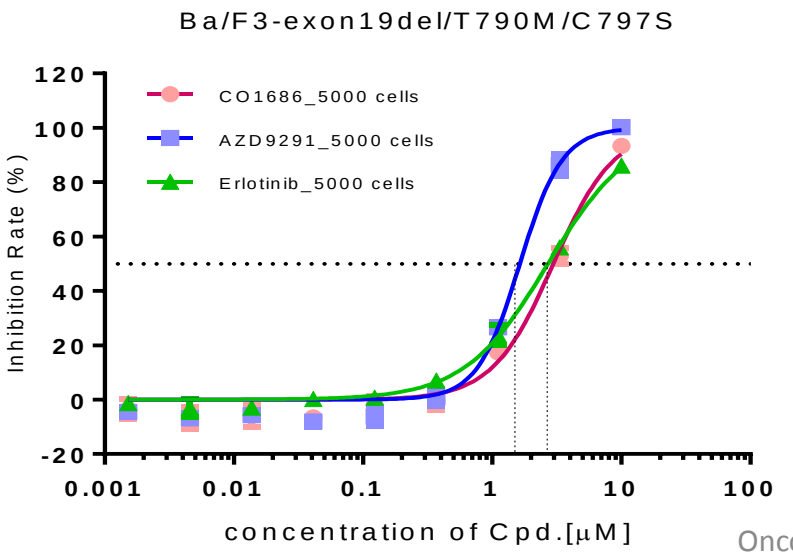
Engineered Ba/F3 cell lines carrying EGFR mutations

Cancer type	Cell line	Model growth curve
Pro-B cells	Ba/F3 EGFR exon19 del/T790M	Yes
	Ba/F3 EGFR exon19 del/T790M/C797S	Yes
	Ba/F3 EGFR L858R/T790M/C797S	Yes
	Ba/F3 EGFR exon19 del/C797S	No
	Ba/F3 EGFR L858R/T790M	No
	Ba/F3 EGFR L858R/C797S	No
	Ba/F3 EGFR L858R	No
	Ba/F3 EGFR exon19 deletion	No
	Ba/F3 exon20_V769_D770insASV	Yes
	Ba/F3 exon20_D770_N771insSVD	Yes
	Ba/F3 exon20_H773_V774insNPH	Yes

Engineered Ba/F3 cells carrying with EGFR mutations-> Cell Proliferation Assay





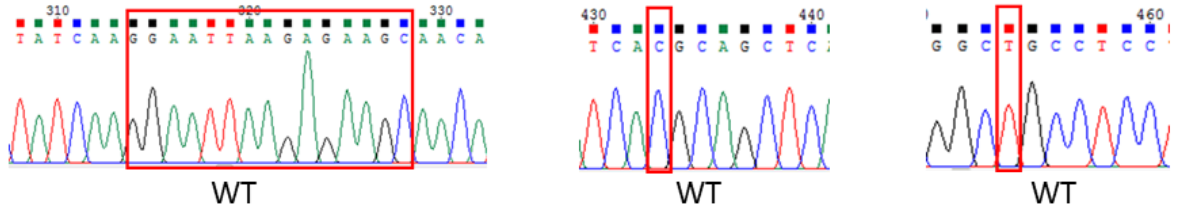


Cell line	Compound	IC50 (nM)
Ba/F3 (Exon19del/T790M)	Osimertinib	4
	Erlotinib	2187
	Gefitinib	2522
Ba/F3 Triple mutant	CO1686	2720
	AZD9291	1537
	Erlotinib	2612

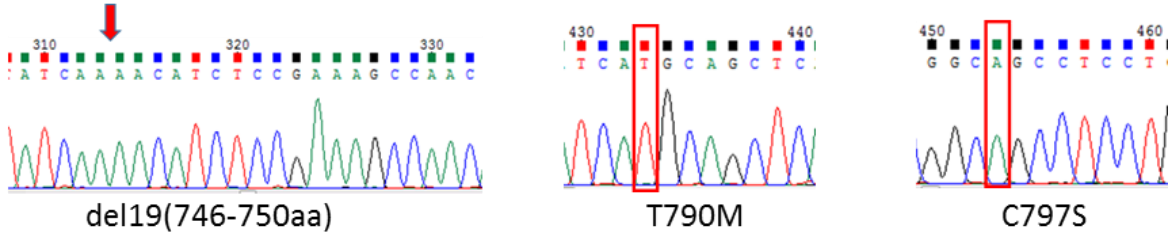
Ba/F3 EGFR-del19/T790M/C797S cell line

Validation with sequencing

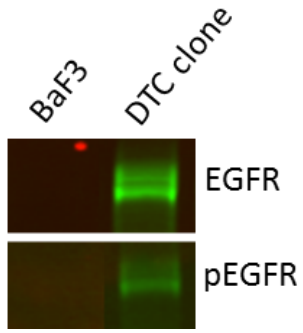
EGFR WT



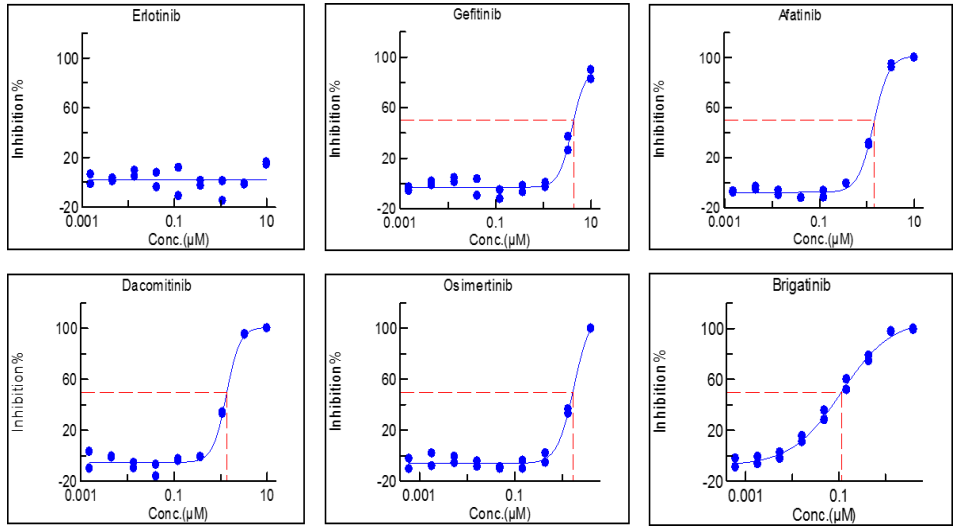
EGFR del19/T790M/C797S (DTC)



Validation with western blot



Validation with CellTiter Glo assays



Ref. Cpmd	DT	DTC
Erlotinib	4.02±0.038	>10
Gefitinib	1.98±0.87	4.17±0.30
Afatinib	0.036±0.0079	1.39±0.014
Dacomitinib	0.13±0.074	1.45±0.17
Osimertinib	0.0033±0.0021	1.75±0.015
Brigatinib	0.072±0.026	0.20±0.13

IC50 of EGFR TKIs in Ba/F3 (EGFR exon19 del/C797S) cells and Ba/F3 (L858R/T790M/C797S) cells

Clone	Reference compound IC50 (uM)					
	Erlotinib	Gefitinib	Afatinib	Dacomitinib	Osimertinib	Brigatinib
Ba/F3 EGFR exon19 del/C797S	0.0035±0.000095	0.0031±0.00085	0.0031±0.000092	<0.0030, 0.0019	1.147±0.116	0.169±0.090
Ba/F3 EGFR L858R/T790M/C797S	>10, 14.654	11.480±2.464	2.214±0.827	2.066±0.719	1.566±0.276	1.044±0.0079

IC50 of EGFR TKIs in Ba/F3 (L858R/T790M, L858R/C790S, L858R and exon19 deletion) cell lines

Cell line	#	Reference compound IC50 (μM)						
		Erlotinib	Gefitinib	Afatinib	Dacomitinib	Osimertinib	Brigatinib	Poziotinib
L858R/T790M	23A clones	>10,>10	>10,8.822	0.151±0.057	0.458±0.003	0.009±0.002	0.408±0.169	0.027±0.004
L858R/C797S	9x pool	0.013±0.004	0.011±0.004	0.011±0.002	0.008±0.003	>1	0.437±0.048	NA
L858R	9x pool	0.006±0.002	0.008±0.002	0.00014±0.00005	0.00012±0.00005	0.004±0.002	0.217±0.067	NA
exon19 deletion	9x pool	0.006±0.002	0.005±0.002	0.0002±0.0001	0.0001±0.0001	0.003±0.002	0.097±0.052	NA

