Genetically engineered Osimertinib-resistant cell lines and models

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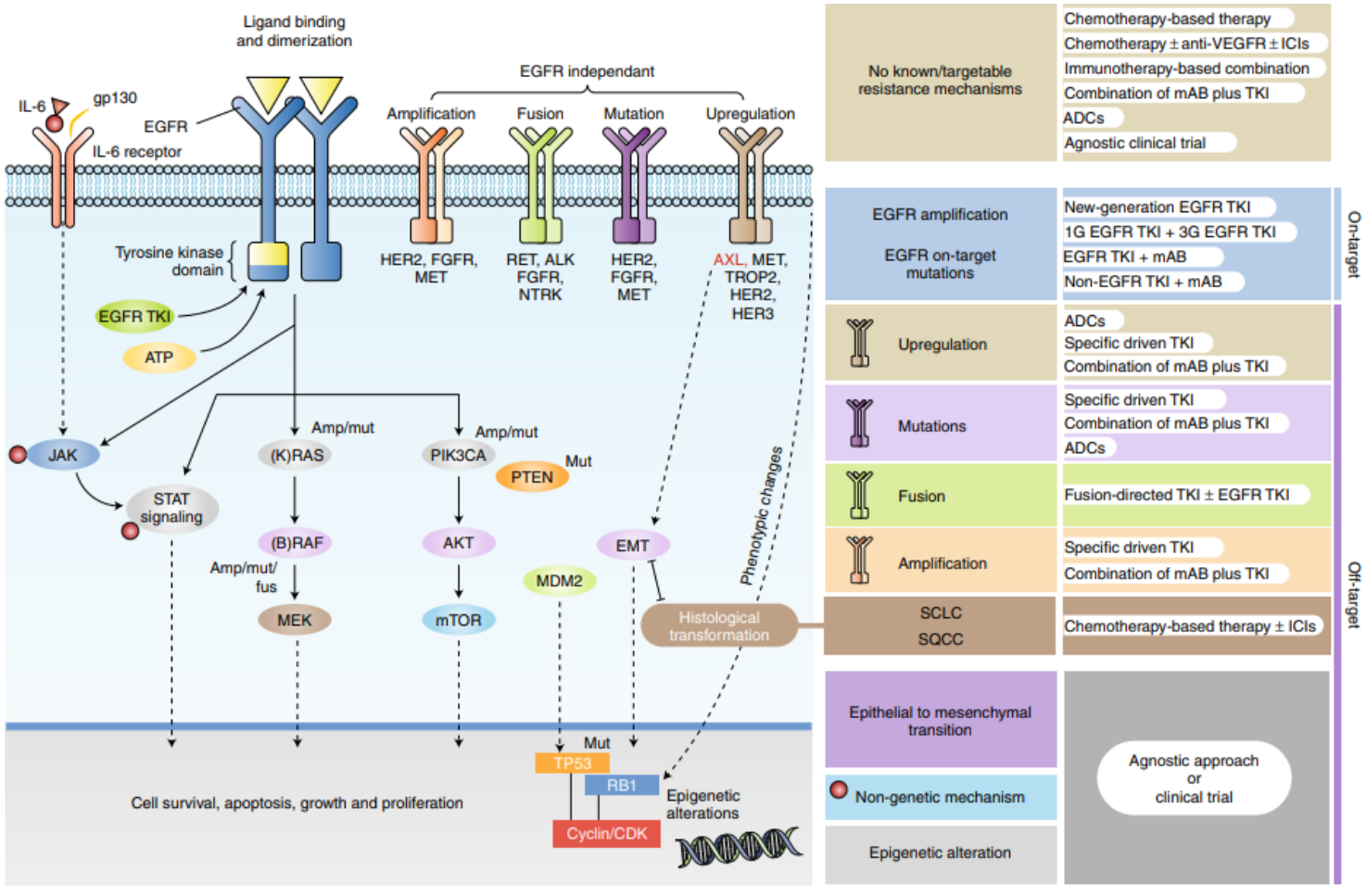
2023.10

Outline

- Mechanisms of resistance to EGFR TKIs
- Genetically engineered Osimertinib-resistant cancer cell lines and models
- Genetically engineered Osimertinib-resistant Ba/F3 cell lines and models

