

WuXi AppTec Genetically Engineered Cell Related Service



WuXi AppTec Research Service Division, Oncology & Immunology Unit



2021.01

OncoWuXi Newsletter

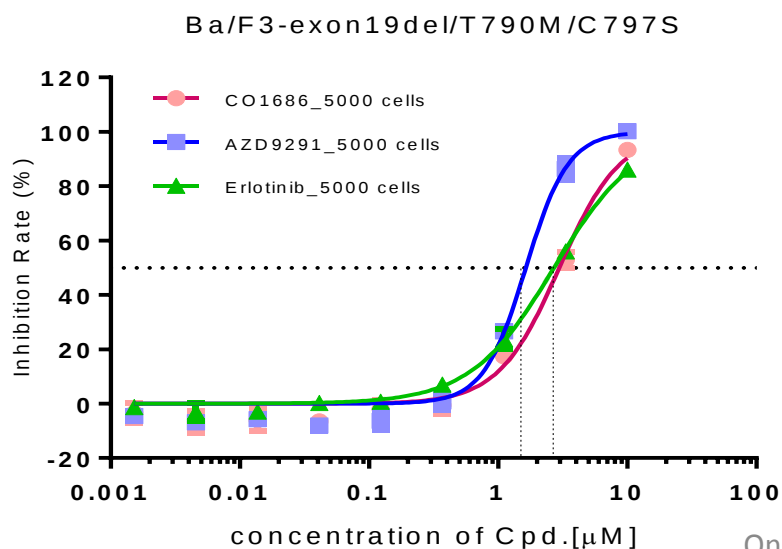
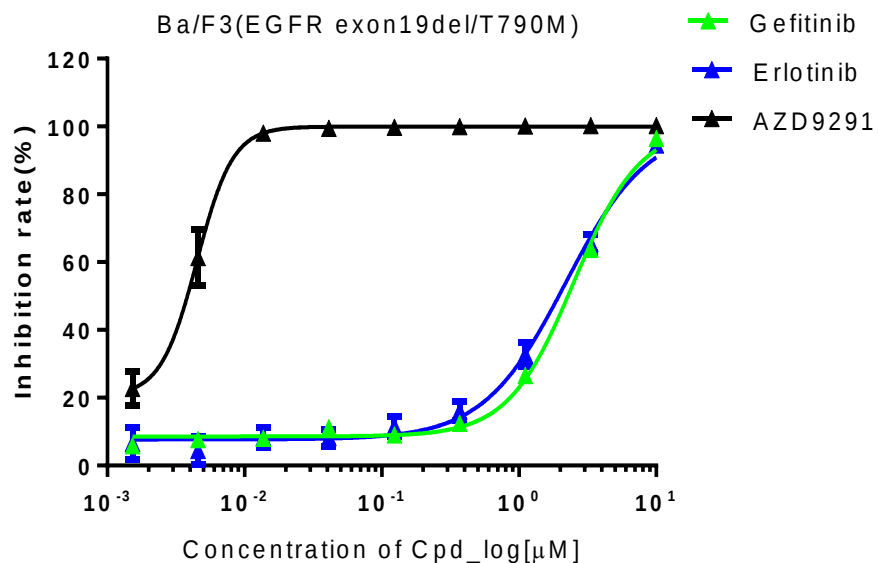
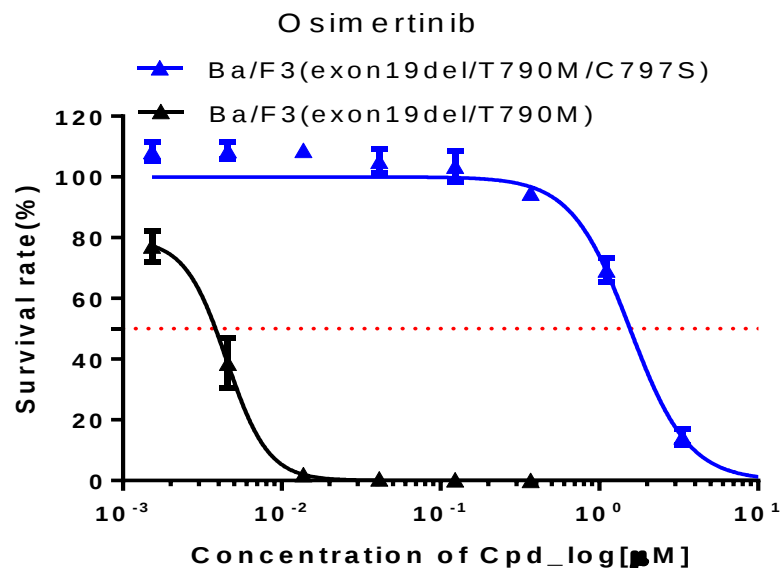
- [Genetically engineered Ba/F3 cell lines carrying EGFR mutations and case study](#)
- [Genetically engineered Ba/F3 cell lines carrying RET mutations and case study](#)
- [Genetically engineered Ba/F3 cell lines carrying FLT3 mutations and case study](#)
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Genetically engineered Ba/F3 cell lines carrying EGFR mutations

Cell type	Cell line	Model growth curve	Drug validation
Pro-B cells	Ba/F3 EGFR exon19 del/T790M	Yes	Yes
	Ba/F3 EGFR exon19 del/T790M/C797S	Yes	Yes
	Ba/F3 EGFR L858R/T790M/C797S	Yes	Yes
	Ba/F3 EGFR L858R/T790M	Yes	Ongoing
	Ba/F3 EGFR L858R/C797S	Yes	Ongoing
	Ba/F3 EGFR L858R	Yes	Ongoing
	Ba/F3 EGFR exon19 deletion	Yes	Ongoing
	Ba/F3 EGFR exon20_V769_D770insASV	Yes	Yes
	Ba/F3 EGFR exon20_D770_N771insSVD	Yes	Yes
	Ba/F3 EGFR exon20_H773_V774insNPH	Yes	Yes

Engineered Ba/F3 cells carrying EGFR mutations

Cell Proliferation Assay

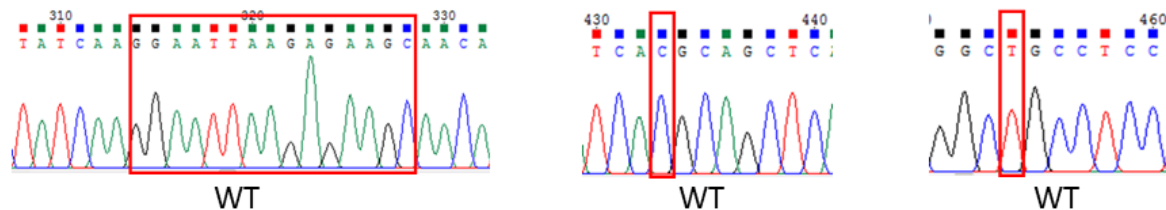


Cell line	Compound	IC50 (nM)
Ba/F3 (Exon19del/T790M)	Osimertinib	4
	Erlotinib	2187
	Gefitinib	2522
Ba/F3 Triple mutant	CO1686	2720
	AZD9291	1537
	Erlotinib	2612

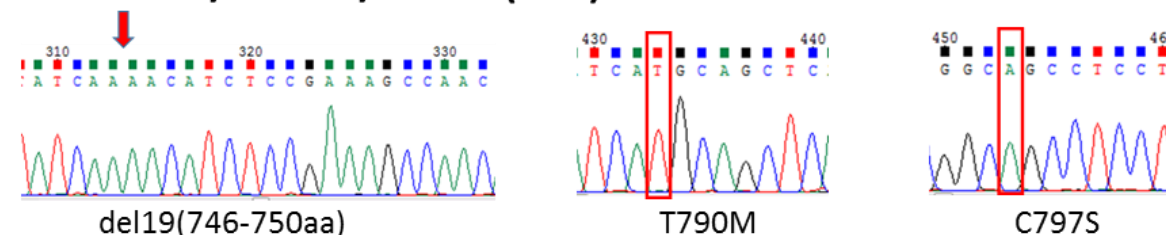
Ba/F3 EGFR-del19/T790M/C797S cell line

Validation with sequencing

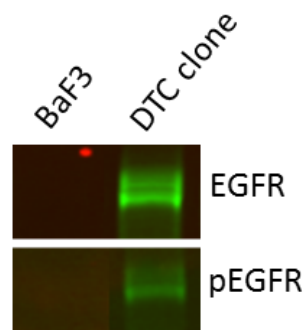
EGFR WT



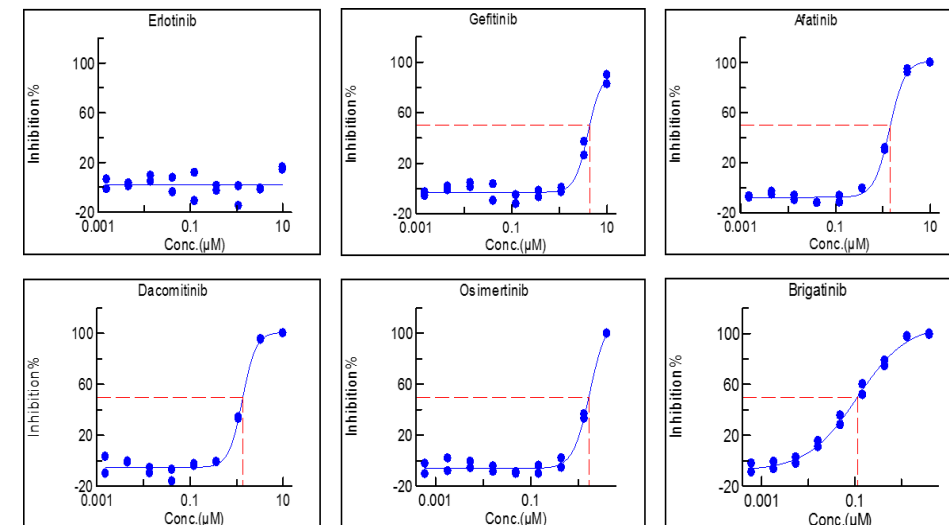
EGFR del19/T790M/C797S (DTC)



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) cell lines and Ba/F3 (L858R/T790M/C797S) cell lines

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	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	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