IC50 of EGFR TKIs in Ba/F3 EGFR exon20 insertion cell lines

Cell Line	Erlotinib (μM)	Gefitinib (μM)	Afatinib (μM)	Dacomitinib (μM)	Osimertinib (μM)	Brigatinib (μM)	Poziotinib (μM)
V769_D770 insASV	2.516±0.22	3.306±0.996	0.075±0.016	0.097±0.021	0.14±0.056	2.121±1.407	0.0027±0.001
D770_N771 insSVD	1.622±0.143	2.514±0.513	0.054±0.008	0.07±0.013	0.083±0.034	1.338±0.189	0.0019±0.0006
H773_V774 insNPH	1.591±0.014	2.592±0.338	0.082±0.006	0.093±0.001	0.153±0.002	1.754±0.185	0.0056±0.0003
Ba/F3	>10	5.006±1.937	1.336±0.223	1.242±0.081	1.816±0.151	1.557±0.022	>10

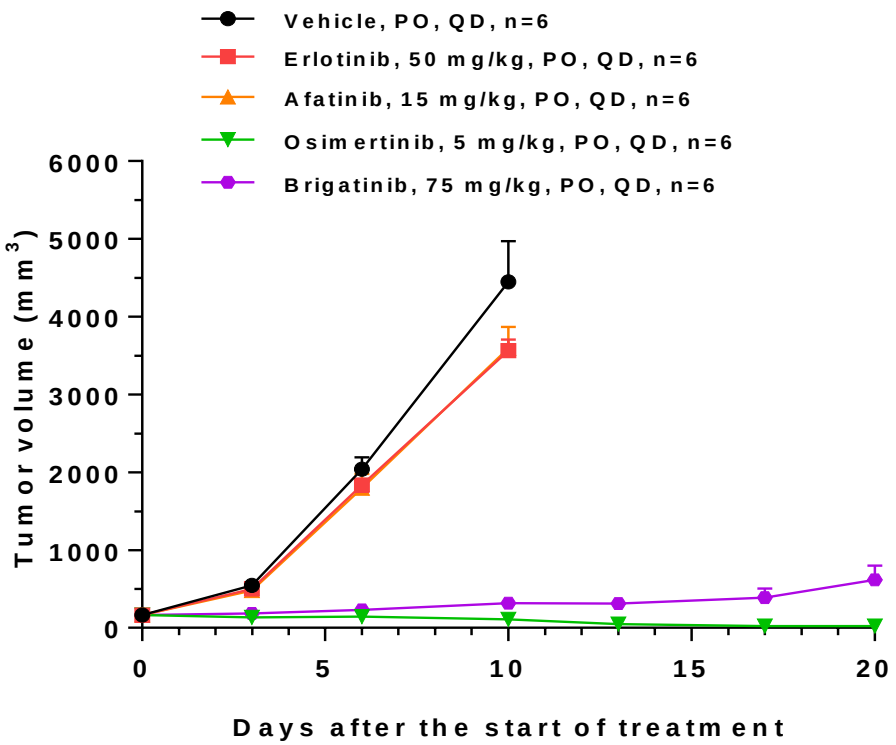
In vivo models derived from Ba/F3 cell lines carrying EGFR mutations

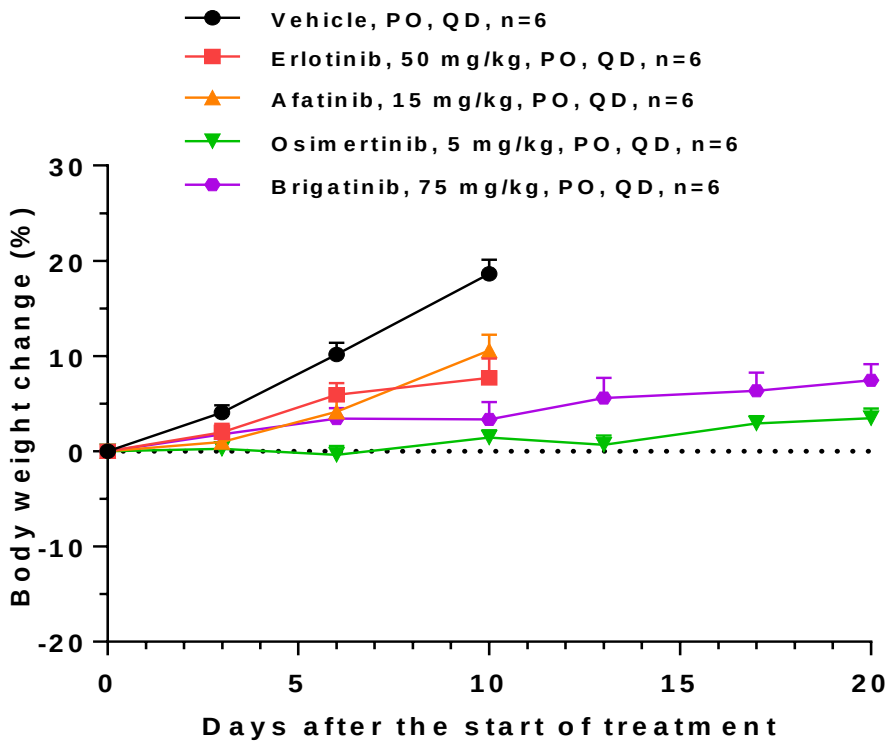
Cancer type	Model ID	Tumor growth curve	Drug tested and Dosage (mg/kg)	TGI (%)	Model genomics
Pro-B cells	Ba/F3-exon 19 del/T790M	Yes	Erlotinib (50) Afatinib (15) Osimertinib (5) Brigatinib (75)	20.68 19.92 101.29 96.49	EGFR-exon 19 del/T790M
	Ba/F3-exon 19 del/T790M/C797S	Yes	Erlotinib (50) Afatinib (15) Osimertinib (5) Brigatinib (75)	8.02 20.4 -7.06 91.19	EGFR-exon 19 del/T790M/C797S
	Ba/F3 (L858R/T790M/C797S)	Yes	Erlotinib (50) Afatinib (15) Osimertinib (5) Brigatinib (75)	0.07 -4.02 4.49 41.79	EGFR L858R/T790M/C797S

In vivo models derived from Ba/F3 cell lines carrying EGFR mutations

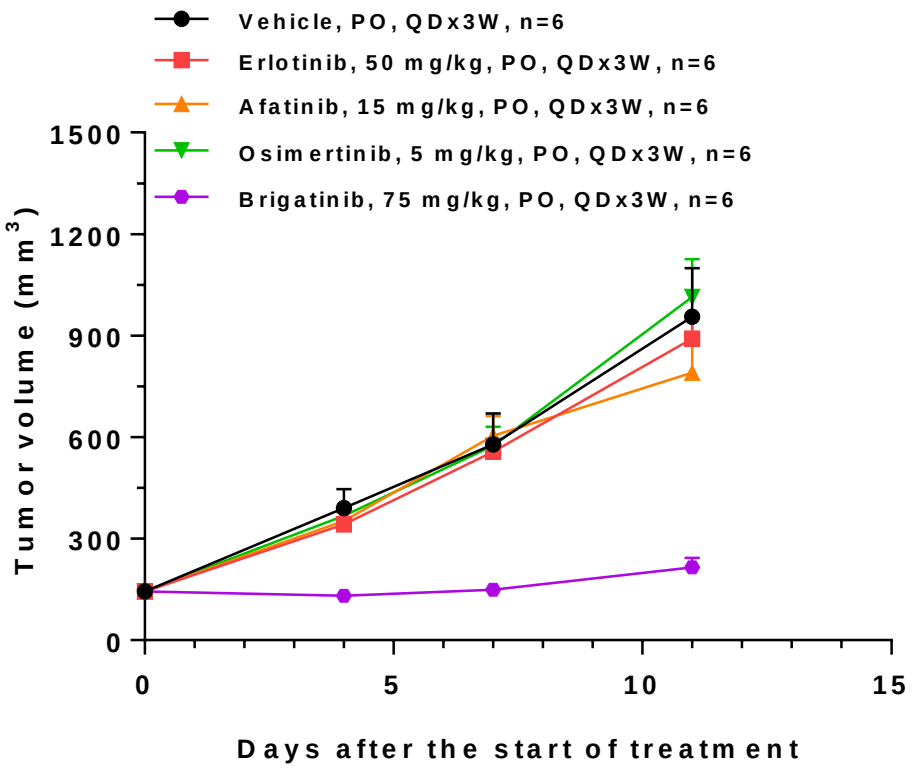
Cancer type	Model ID	Tumor growth curve	Drug tested and Dosage (mg/kg)	TGI (%)	Model genomics
Pro-B cells	Ba/F3 (EGFR exon20ins ASV)	Yes	Erlotinib (50) Afatinib (15) Osimertinib (5/25) Poziotinib (5/1/0.5)	12.72 11.03 10.60 47.03	EGFR exon20ins ASV
	Ba/F3 (EGFR exon20ins SVD)	Yes	Erlotinib (50) Afatinib (15) Osimertinib (5/25) Poziotinib (5/1/0.5)	4.27 -4.00 11.97 61.35	EGFR exon20ins SVD
	Ba/F3 (EGFR exon20ins NPH)	Yes	Erlotinib (50) Afatinib (15) Osimertinib (5) Poziotinib (0.5)	12.67 4.63 12.04 39.83	EGFR exon20ins NPH