EGFR signaling pathway, resistance mechanisms and therapy strategy

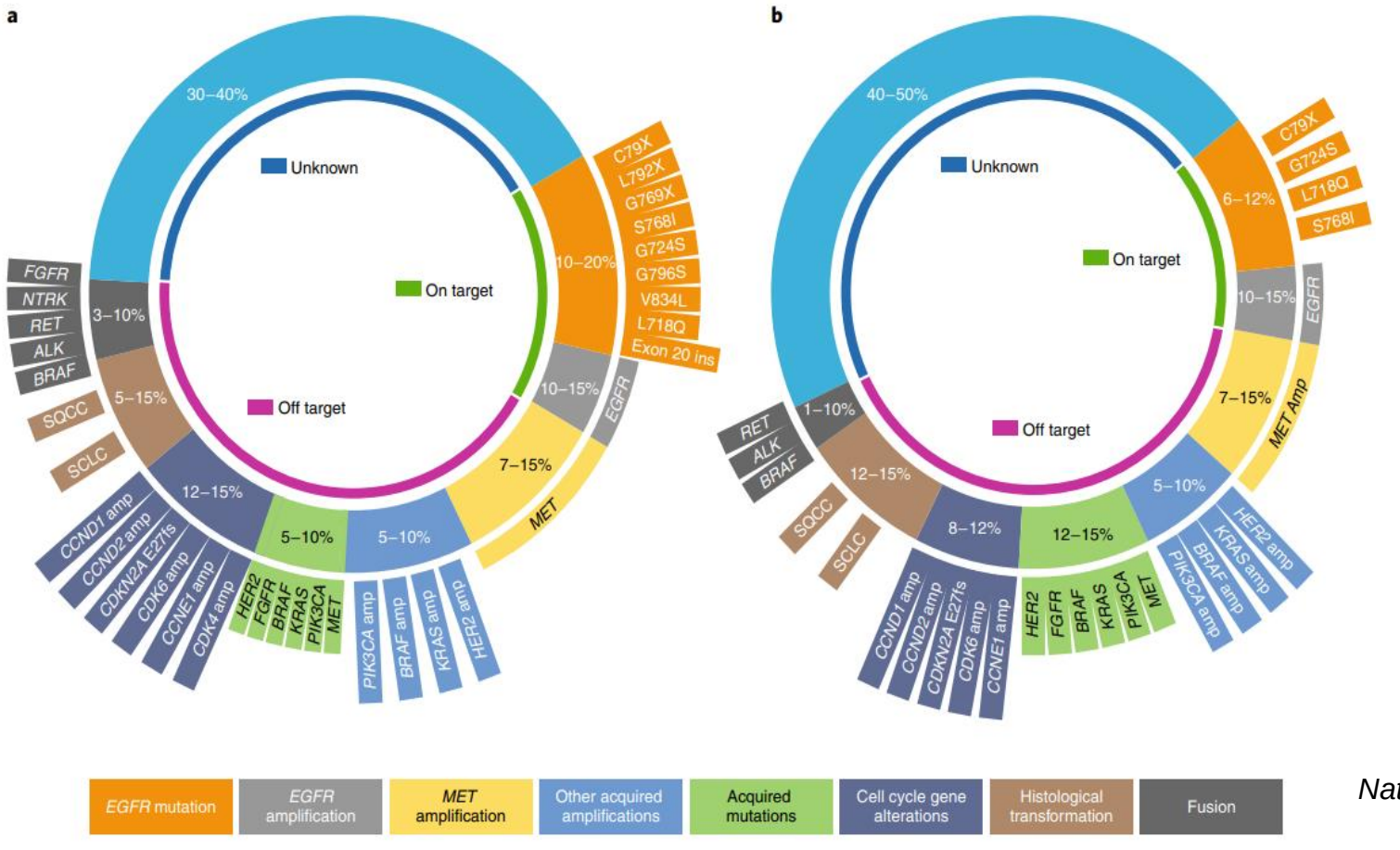


- Gene amplification and mutation are the main mechanisms of acquired resistance to EGFR TKIs.
- Rewiring of cell signaling irrespective of EGFR is also a key mechanism to circumvent EGFR TKI therapy in the absence of EGFR mutations.
- Combinations of EGFR TKIs with different drugs (including other TKIs, monoclonal antibodies, chemotherapy and vaccines) might be strategies for overcoming the acquired resistance to third-generation inhibitors.