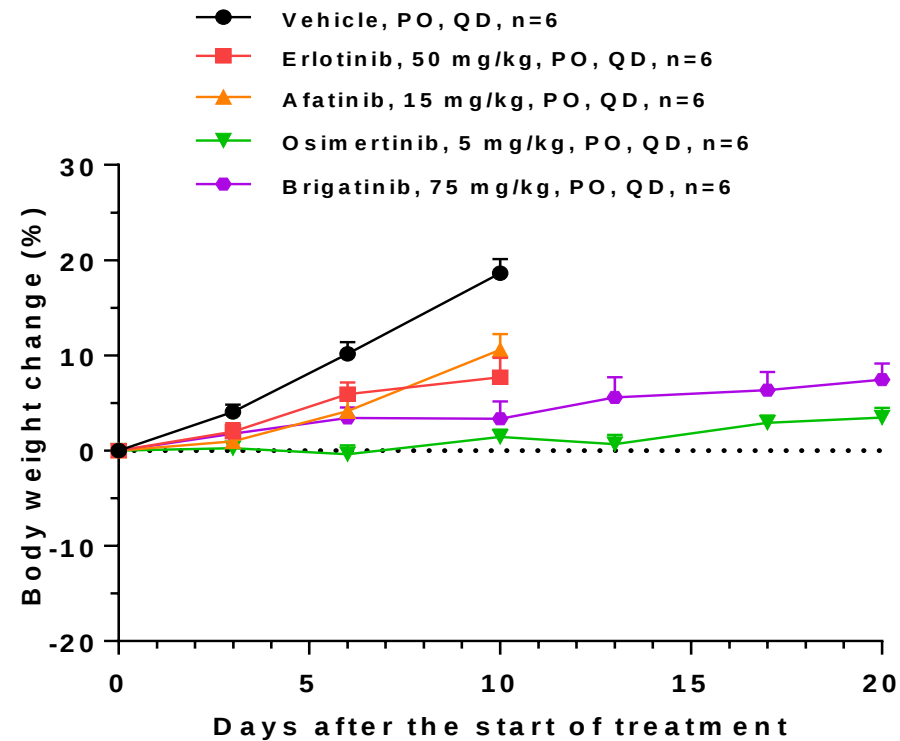
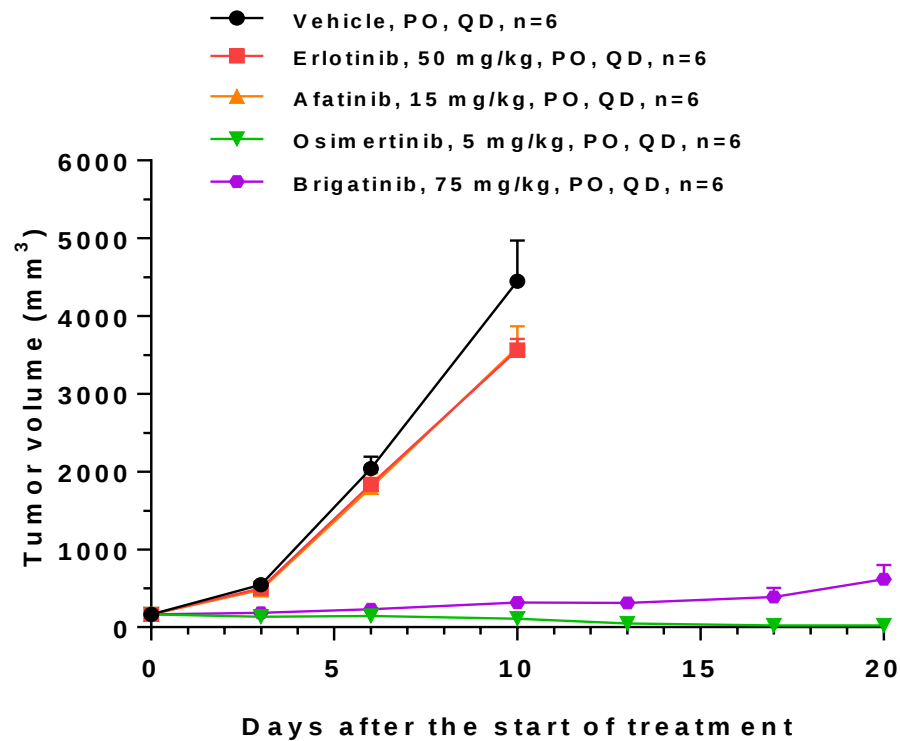
IC50 of EGFR TKIs in Ba/F3 EGFR exon20 insertion cell lines

Cell line	Reference compound IC50 (μM)						
	Erlotinib	Gefitinib	Afatinib	Dacomitinib	Osimertinib	Brigatinib	Poziotinib
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

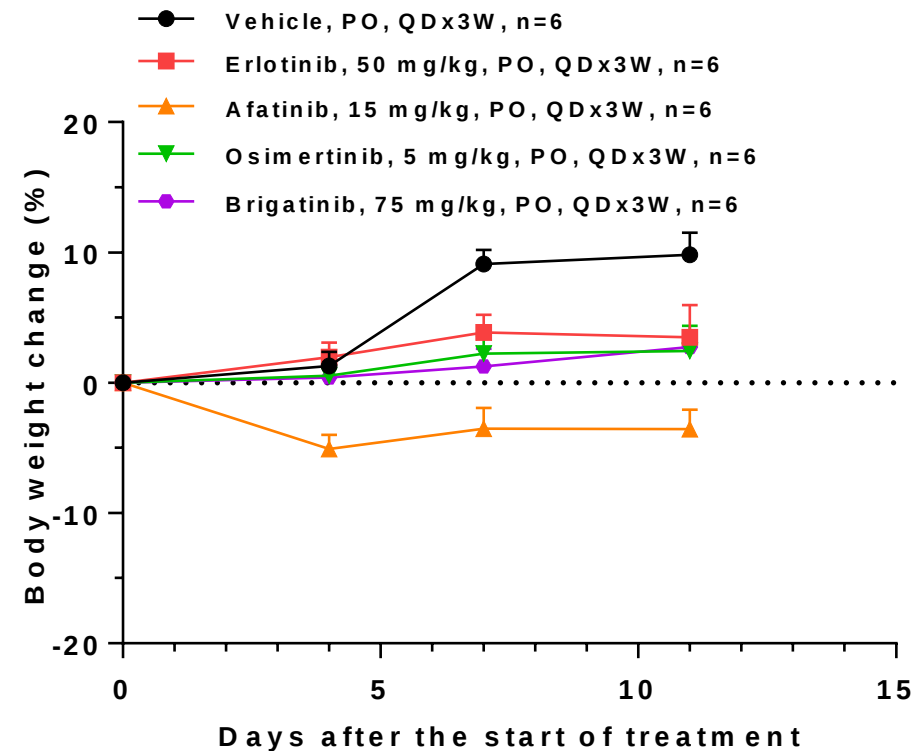
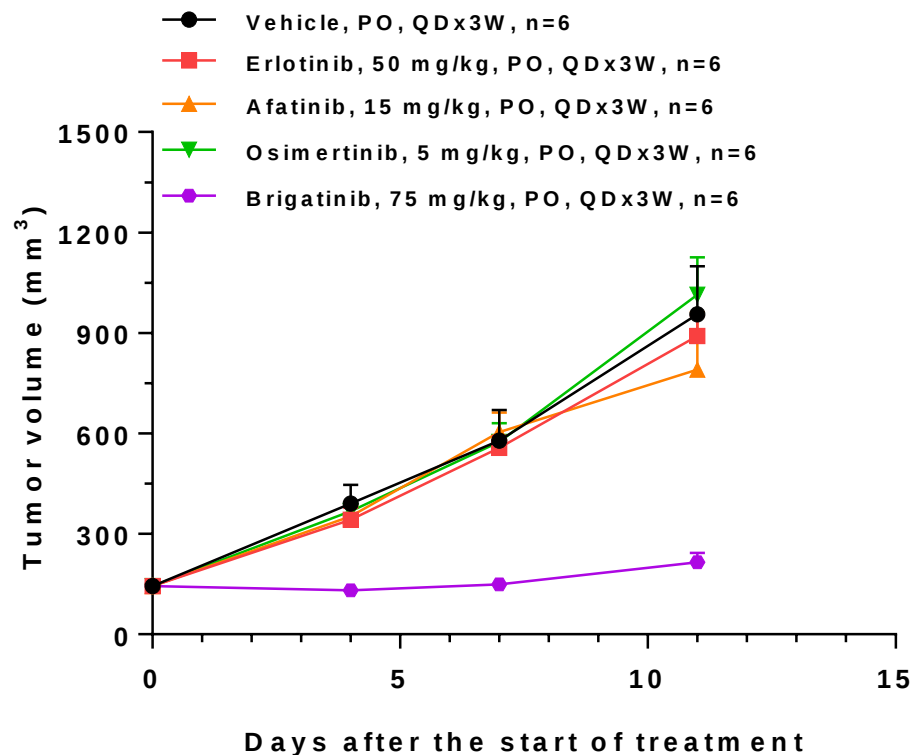
In vivo models derived from Ba/F3 cell lines carrying EGFR mutations

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

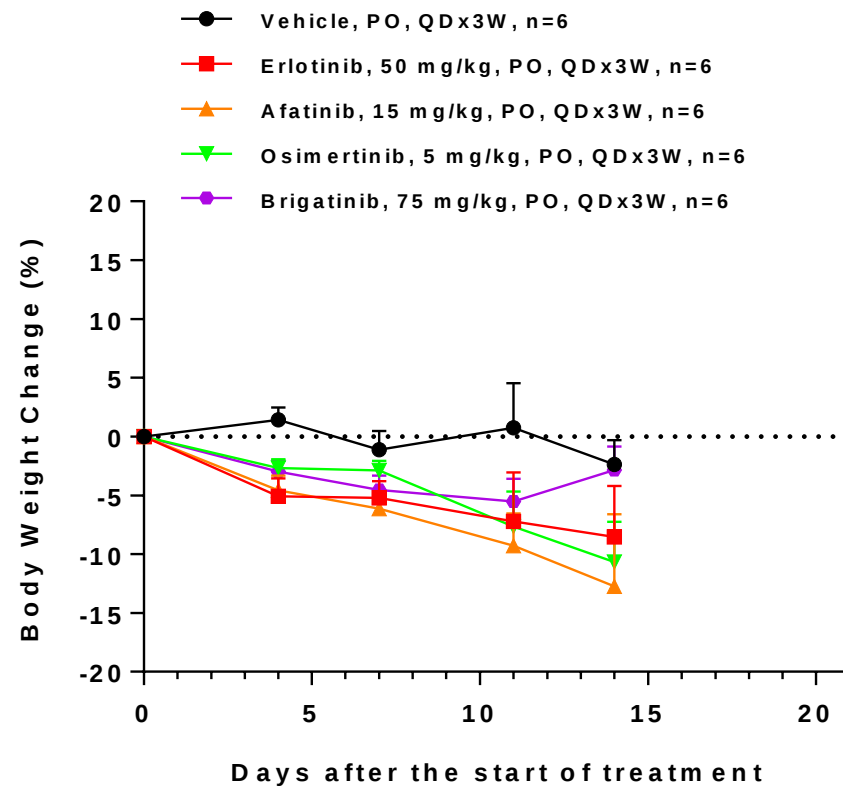
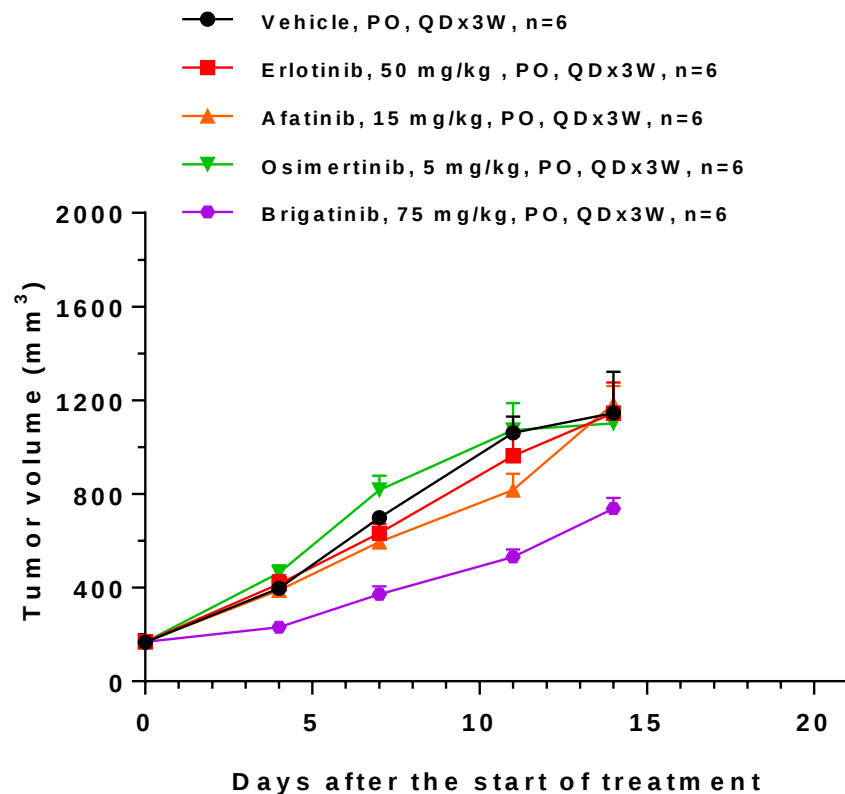
Erlotinib, Afatinib, Osimertinib & Brigatinib in Ba/F3 (exon19 del/T790M) model