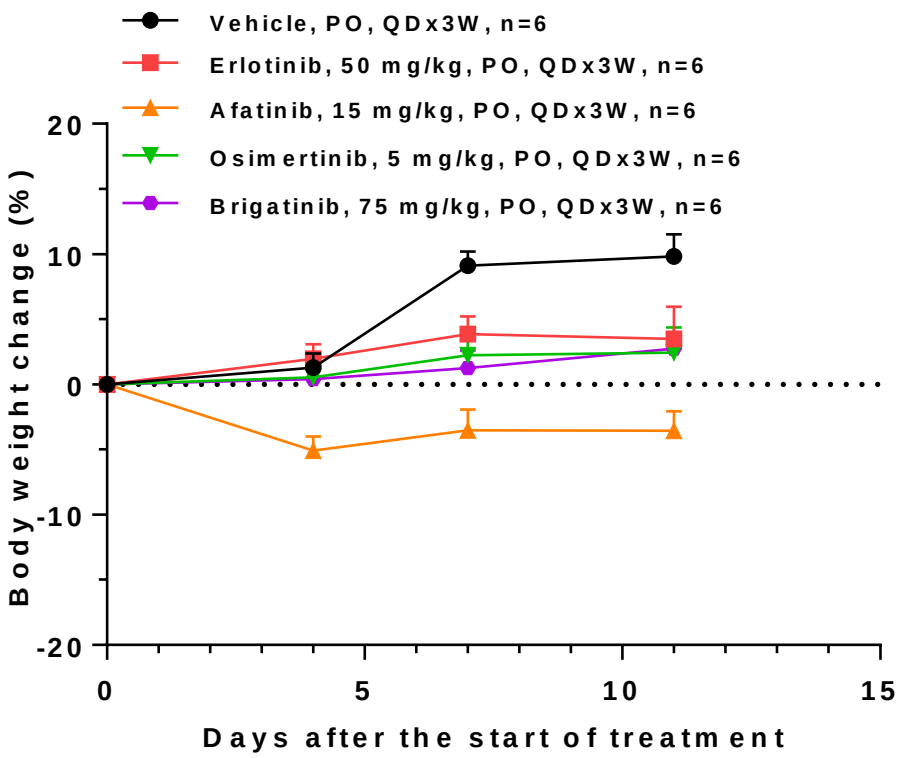
Erlotinib, Afatinib, Osimertinib & Brigatinib in Ba/F3 (exon19 del/T790M) model



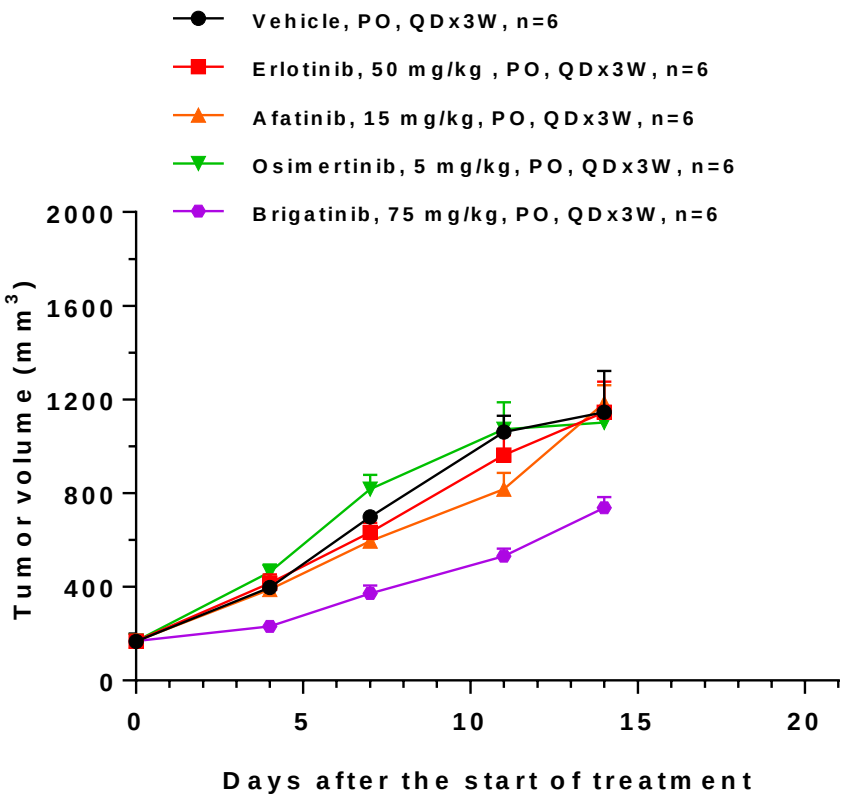


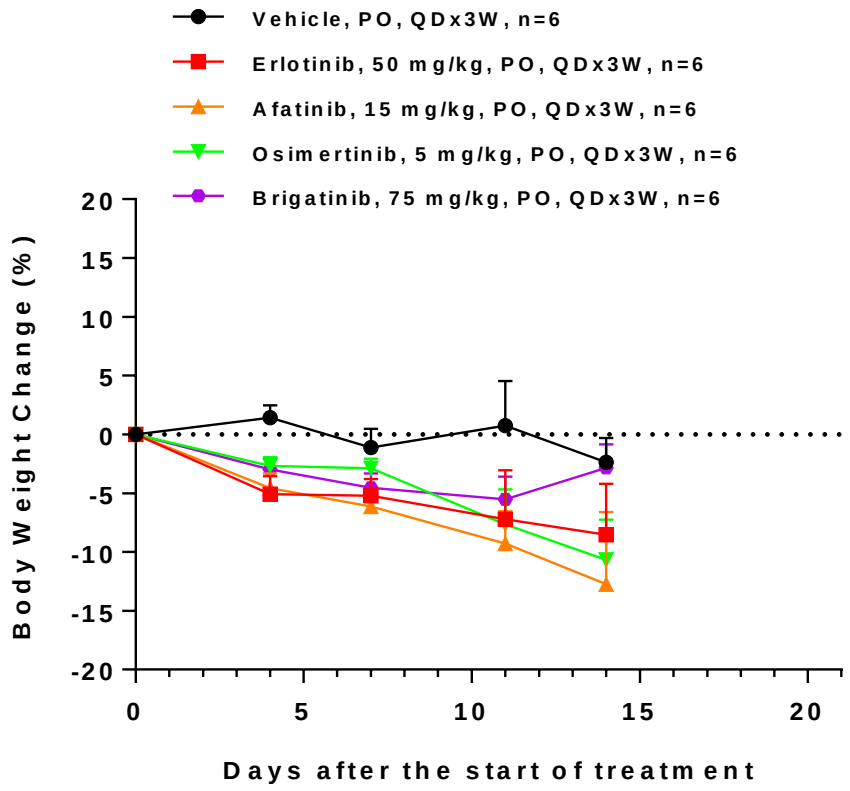
Erlotinib, Afatinib, Osimertinib & Brigatinib in Ba/F3 (exon19 del/T790M/C797S) model