Nat Cancer. 2021 Apr;2(4):377-391.

Mechanisms of resistance to Osimertinib

Resistance mechanisms arising after second-line (a) and first-line (b) Osimertinib therapy



- The most common tertiary EGFR mutation is C797S in exon 20 and it accounts for 15%-26% of cases of resistance to second-line Osimertinib treatment. Other uncommon mutations in exon 18 and exon 20 also lead to resistance to Osimertinib.
- In EGFR off-target alterations, MET amplification is one of the most common mechanisms of acquired resistance to Osimertinib second- and first-line therapy.

Nat Cancer. 2021 Apr;2(4):377-391.

Genetically engineered human cancer cell lines carrying EGFR C797S mutation

IC50 of EGFR TKIs in parental vs. genetically engineered cell lines

Cancer type	Cell line	Model Genetics	Reference compound IC50 (μM)			
			Erlotinib	Afatinib	Osimertinib	BI-4020
Lung cancer	PC-9	Carrying (EGFR delE746-A750)	/	/	0.078	0.032
	PC-9 EGFR DTC	EGFR exon 19 deletion/T790M/C797S	4.483	/	4.951	0.047
Lung cancer	NCI-H1975	Carrying (EGFR L858R/T790M)	/	/	0.018	0.250
	NCI-H1975 EGFR DTC	EGFR exon 19 deletion/T790M/C797S	9.5850	/	3.2920	0.180
	NCI-H1975 EGFR LTC	EGFR L858R/T790M/C797S	3.9480	2.1790	4.4580	0.372

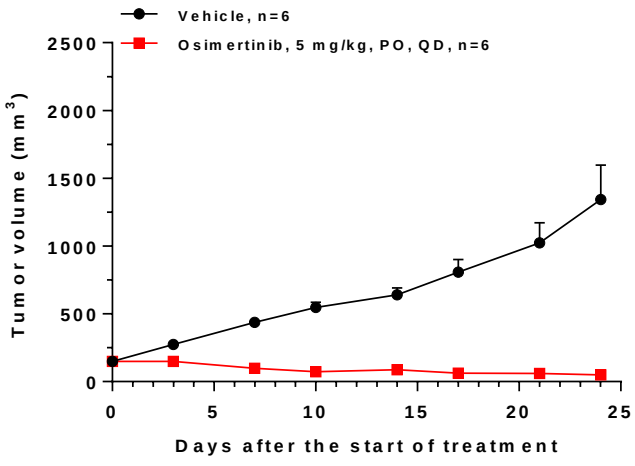
Genetically engineered human cancer models carrying EGFR C797S mutation

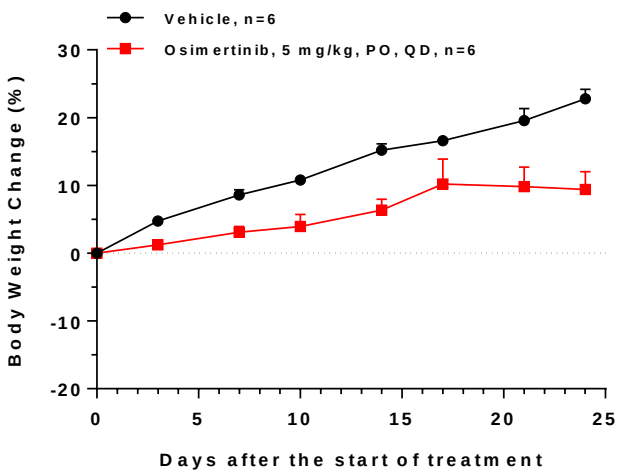
In vivo efficacy of EGFR TKIs in parental vs. genetically engineered models