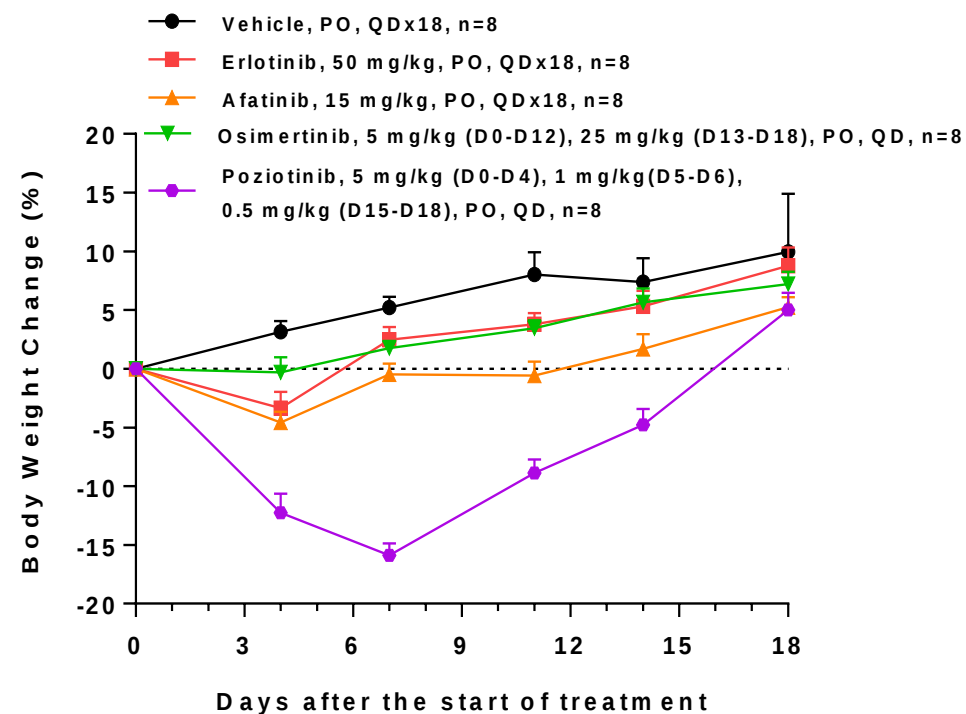
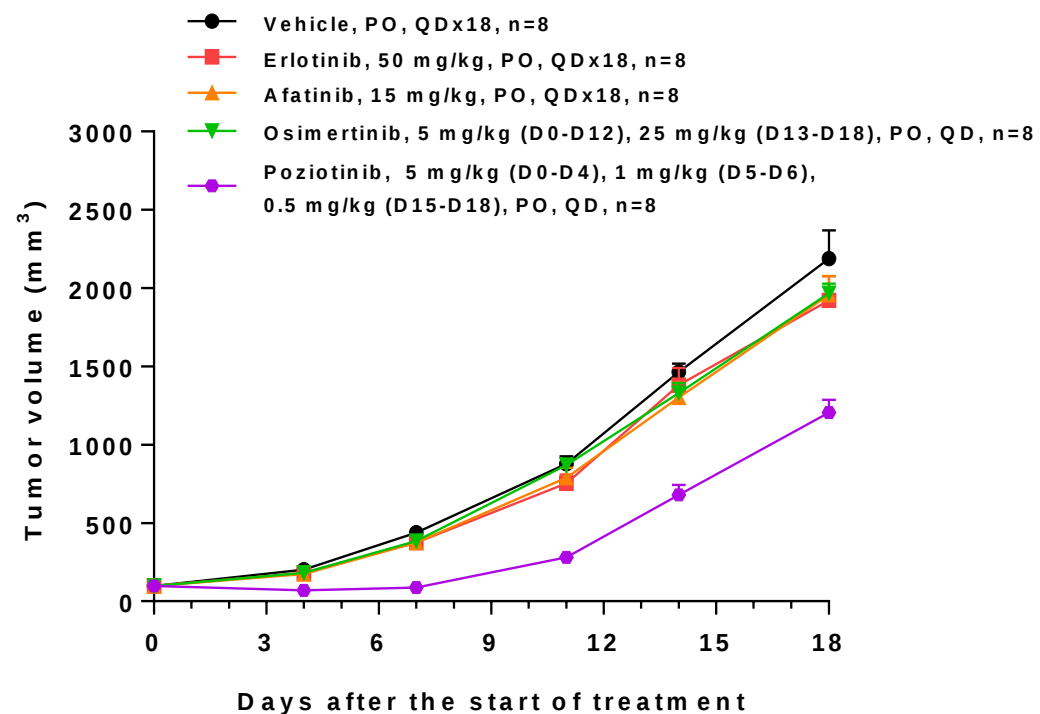
Erlotinib, Afatinib, Osimertinib & Brigatinib in Ba/F3 (exon19 del/T790M/C797S) model



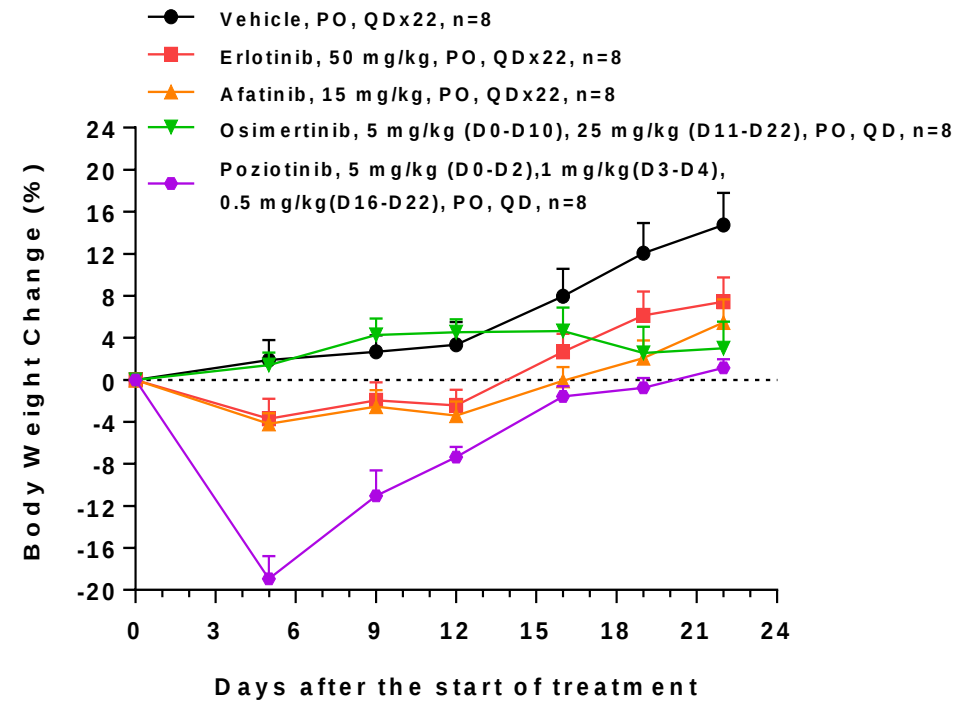
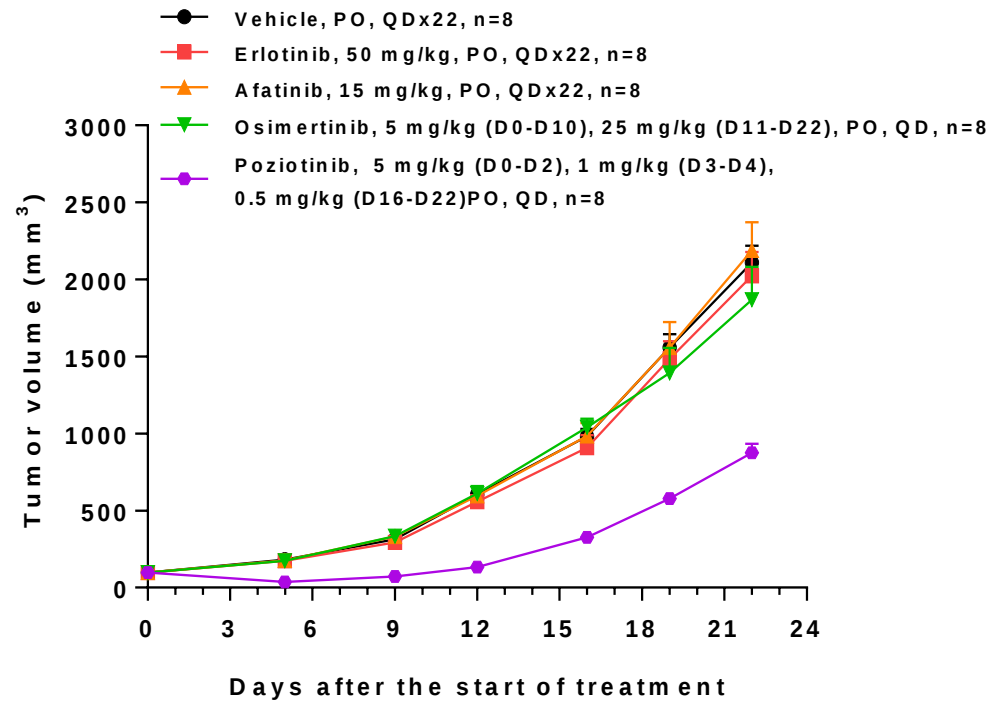
Erlotinib, Afatinib, Osimertinib & Brigatinib in Ba/F3 (L858R/T790M/C797S) model



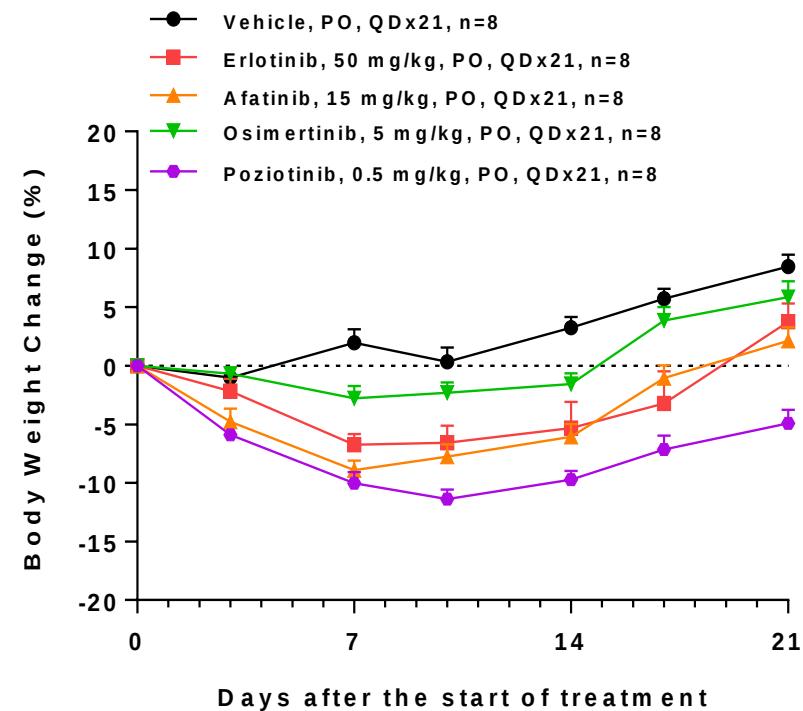
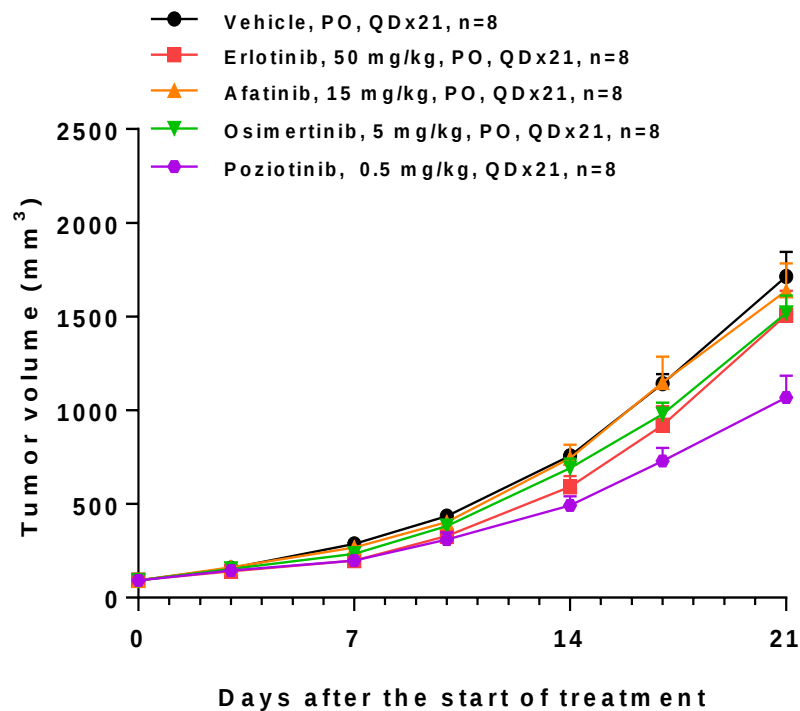
Anti-tumor efficacy of Erlotinib, Afatinib, Osimertinib and Poziotinib in the Ba/F3 (EGFR exon20ins ASV) model