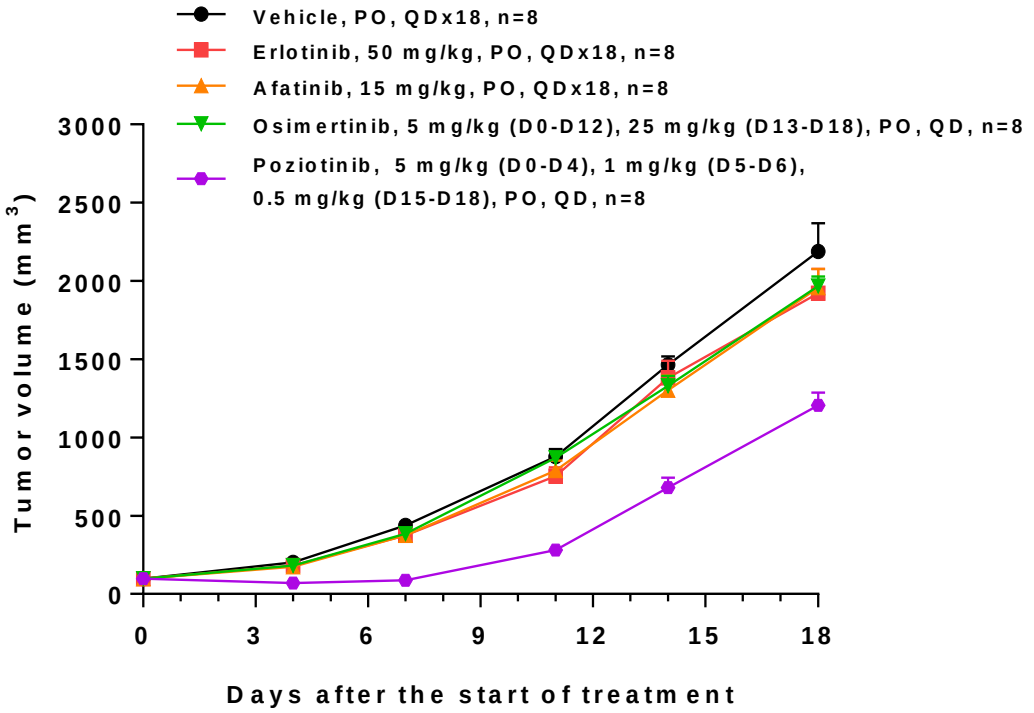


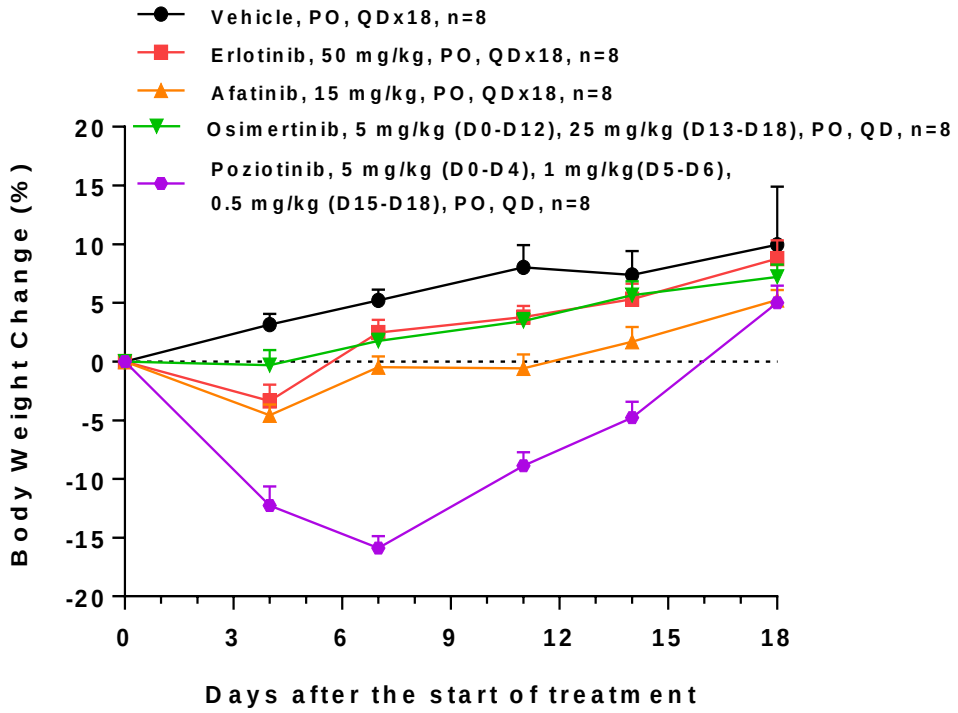
Erlotinib, Afatinib, Osimertinib & Brigatinib in Ba/F3 (L858R/T790M/C797S) model



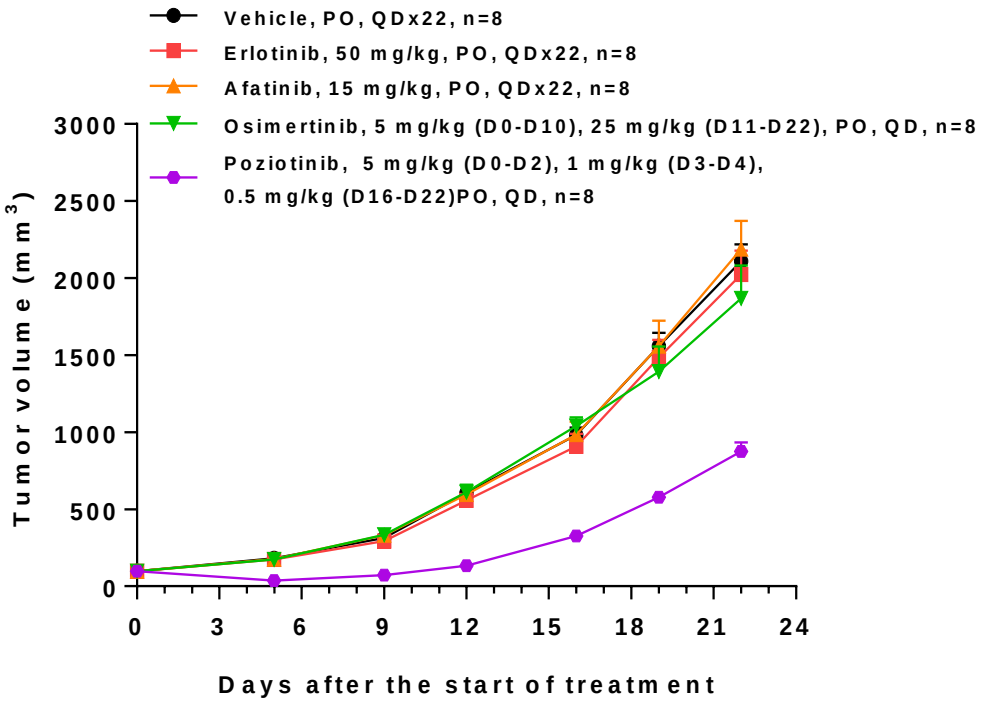


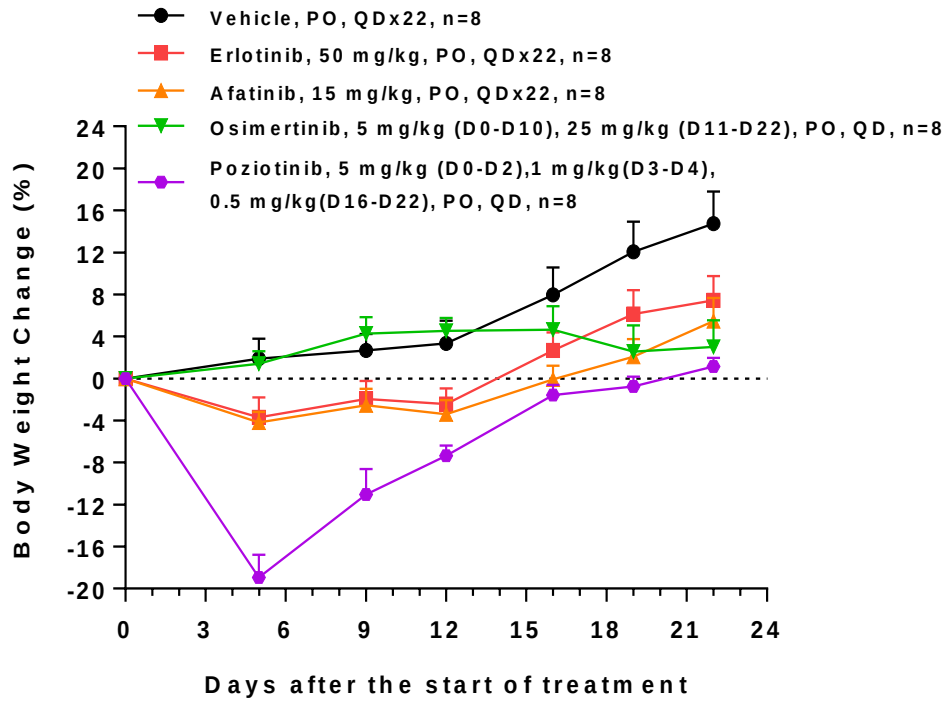
Anti-tumor efficacy of Erlotinib, Afatinib, Osimertinib and Poziotinib in the Ba/F3 (EGFR exon20ins ASV) model