Cancer type	Cell line	Inoculation method	Drugs tested	Dosage (mg/kg)	TGI (%)	MST (day)
Lung cancer	PC-9	subcutaneous	Osimertinib	5	108	/
	PC-9 EGFR DTC	subcutaneous	Osimertinib BI-4020	10 10	21.61 106	/
	PC-9-luc	intracranial	Vehicle Osimertinib Osimertinib BI-4020 Brigatinib	- 10 25 10 75	/	17 43.5 Not reach 26.5 26
	PC-9 EGFR DTC	intracranial	Vehicle Osimertinib Osimertinib BI-4020 Brigatinib	- 10 25 10 75	/	13.5 19 18 23 13.5
Lung cancer	NCI-H1975	subcutaneous	Osimertinib	1 5	51 108	/
	NCI-H1975 EGFR DTC	subcutaneous	Erlotinib Brigatinib Osimertinib	50 50 2.5	12.46 22.44 30.13	/
	NCI-H1975 EGFR LTC	subcutaneous	Osimertinib	10	4.72	/

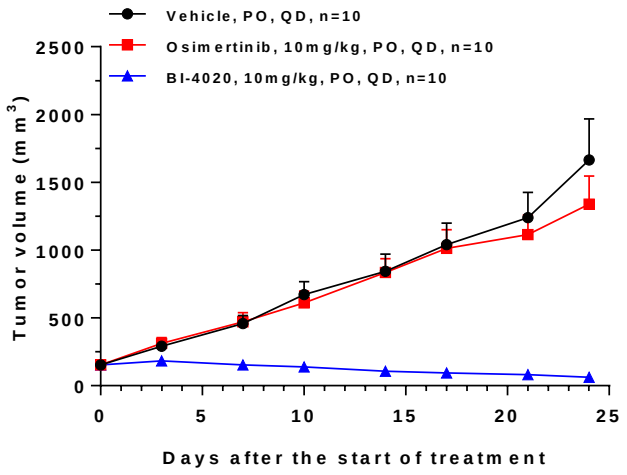
EGFR TKIs in PC-9 vs. genetically engineered PC-9 subcutaneous model