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



Genetically engineered Ba/F3 cell lines carrying RET mutations

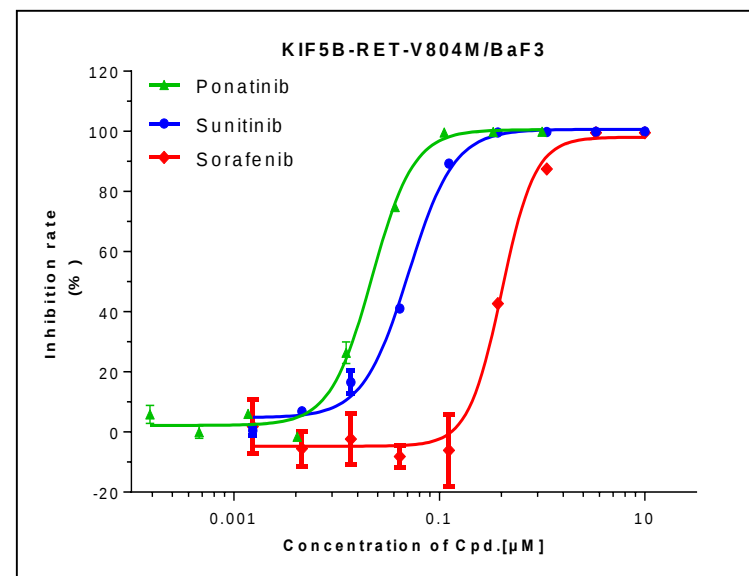
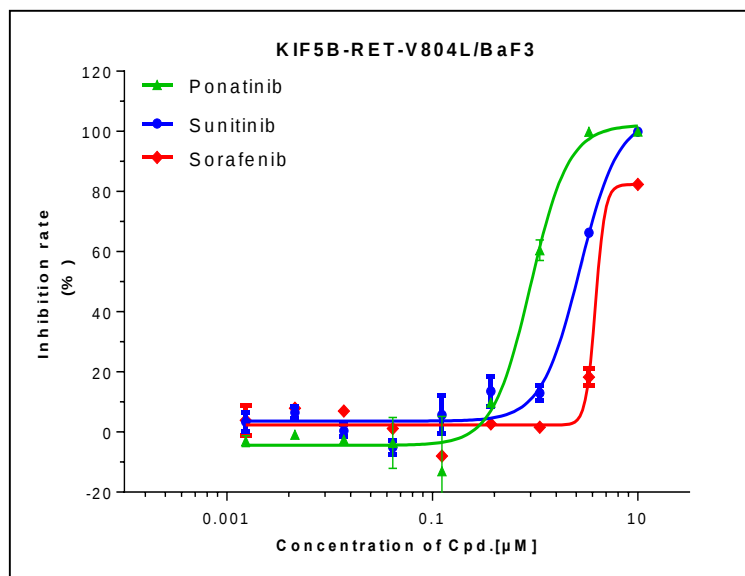
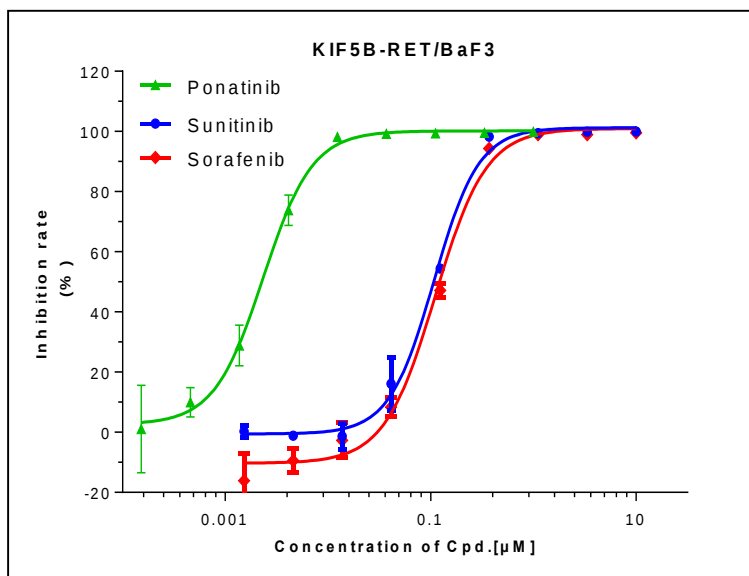
Cell type	Cell line	<i>In vitro</i> validation	<i>In vivo</i> validation
Pro-B cells	Ba/F3 KIF5B-RET-WT	Ready	Ongoing
	Ba/F3 KIF5B-RET-V804L	Ready	Ongoing
	Ba/F3 KIF5B-RET-V804M	Ready	Ongoing
	Ba/F3 CCDC6-RET-WT	Ready	Ready
	Ba/F3 CCDC6-RET-V804M	Ready	Ready
	Ba/F3 CCDC6-RET-V804L	Ready	Ready
	Ba/F3 KIF5B-RET-G810R	Ongoing	Ongoing
	Ba/F3 KIF5B-RET-G810S	Ready	Ongoing

Relative IC50 of RET inhibitors in Ba/F3 CCDC6-RET cell lines

Cell Name	Compound	Re IC50 Ave±SD
Ba/F3-CCDC6-RET-WT	Cabozatinib (XL 184)	0.454 ± 0.078
	Vandetanib (ZD6474)	0.470 ± 0.045
	Lenvatinib (E7080)	0.190 ± 0.021
	Sunitinib	0.394 ± 0.049
	Regorafenib (BAY 73-45)	0.210 ± 0.026
	Pralsetinib (BLU-667)	0.016 ± 0.003
Ba/F3-CCDC6-RET-V804M	Cabozatinib (XL 184)	3.028 ± 0.535
	Vandetanib (ZD6474)	4.752 ± 0.212
	Lenvatinib (E7080)	2.005 ± 0.774
	Sunitinib	0.263 ± 0.040
	Regorafenib (BAY 73-45)	1.577 ± 0.264
	Pralsetinib (BLU-667)	0.016 ± 0.009
Ba/F3-CCDC6-RET-V804L	Cabozatinib (XL 184)	2.825 ± 0.268
	Vandetanib (ZD6474)	4.285 ± 0.178
	Lenvatinib (E7080)	3.542 ± 0.717
	Sunitinib	0.494 ± 0.052
	Regorafenib (BAY 73-45)	2.124 ± 0.368
	Pralsetinib (BLU-667)	0.01 ± 0.005

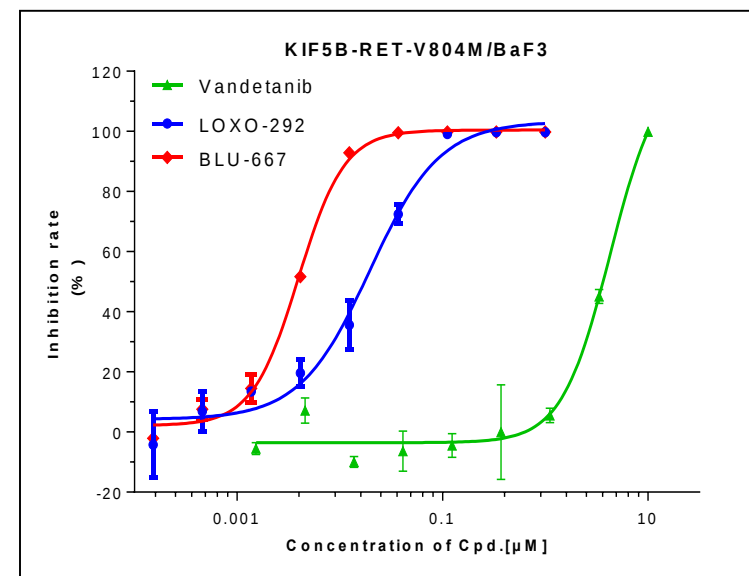
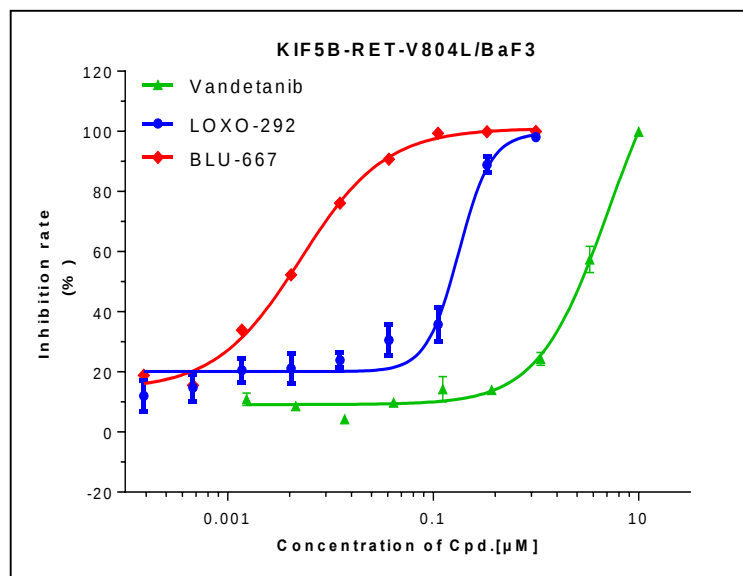
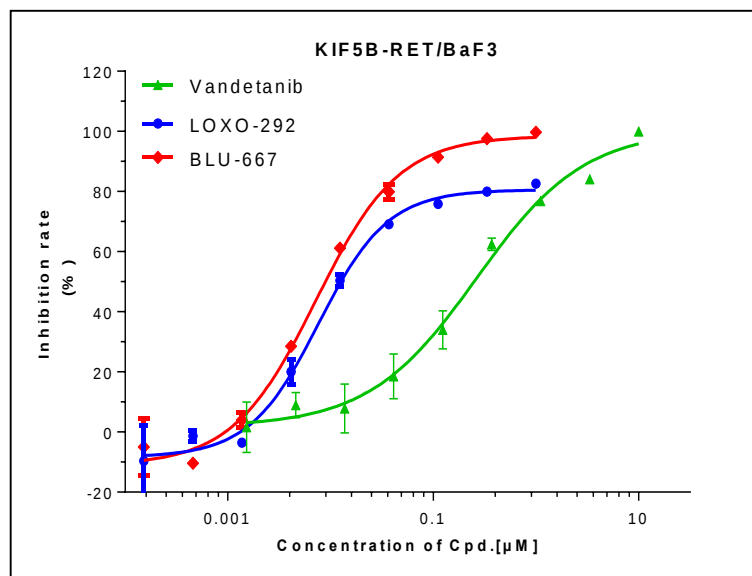
RET inhibitors	Note
Cabozatinib (XL 184)	1 st generation
Vandetanib (ZD6474)	1 st generation
Lenvatinib (E7080)	1 st generation
Sunitinib	1 st generation
Regorafenib (BAY 73-45)	1 st generation
Pralsetinib (BLU-667)	2 nd generation