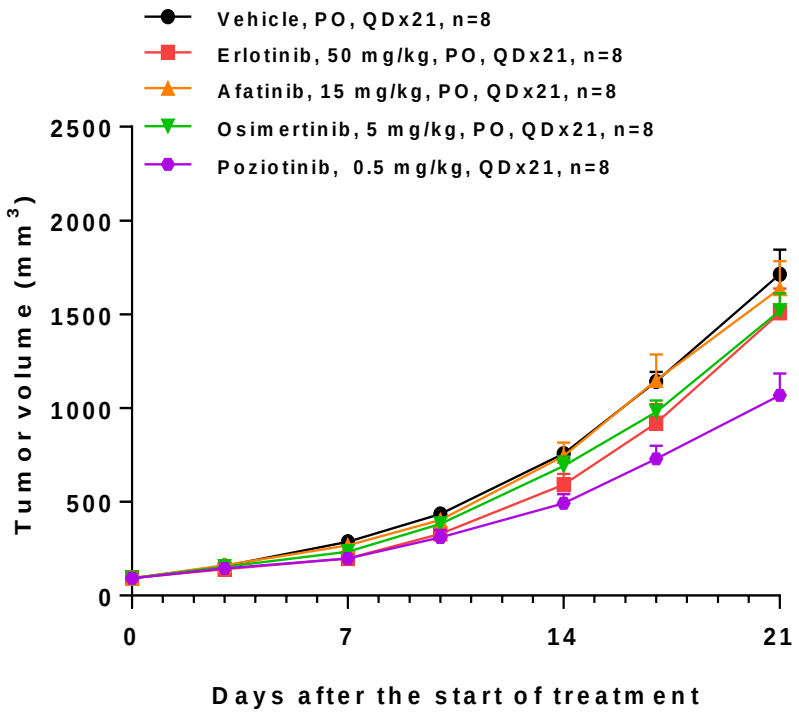


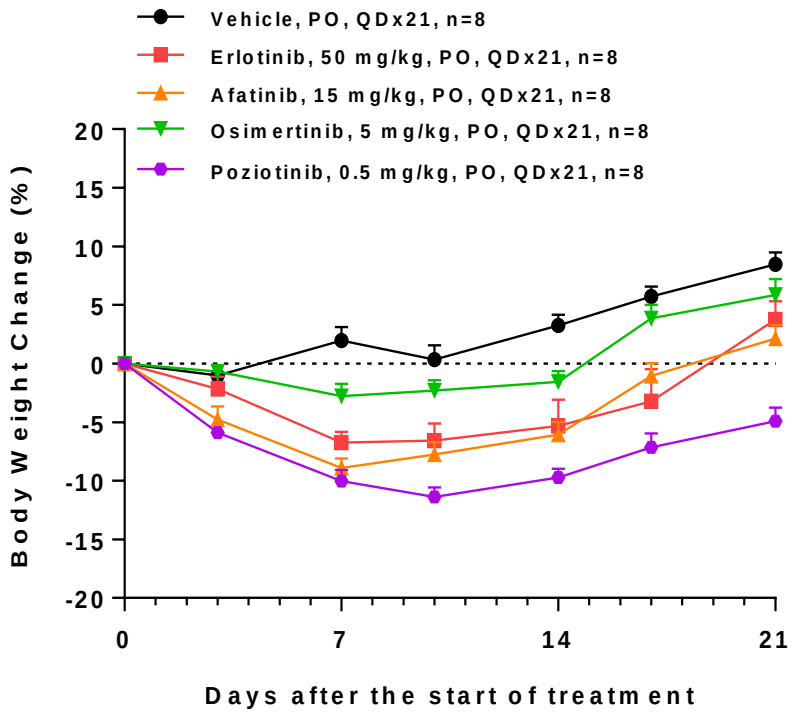
Anti-tumor efficacy of Erlotinib, Afatinib, Osimertinib and Poziotinib in the Ba/F3 (EGFR exon20ins SVD) model





Anti-tumor efficacy of Erlotinib, Afatinib, Osimertinib and Poziotinib in the Ba/F3 (EGFR exon20ins NPH) model





TRK fusion engineered cell lines *in vitro* validation

Clone	Sequencing confirmed	LOXO-101					
		Ab IC50 (uM)	Re IC50 (uM)	Top	Bottom	Slope	Max Inh%
ETV6–NTRK3							
ET-G623R-2A	yes	>1	>1	32.09	-3.76	1.18	27.87
ET-G696A-12A	yes	0.082	0.085	100.81	2.12	1.57	97.84
ET-WT-16A	yes	0.011	0.0082	79.14	4.84	1.47	82.87
TPM3-NTRK1							
TT-G595R-13	yes	>1	>1	3.87	-27.30	0.43	10.96
TT-G667C-5A	yes	1.942	2.216	117.35	-8.96	1.01	94.21
TT-WT-9	yes	0.013	0.013	98.54	-0.05	1.18	96.65
LMNA-NTRK1							
LT-G595R-1	yes	>1	>1	10.15	-6.24	-22.68	10.15
LT-G667C-12	yes	>1	>1	45.14	-5.84	1.06	26.77
LT-WT-10	yes	0.026	0.025	99.73	-3.44	1.22	97.40
Parental							
BaF3-parental	NA	>10	>10	21.70	-1.17	1.77	20.35

TRK fusion engineered cell lines *in vitro* validation

Clone	Sequencing confirmed	Entrectinib (RXDX-101)					
		Ab IC50 (uM)	Re IC50 (uM)	Top	Bottom	Slope	Max Inh%
ETV6–NTRK3							
ET-G623R-2A	yes	0.323	0.331	102.38	-0.28	1.72	89.80
ET-G696A-12A	yes	0.0030	0.0031	98.61	4.90	2.19	98.67
ET-WT-16A	yes	0.00072	0.00058	86.93	2.80	1.18	90.94
TPM3-NTRK1							
TT-G595R-13	yes	0.591	0.590	100.00	-0.20	3.00	84.01
TT-G667C-5A	yes	0.054	0.055	99.79	2.63	1.59	99.67
TT-WT-9	yes	0.00066	0.00061	97.52	-3.58	1.62	97.82
LMNA-NTRK1							
LT-G595R-1	yes	0.439	0.419	94.68	-3.13	3.74	92.01
LT-G667C-12	yes	0.058	0.058	94.58	5.59	1.79	94.94
LT-WT-10	yes	0.0013	0.0015	98.13	9.39	2.08	97.91
Parental							
BaF3-parental	NA	1.213	1.242	103.02	-0.52	2.05	99.95

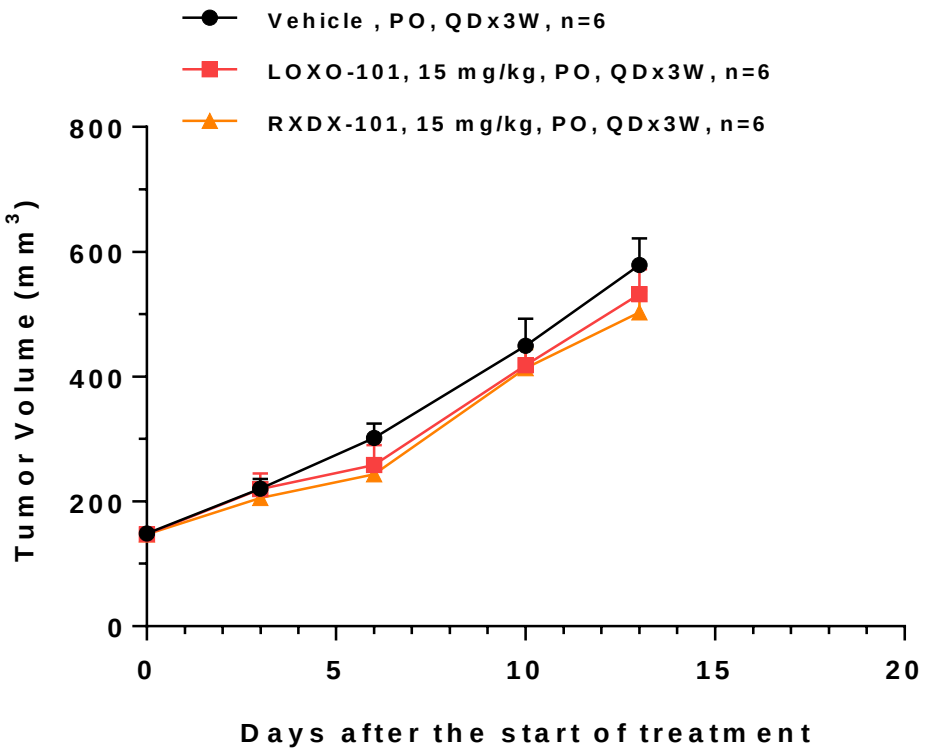
In vivo models derived from Ba/F3 cell lines carrying TRK fusion