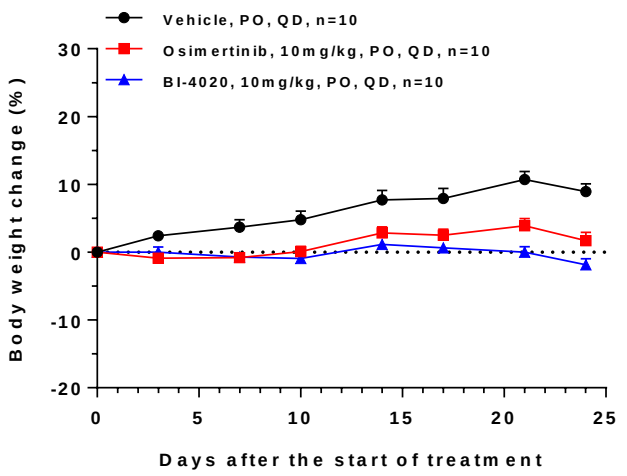
PC-9



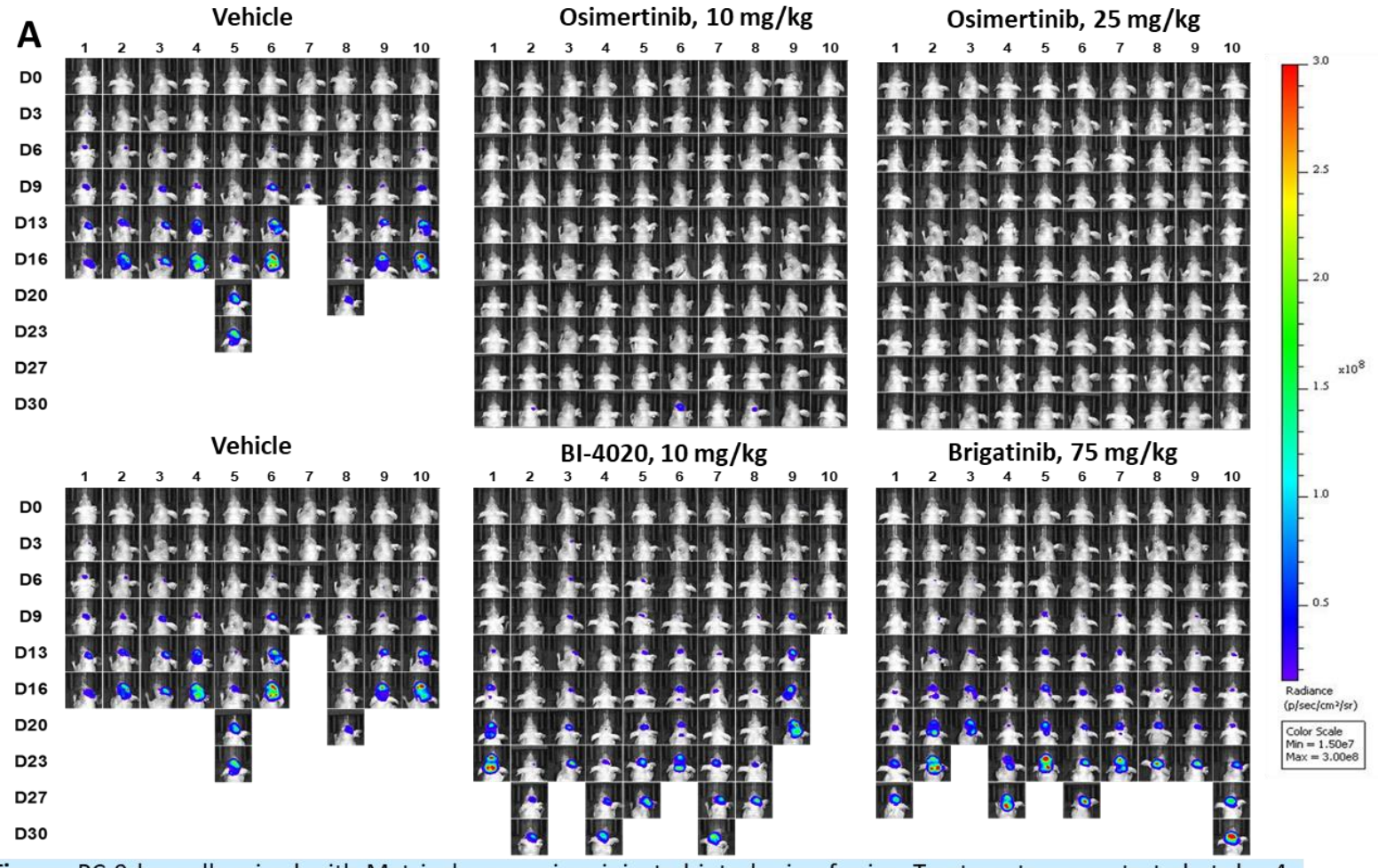


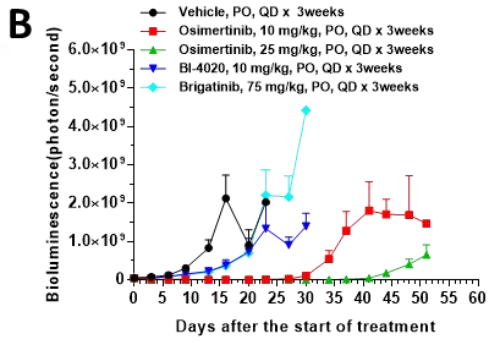
PC-9 EGFR-Del19/T790M/C797S

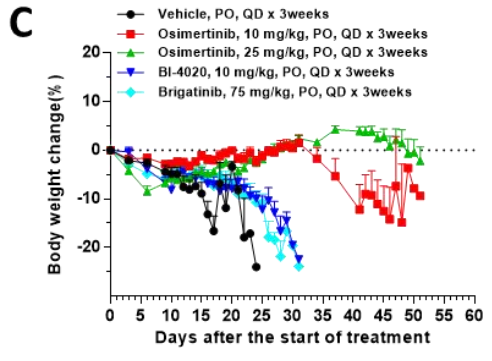