ReIC50 of RET inhibitors in Ba/F3 KIF5B-RET cell lines



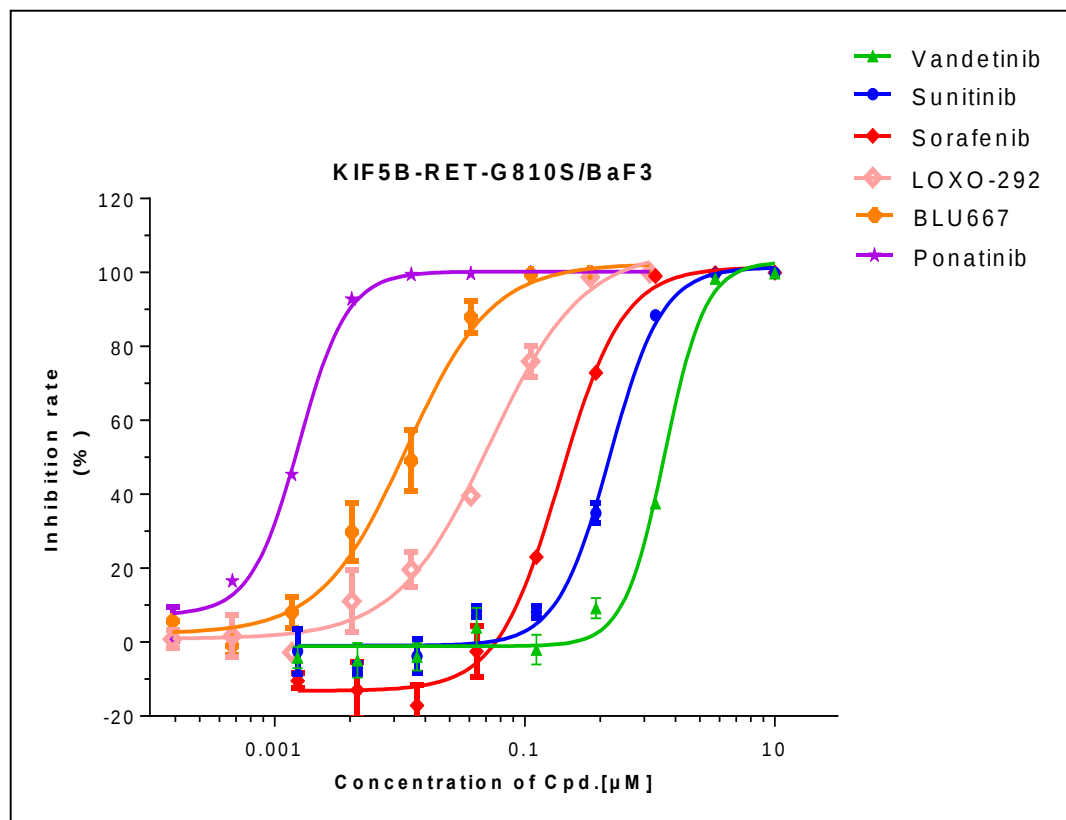
Cell Name	Compound	ReIC50 (μ M)
Ba/F3-KIF5B-RET-WT	Ponatinib	0.0023
	Sunitinib	0.1075
	Sorafenib	0.1107
Ba/F3-KIF5B-RET-V804L	Ponatinib	0.9040
	Sunitinib	2.722
	Sorafenib	~3.864
Ba/F3-KIF5B-RET-V804M	Ponatinib	0.0222
	Sunitinib	0.0499
	Sorafenib	0.4032

ReIC50 of RET inhibitors in Ba/F3 KIF5B-RET cell lines



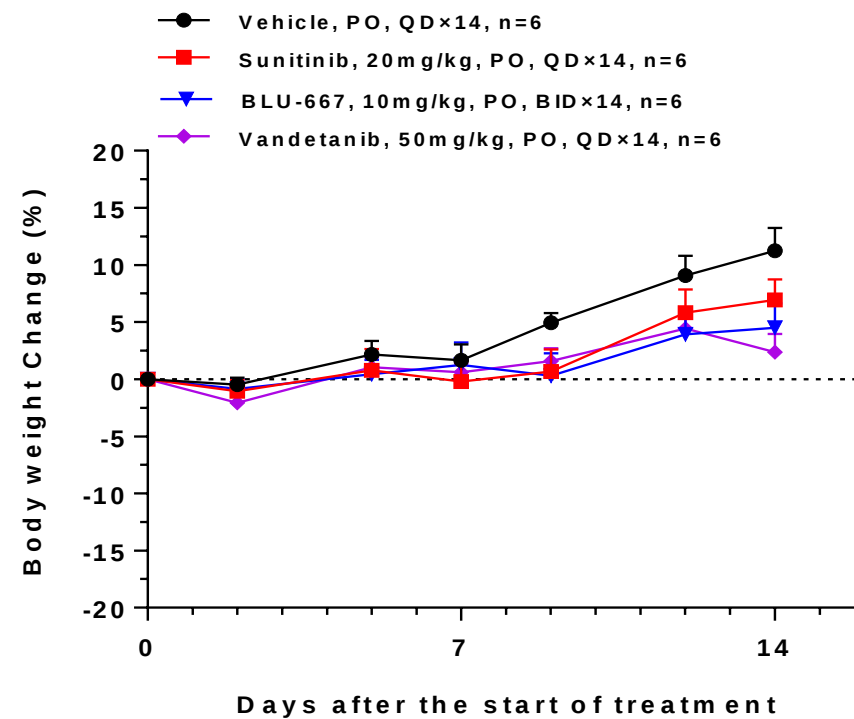
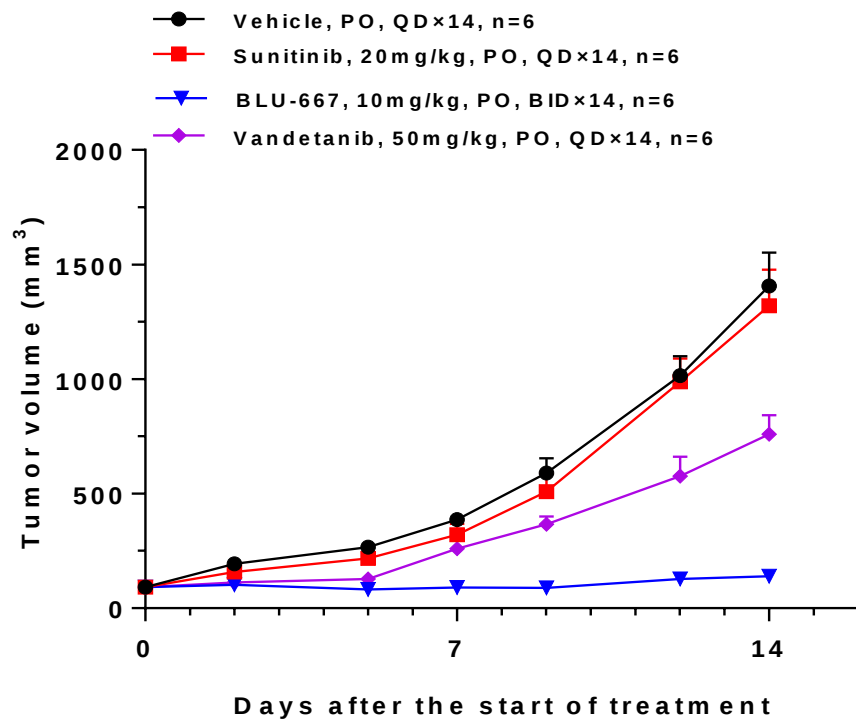
Cell Name	Compound	ReIC50 (μM)
Ba/F3-KIF5B-RET-WT	Vandetanib	0.2635
	LOXO-292	0.0076
	BLU-667	0.0072
Ba/F3-KIF5B-RET-V804L	Vandetanib	5.0810
	LOXO-292	0.1770
	BLU-667	0.0051
Ba/F3-KIF5B-RET-V804M	Vandetanib	4.2580
	LOXO-292	0.0196
	BLU-667	0.0040

ReIC50 of RET inhibitors in Ba/F3 KIF5B-RET cell lines

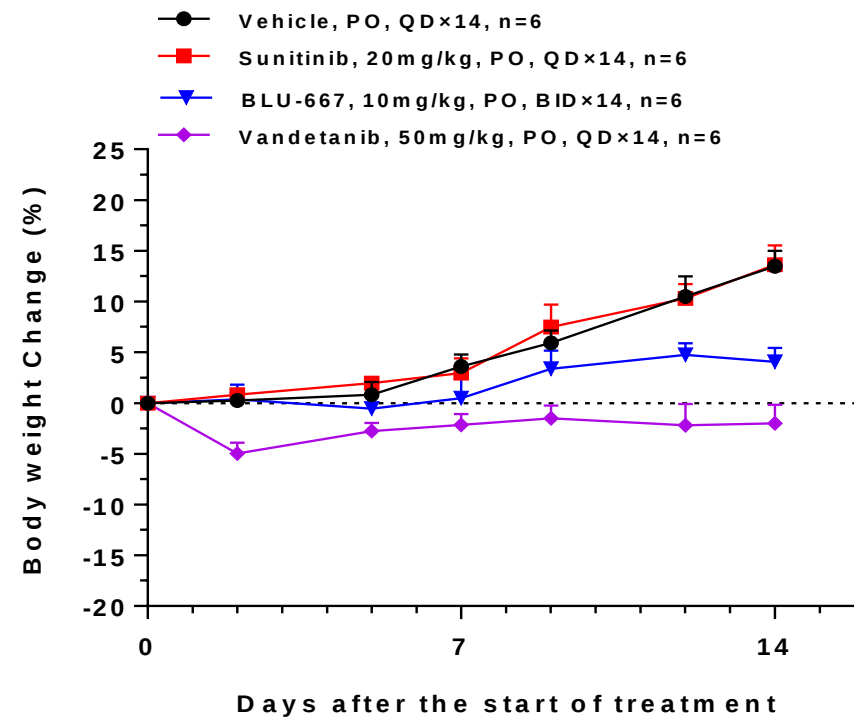
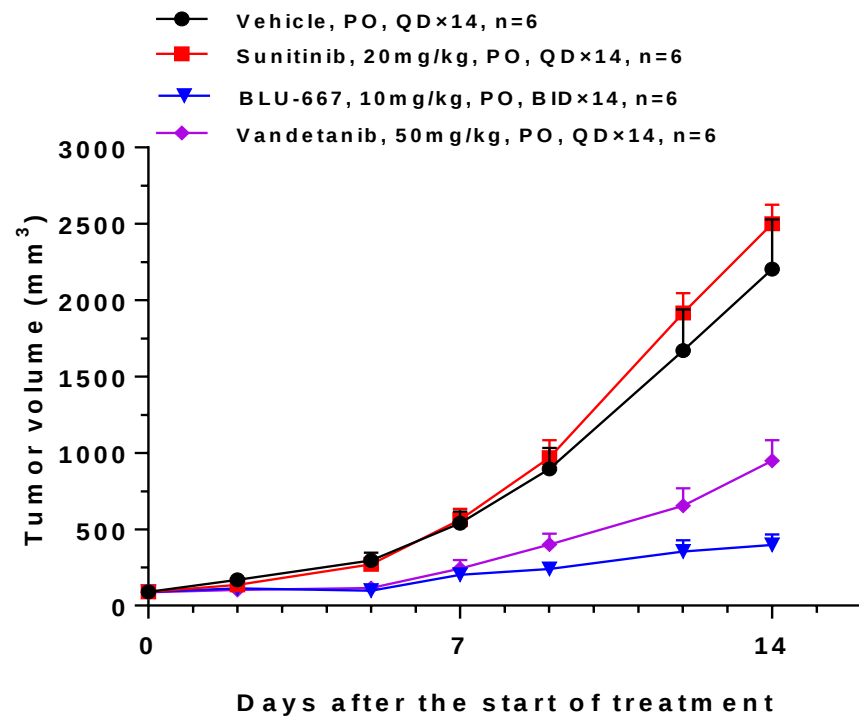


Cell Name	Compound	ReIC50 (μM)
Ba/F3-KIF5B-RET-G810S	Vandetanib	1.315
	Sunitinib	0.478
	Sorafenib	0.191
	LOXO-292	0.052
	BLU-667	0.011
	Ponatinib	0.002