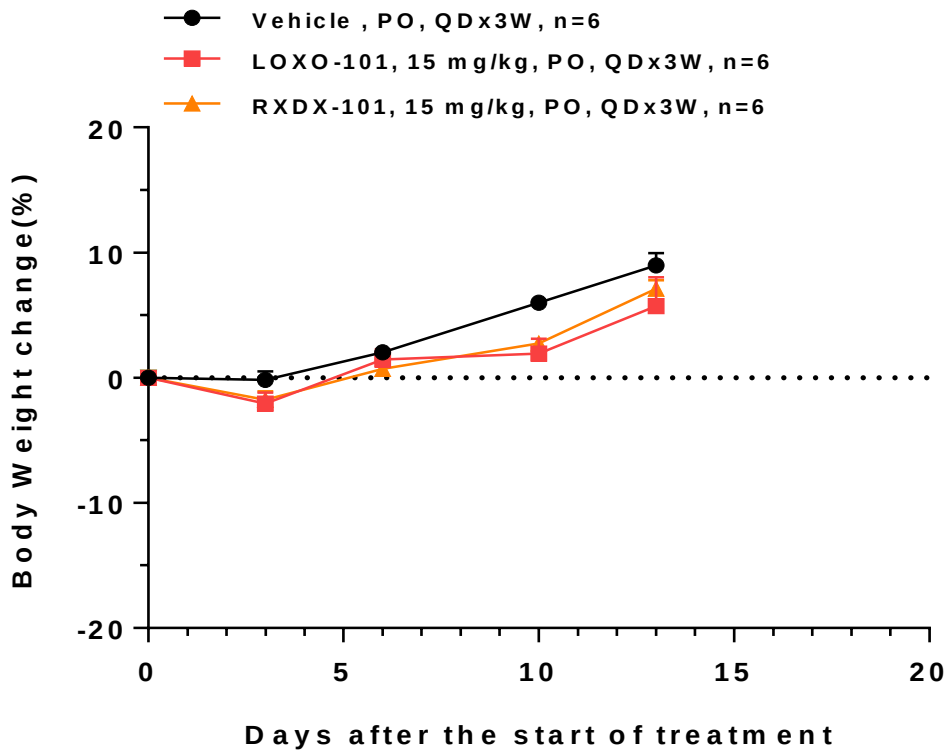
Cancer type	Model ID	Tumor growth curve	Drug tested and Dosage (mg/kg)	TGI (%)	Model genomics
ETV6–NTRK3					
Pro-B cells	ET-G623R-2A	Yes	LOXO-101 (15)	11	ETV6-NTRK3-G623R
			RXDX-101 (15)	18	
	ET-G696A-12A	Yes	LOXO-101 (15)	10	ETV6-NTRK3-G696A
			RXDX-101 (15)	51	
	ET-WT-16A	Yes	LOXO-101 (15)	35	ETV6-NTRK3-WT
			RXDX-101 (15)	109	
TPM3-NTRK1					
Pro-B cells	TT-G595R-13	Yes	LOXO-101 (15)	-2	TPM3-NTRK1-G595R
			RXDX-101 (15)	9	
	TT-G667C-5A	Yes	LOXO-101 (15)	-66	TPM3-NTRK1-G667C
			RXDX-101 (15)	-7	
	TT-WT-9	Yes	LOXO-101 (15)	29	TPM3-NTRK1-WT
			RXDX-101 (15)	115	

In vivo models derived from Ba/F3 cell lines carrying TRK fusion

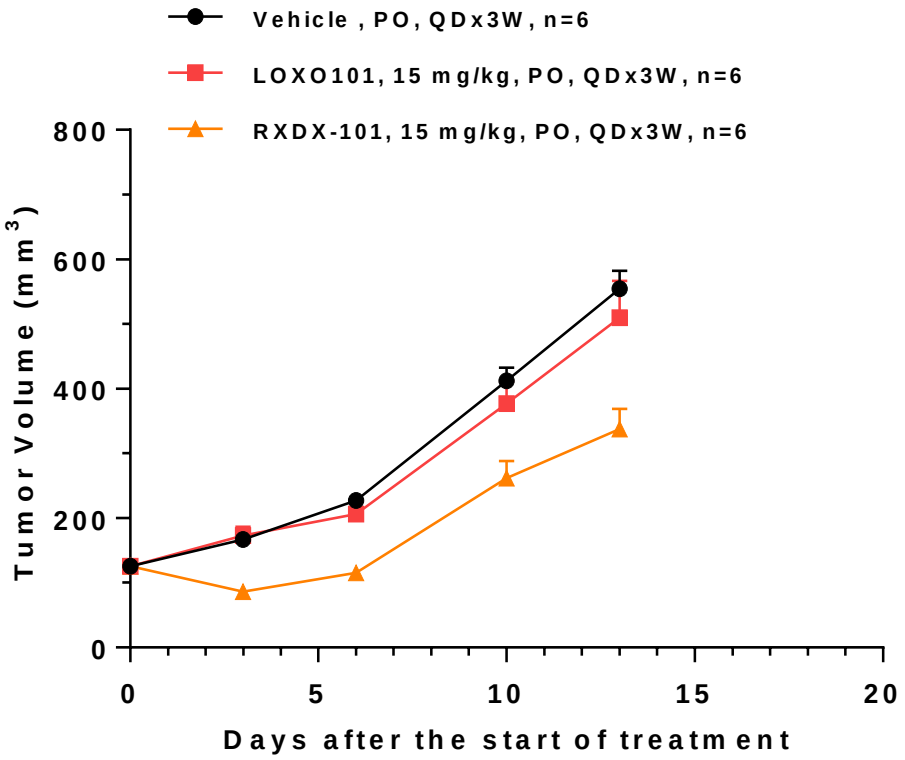
Cancer type	Model ID	Tumor growth curve	Drug tested and Dosage (mg/kg)	TGI (%)	Model genomics
LMNA-NTRK1					
Pro-B cells	LT-G595R-1	Yes	LOXO-101 (15)	-14	LMNA-NTRK1-G595R
			RXDX-101 (15)	2	
	LT-G667C-12	Yes	LOXO-101 (15)	11	LMNA-NTRK1-G667C
			RXDX-101 (15)	-6	
	LT-WT-10	Yes	LOXO-101 (15)	17	LMNA-NTRK1-WT
			RXDX-101 (15)	119	

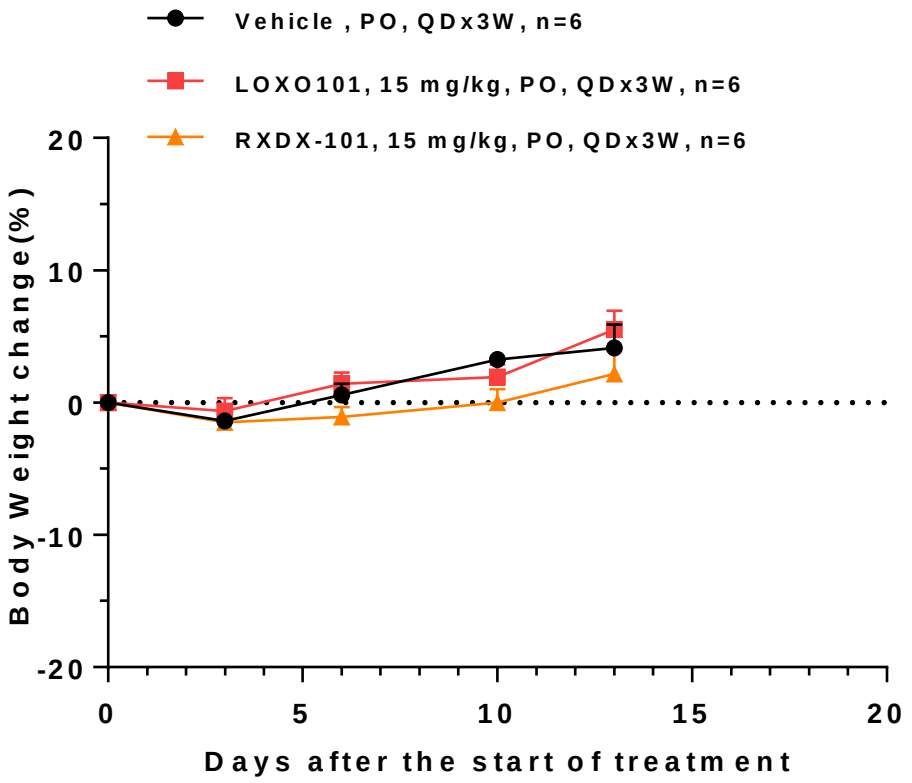
LOXO-101 and RXDX-101 in Ba/F3 (ET-G623R) engineered cell lines