EGFR TKIs in PC-9-luc intracranial model







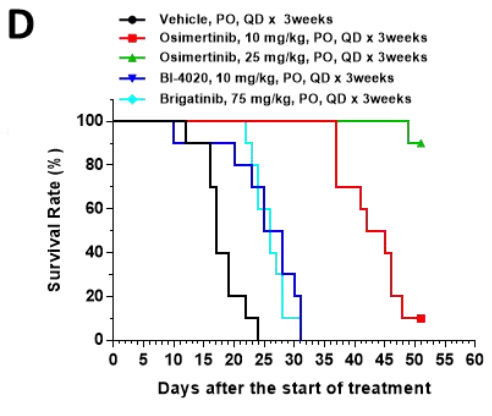
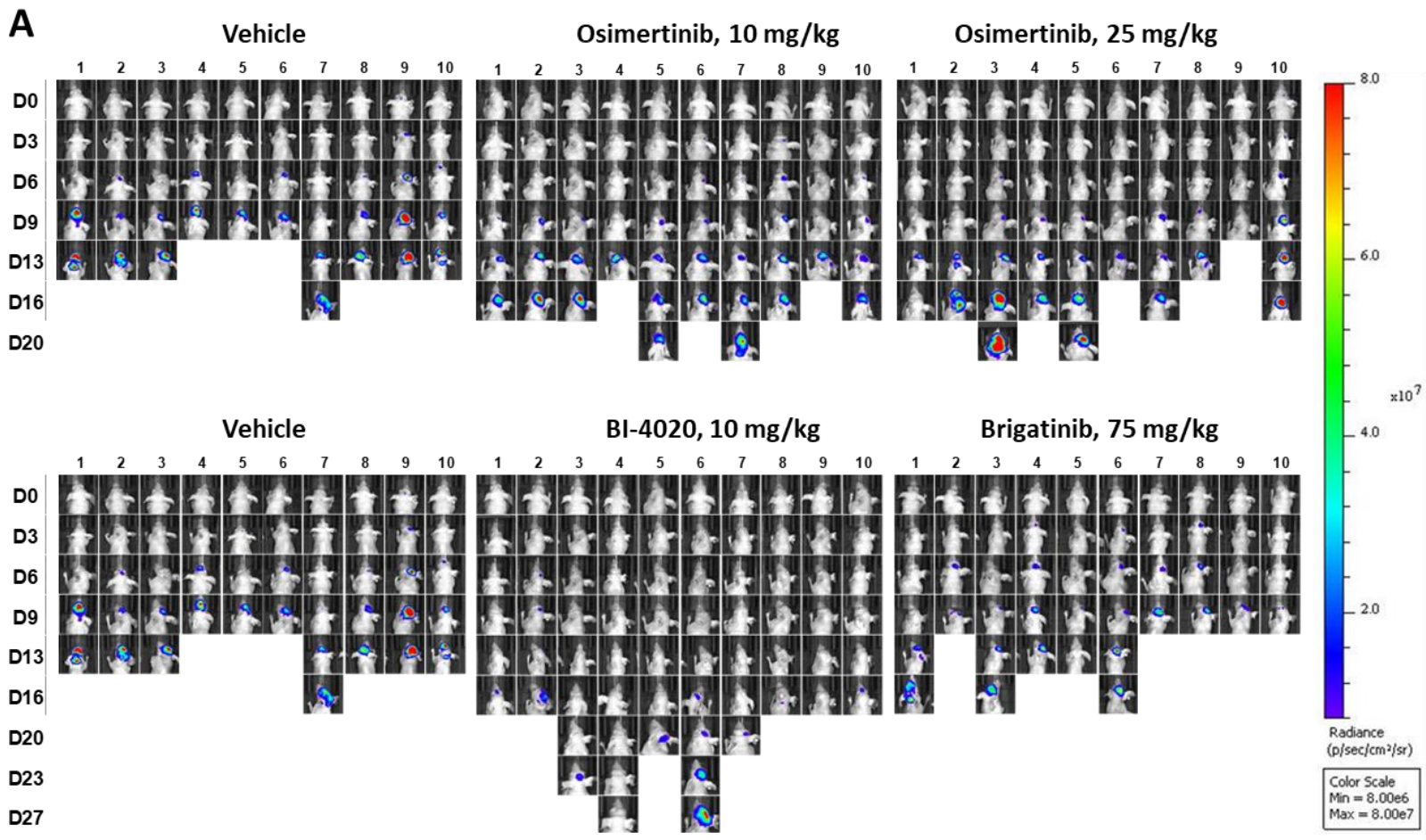
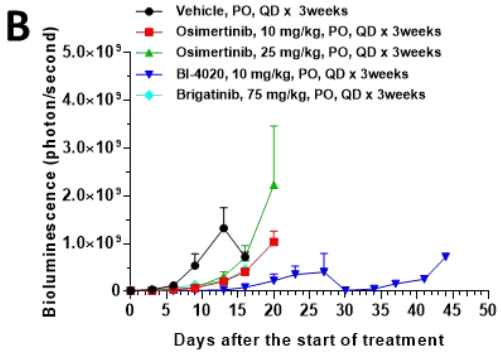
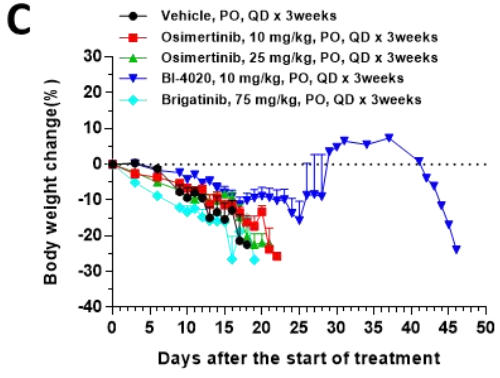