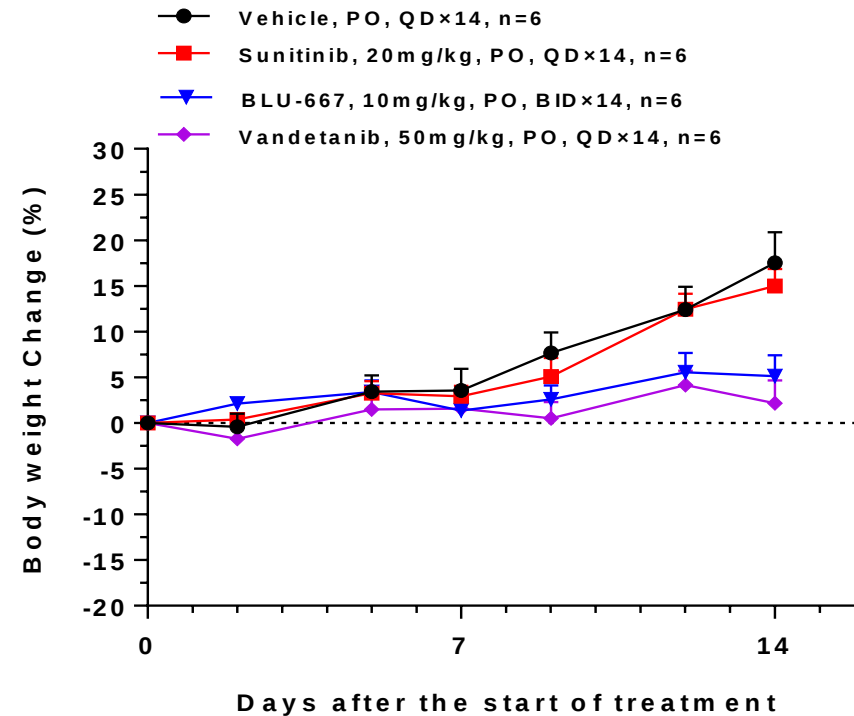
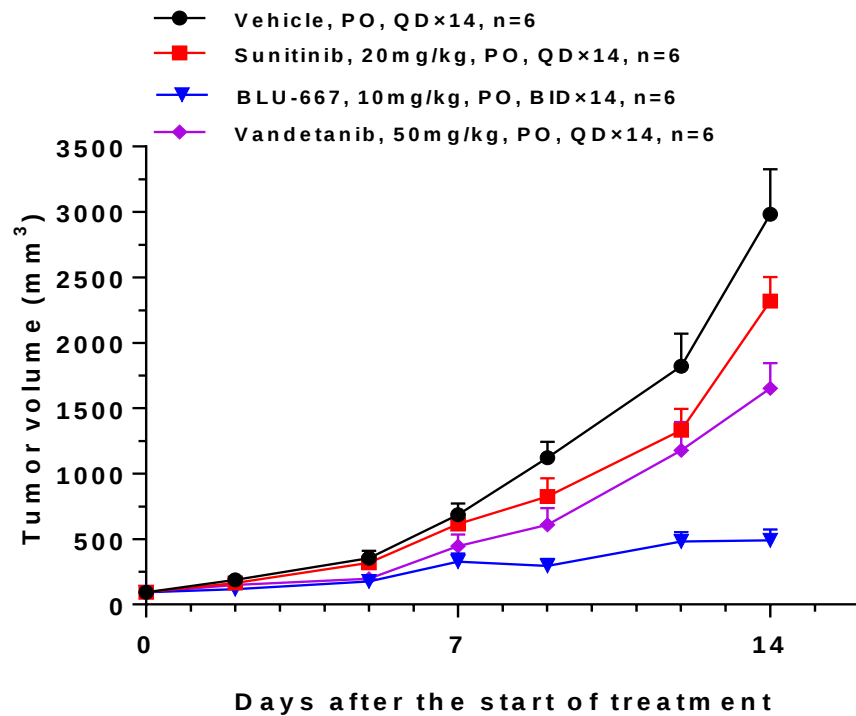
Sunitinib, BLU-667, Vandetanib in Ba/F3 CCDC6-RET model



Sunitinib, BLU-667, Vandetanib in Ba/F3 CCDC6-RET-V804L model



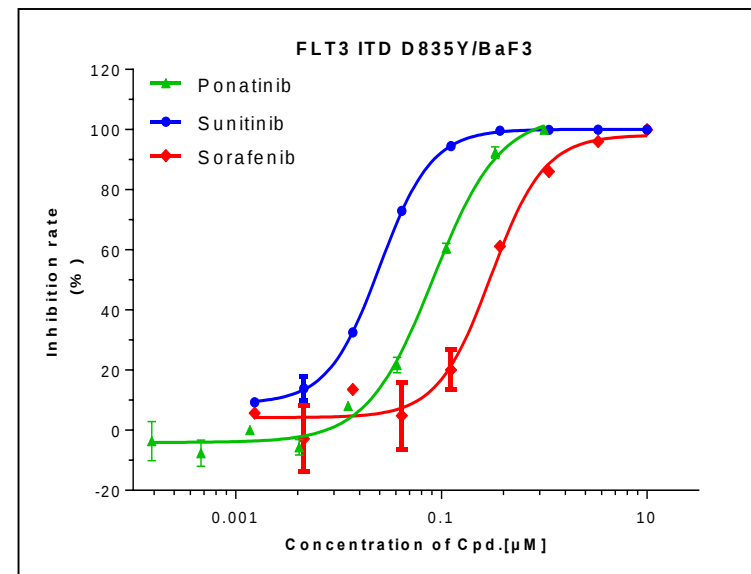
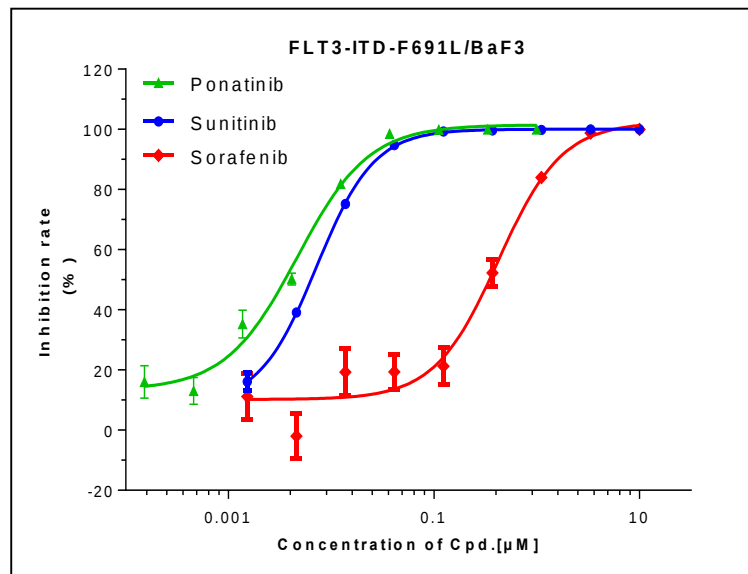
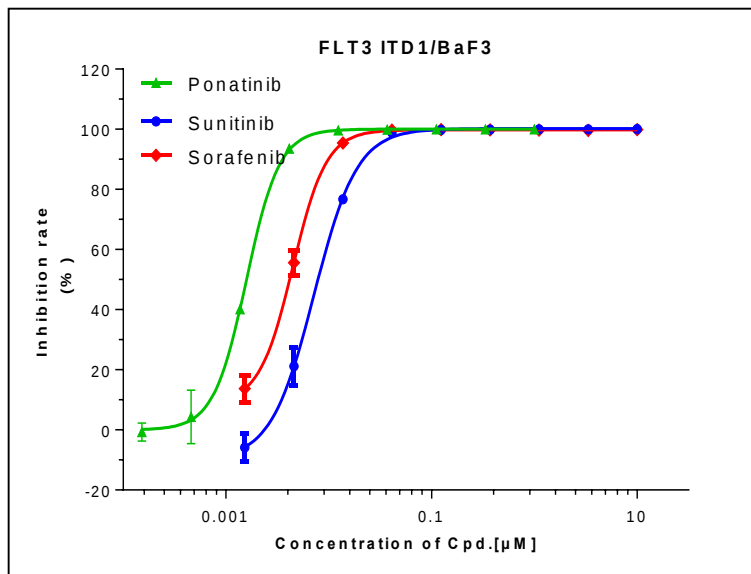
Sunitinib, BLU-667, Vandetanib in Ba/F3 CCDC6-RET-V804M model



Genetically engineered Ba/F3 cell lines carrying FLT3 mutations

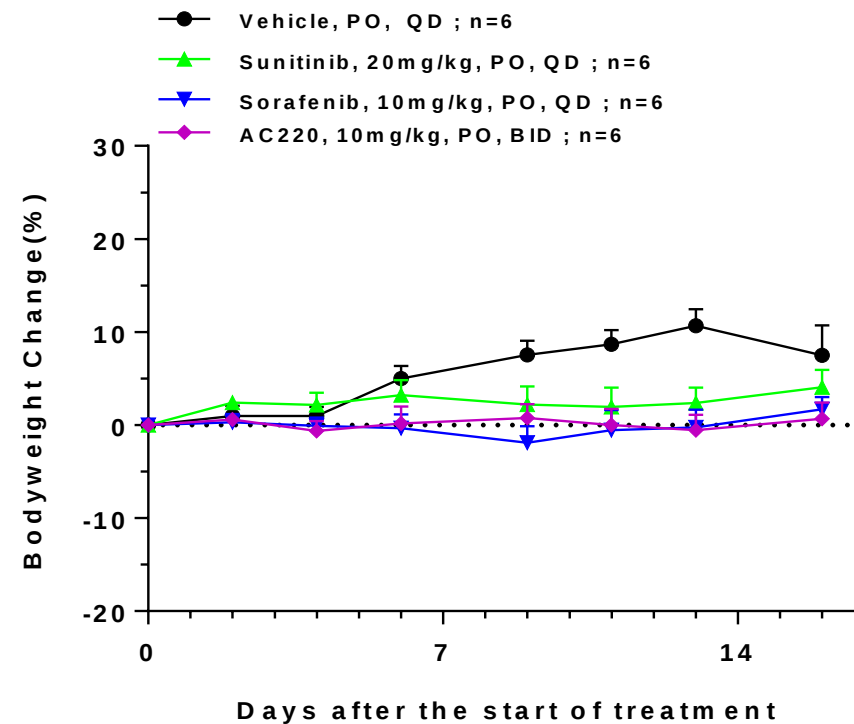
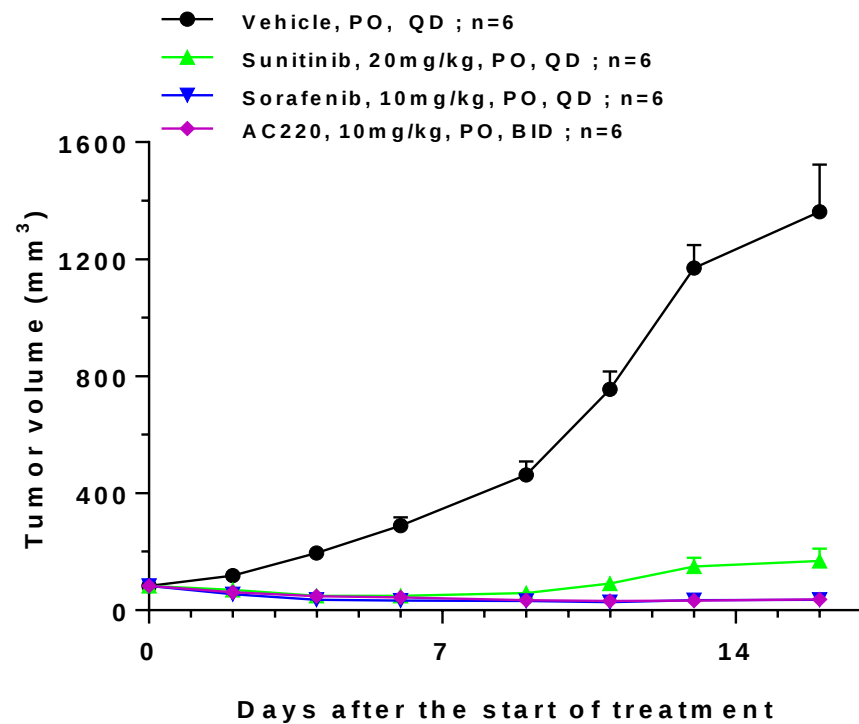
Cell type	Cell line	<i>In vitro</i> validation	<i>In vivo</i> validation
Pro-B cells	Ba/F3 FLT3-ITD	Ready	Ready
	Ba/F3 FLT3 ITD F691L	Ready	Ready
	Ba/F3 FLT3 ITD D835Y	Ready	Ready