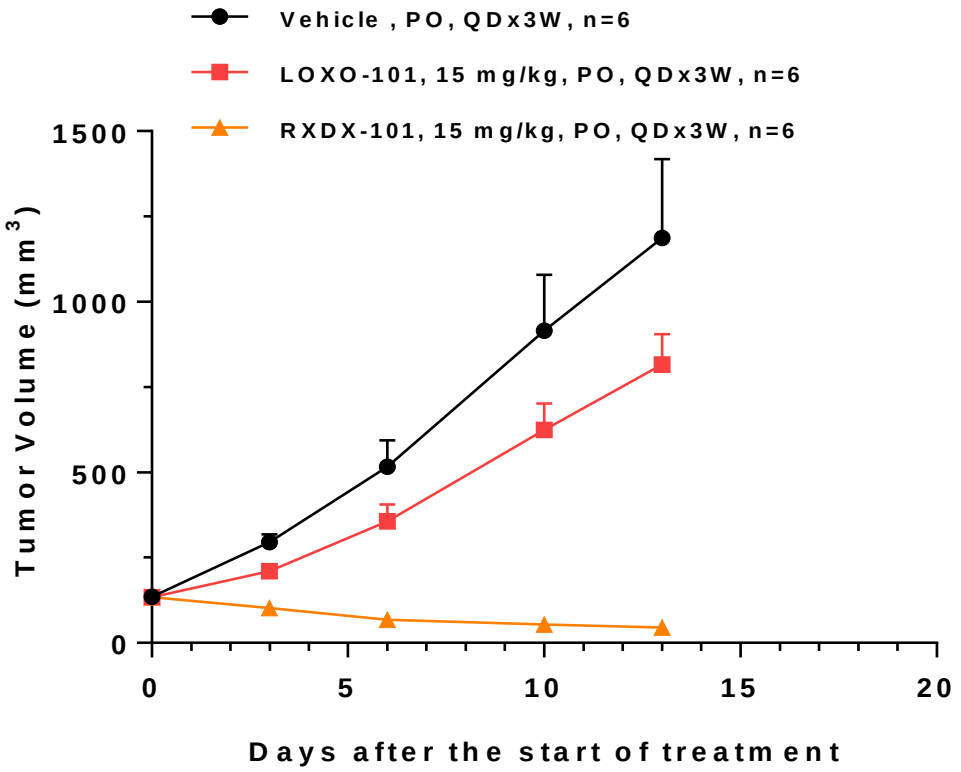


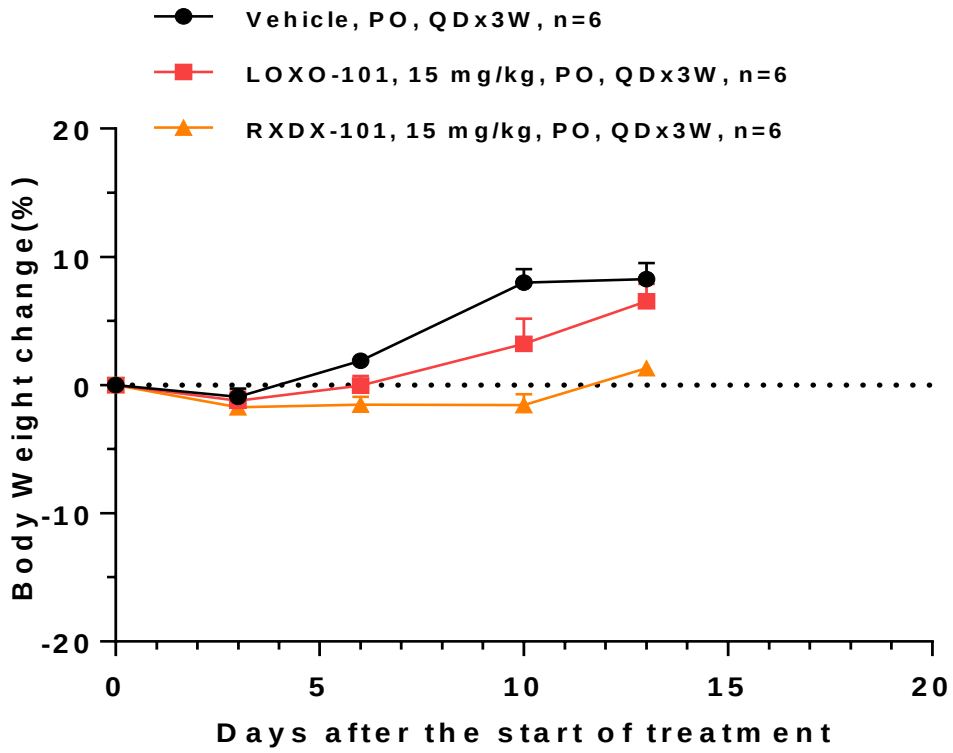
LOXO-101 and RXDX-101 in Ba/F3 (ET-G696A) engineered cell lines



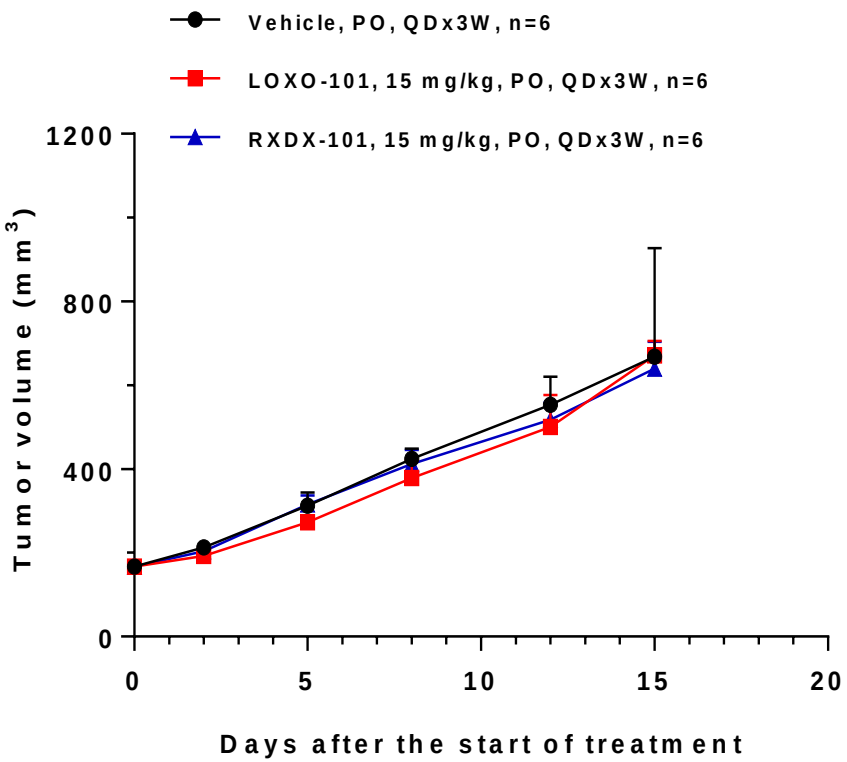


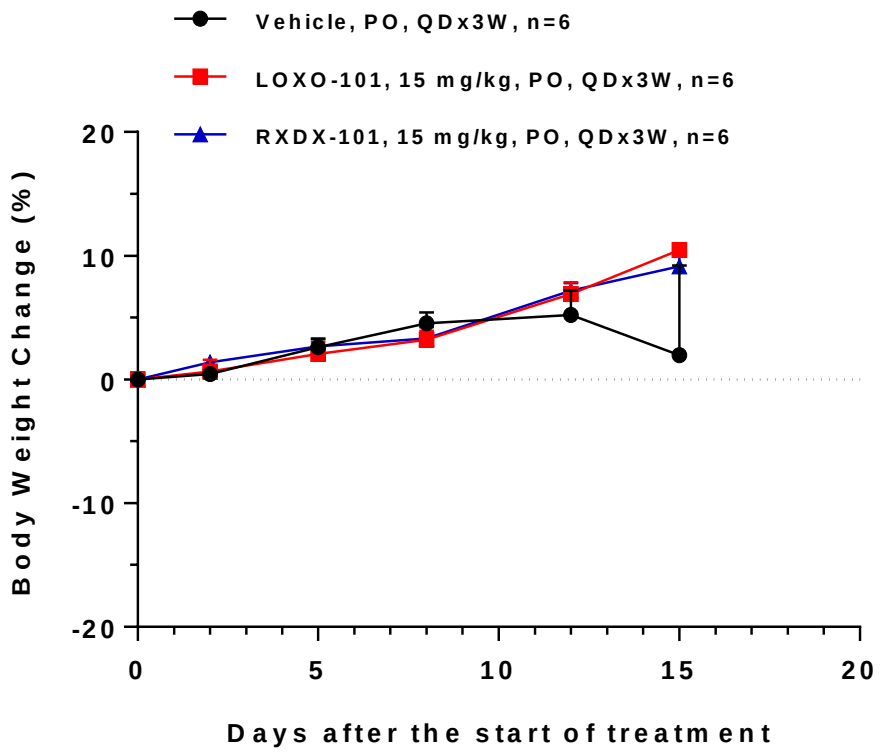
LOXO-101 and RXDX-101 in Ba/F3 (ET-WT) engineered cell lines