ReIC50 of FLT3 inhibitors in Ba/F3 FLT3-ITD lines

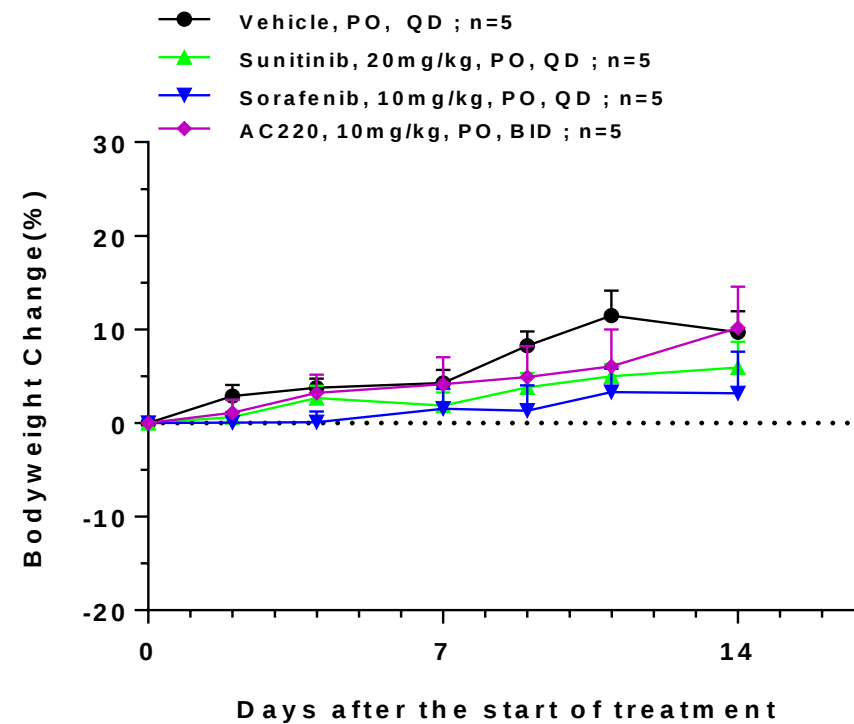
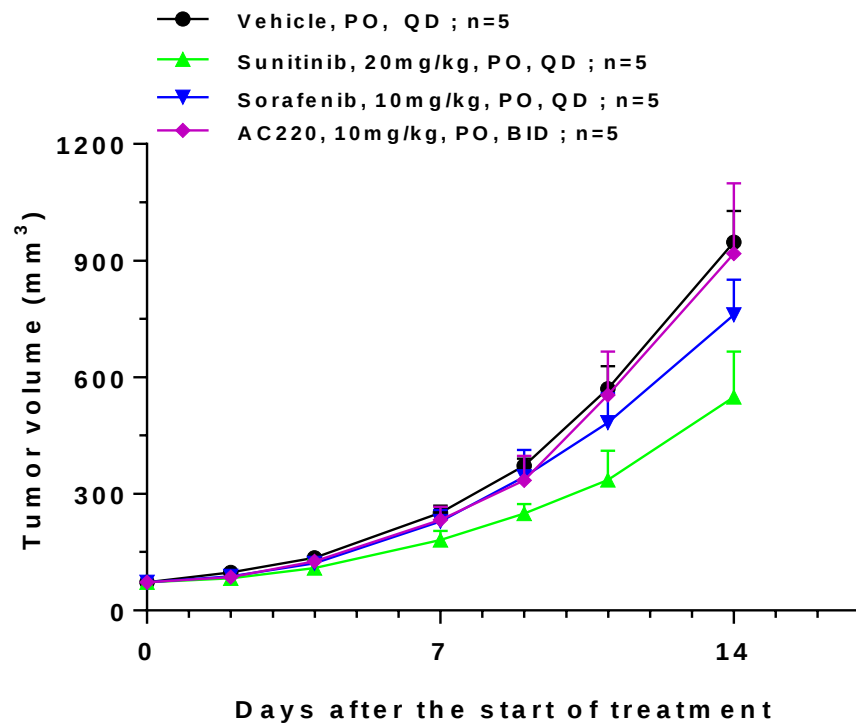


Cell Name	Compound	ReIC50 (μM)
Ba/F3 FLT3-ITD	Ponatinib	0.0016
	Sunitinib	0.0072
	Sorafenib	0.0045
Ba/F3 FLT3-ITD F691L	Ponatinib	0.0046
	Sunitinib	0.0072
	Sorafenib	0.4207
Ba/F3 FLT3-ITD D835Y	Ponatinib	0.0831
	Sunitinib	0.0251
	Sorafenib	0.3024

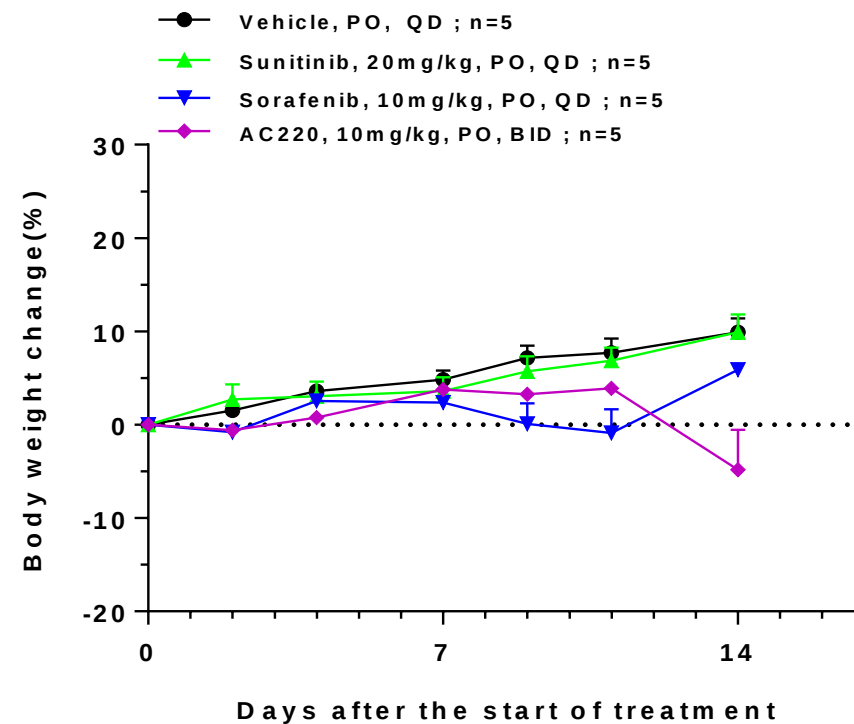
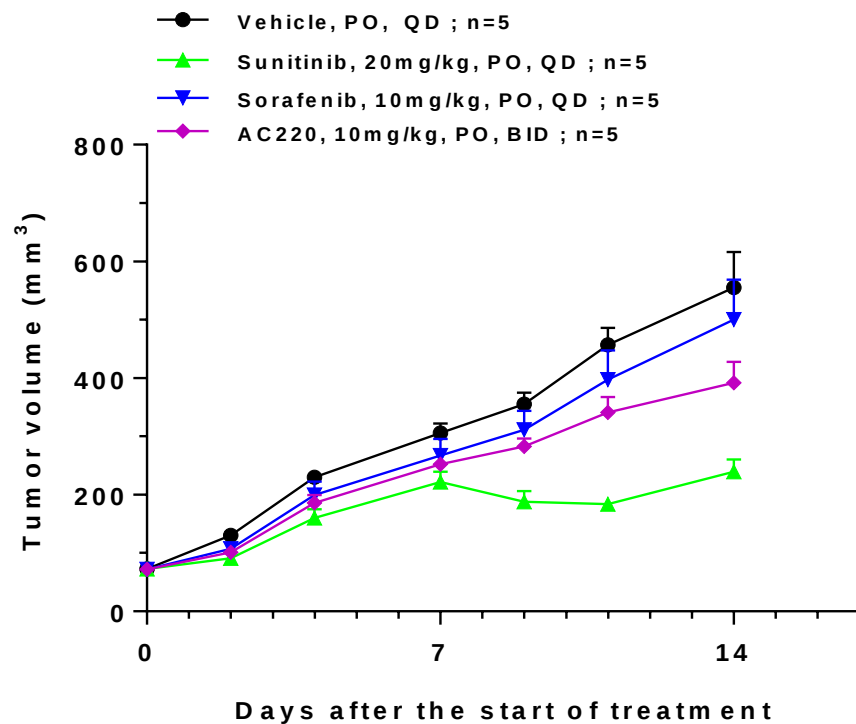
Sunitinib, Sorafenib, AC220 in Ba/F3 FLT3-ITD model



Sunitinib, Sorafenib, AC220 in Ba/F3 FLT3-ITD D835Y model



Sunitinib, Sorafenib, AC220 in Ba/F3 FLT3-ITD F691L model



Genetically engineered Ba/F3 cell lines carrying ROS1 mutations

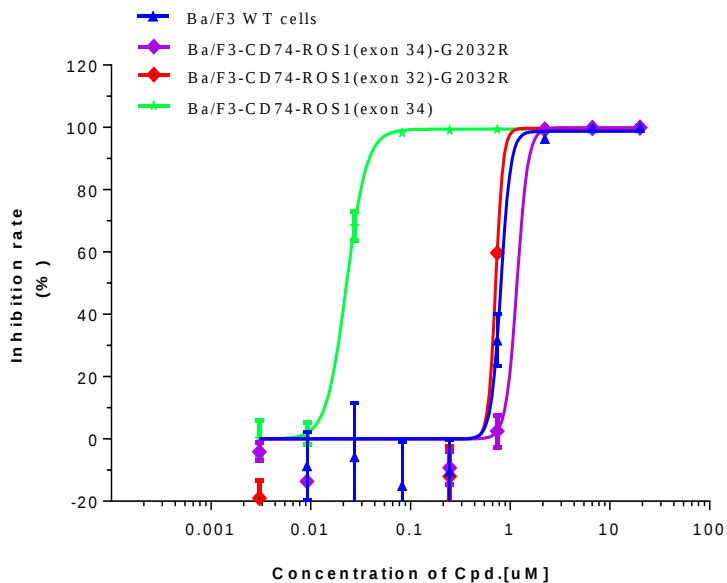
Cell type	Cell line	<i>In vitro</i> validation	<i>In vivo</i> validation
Pro-B cells	Ba/F3 CD74-ROS1 (exon 32)	Ready	Ongoing
	Ba/F3 CD74-ROS1 (exon 32) G2032R	Ready	Ongoing
	Ba/F3 CD74-ROS1 (exon 34)	Ready	Ready
	Ba/F3 CD74-ROS1 (exon 34) G2032R	Ready	Ready
	Ba/F3 CD74-ROS1 (exon 32) L2026M	Ongoing	Ongoing
	Ba/F3 CD74-ROS1 (exon 34) L2026M	Ongoing	Ongoing

ReIC50 of ROS1 inhibitors in Ba/F3 CD74-ROS1 lines

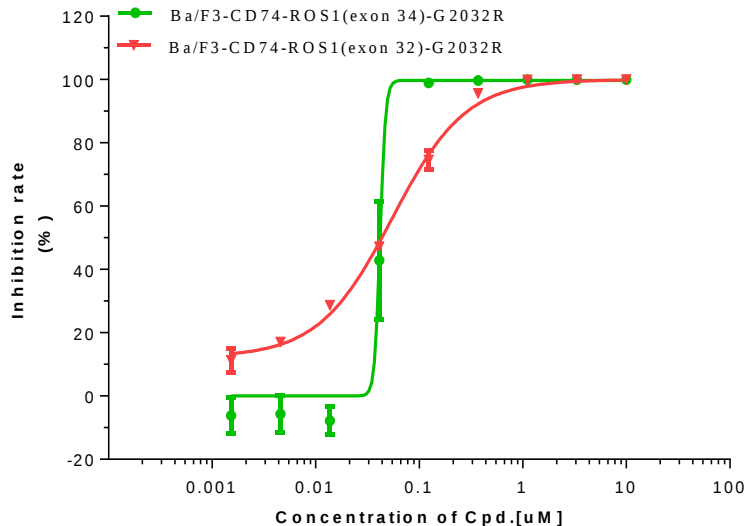
Cell line	Compound ID	IC50 (nM)
Ba/F3 WT	Crizotinib	809
Ba/F3 CD74-ROS1 (exon 34)	Crizotinib	23
Ba/F3 CD74-ROS1 (exon 34) G2032R	Crizotinib	1172
	TPX-0005	42
Ba/F3 CD74-ROS1 (exon 32) G2032R	Crizotinib	716
	TPX-0005	44

CD74-ROS1 (exon 34): fusion point is at the start of exon 34 of ROS1 gene.
 CD74-ROS1 (exon 32): fusion point is at the start of exon 32 of ROS1 gene.