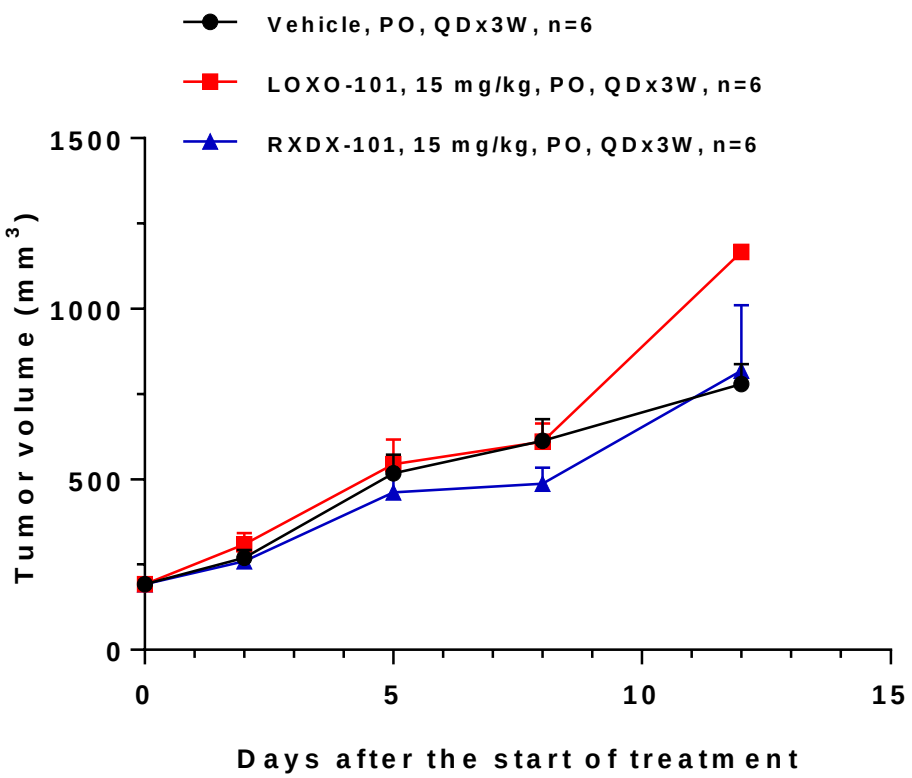


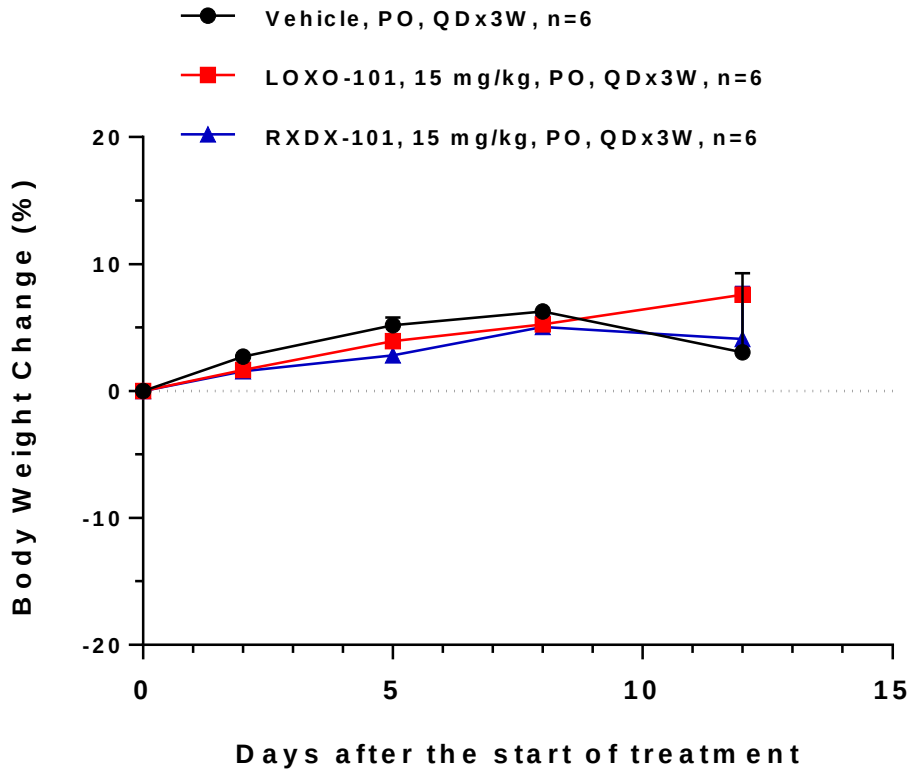
LOXO-101 and RXDX-101 in Ba/F3 (TT-G595R) engineered cell lines



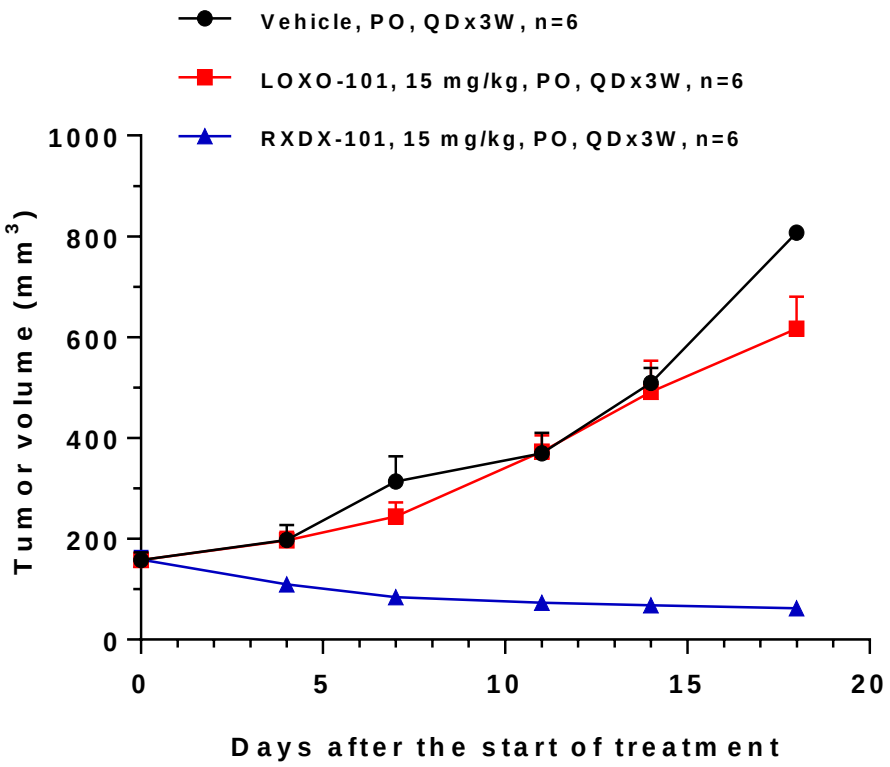


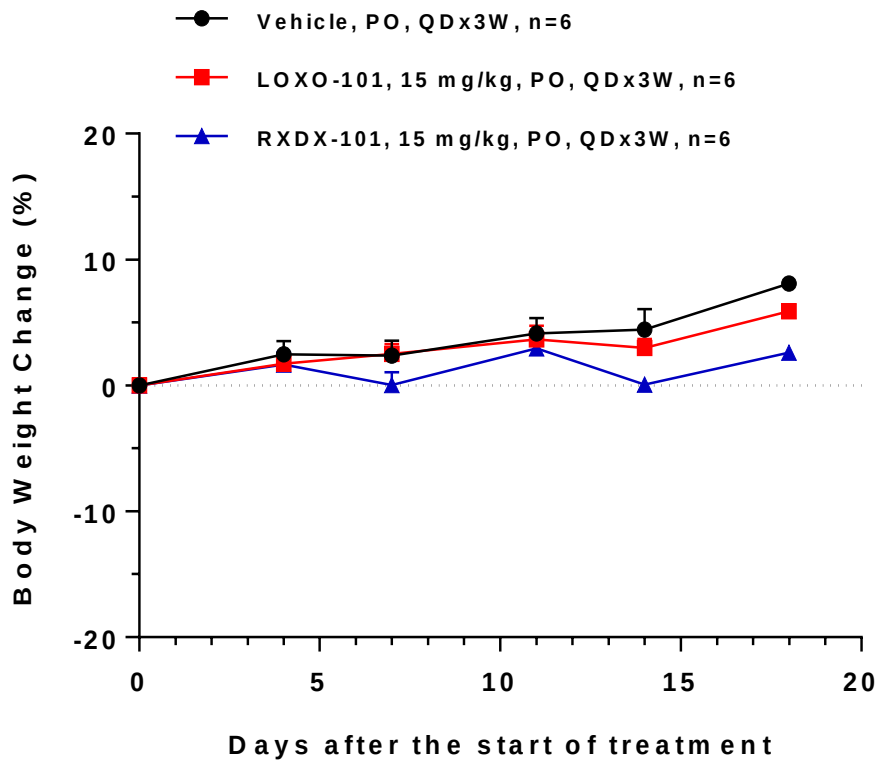
LOXO-101 and RXDX-101 in Ba/F3 (TT-G667C) engineered cell lines





LOXO-101 and RXDX-101 in Ba/F3 (TT-WT) engineered cell lines





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