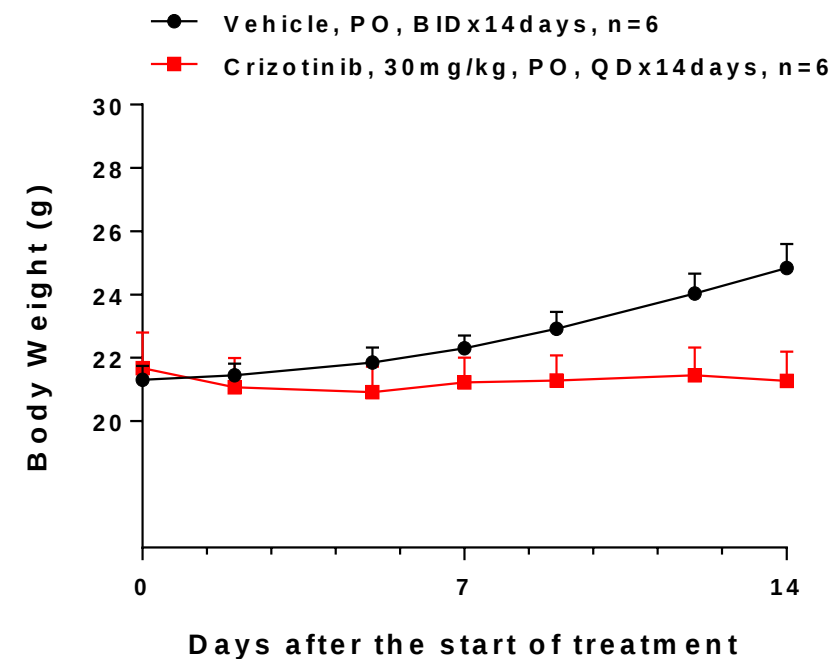
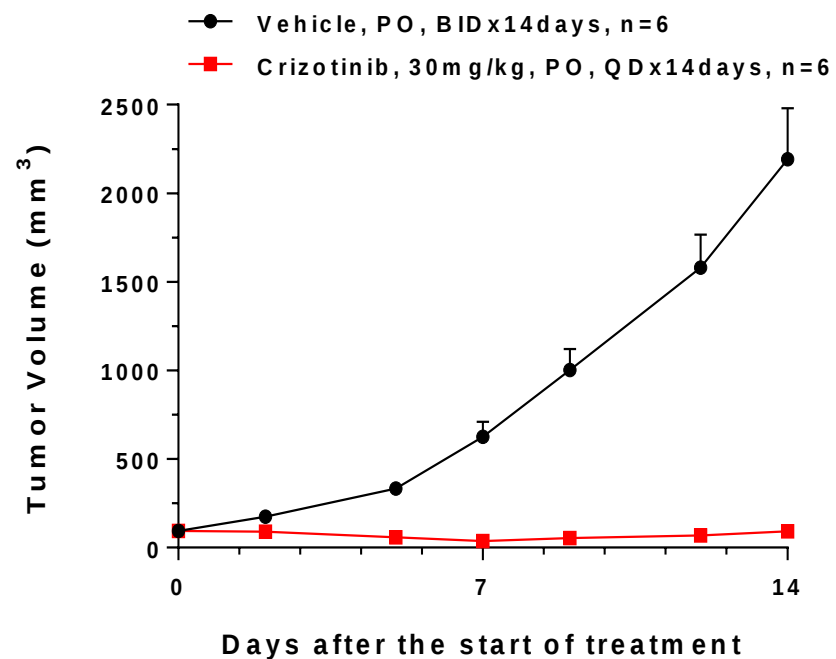
Crizotinib sensitivity test



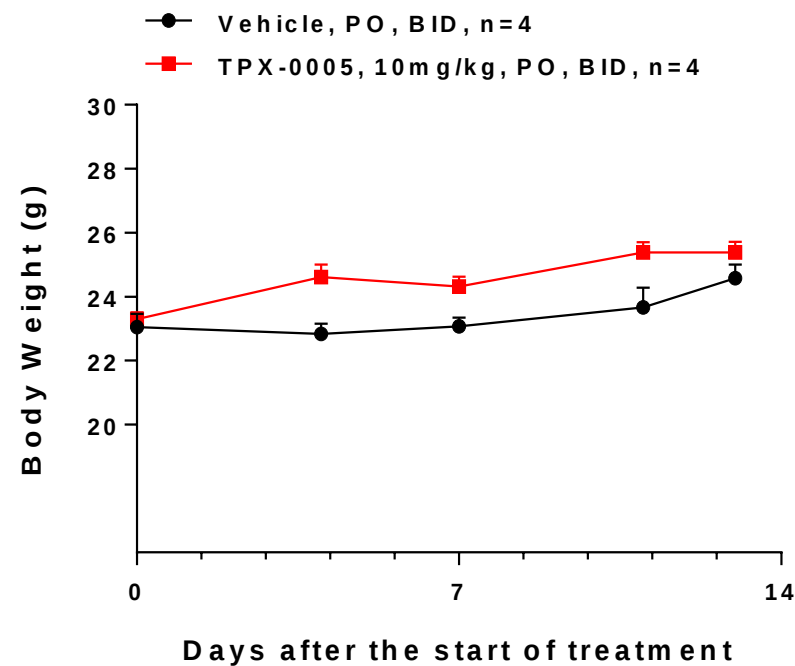
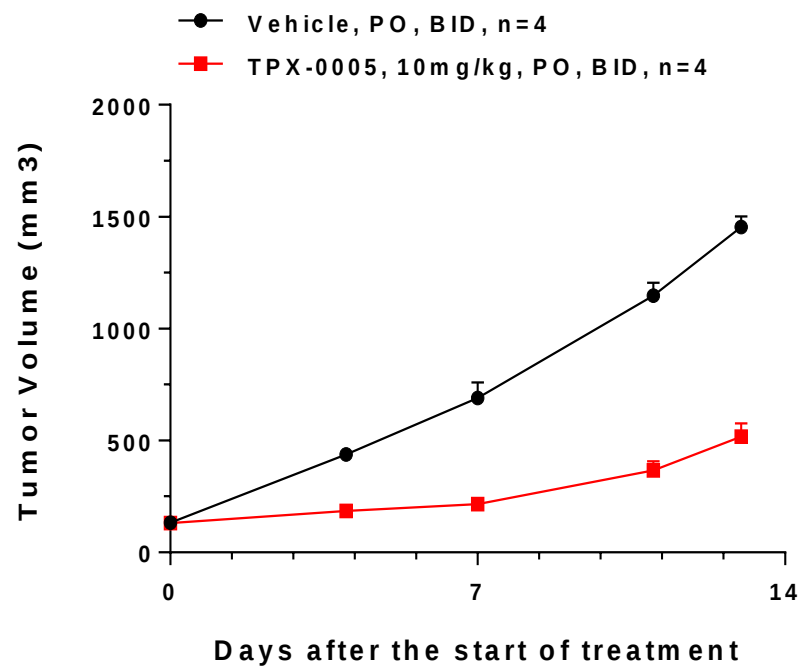
TPX-0005 sensitivity test



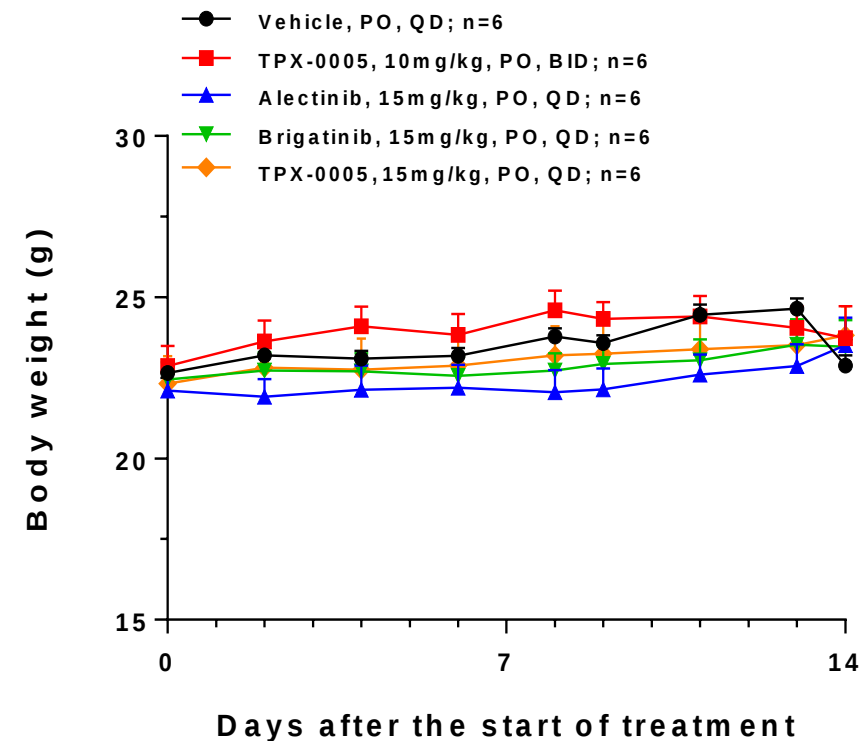
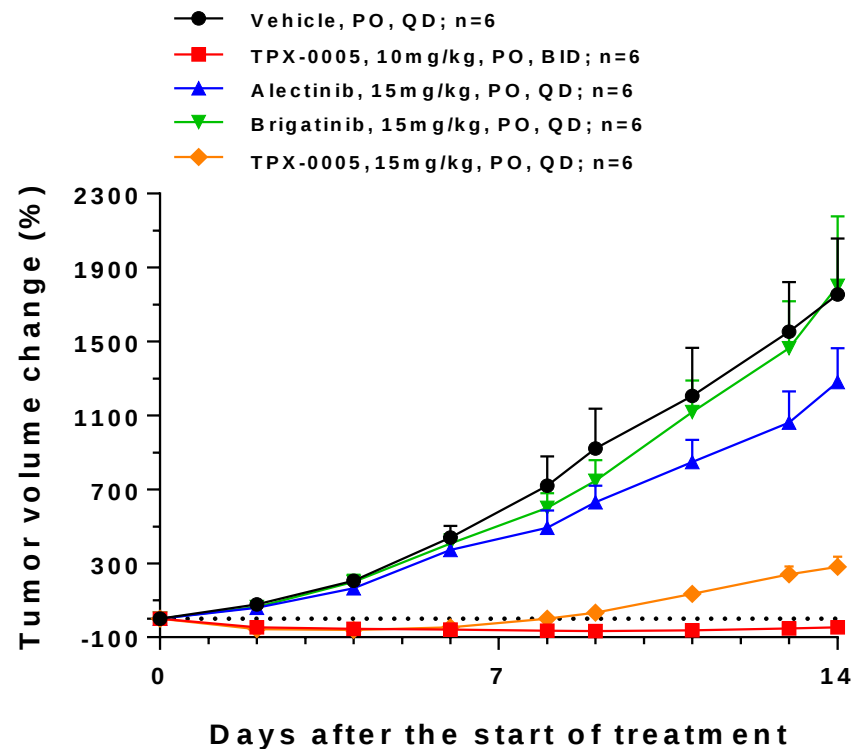
ROS1 TKIs in Ba/F3 CD74-ROS1 (exon 34) model



ROS1 TKIs in Ba/F3 CD74-ROS1 (exon 34) G2032R model



ROS1 TKIs in Ba/F3 CD74-ROS1 (exon 34) G2032R model





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For questions and requests, please email to info_onco@wuxiapptec.com



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