Figure: PC-9-luc cells mixed with Matrigel were micro-injected into brain of mice. Treatments were started at day 4 after inoculation (A) Bioluminescent imaging of mice over 30 days (n=10). (B) Intracranial tumor growth curve as measured by average photon intensity in the experiments. (C) The average body weight curve of mice was used in the experiment. (D) Survival curve till day 51.

EGFR TKIs in PC-9 EGFR-Del19/T790M/C797S-luc intracranial model







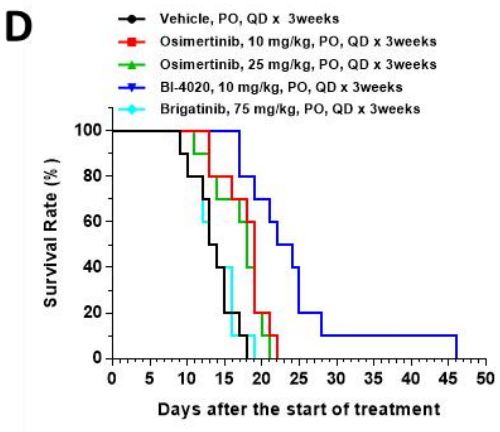
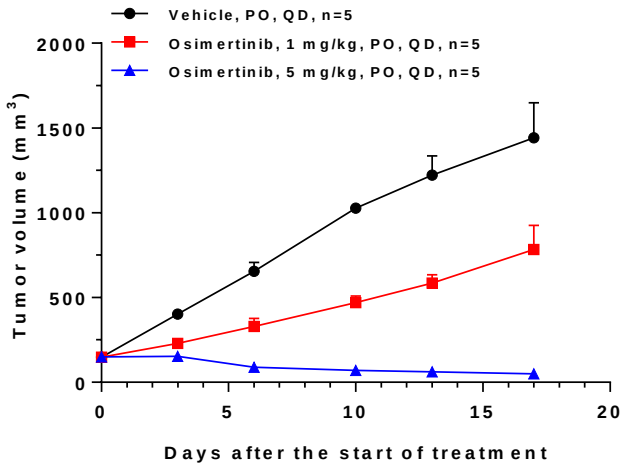
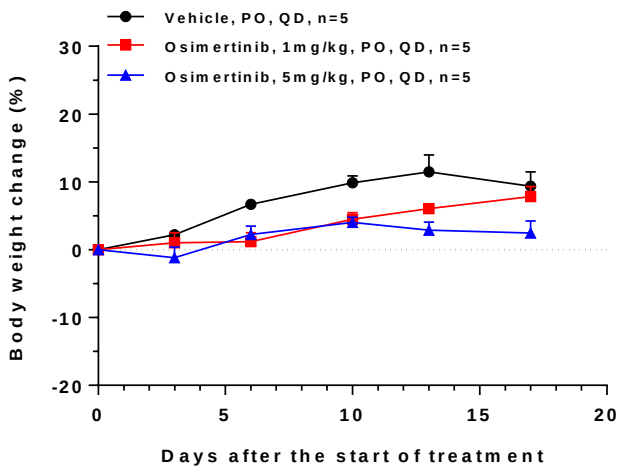


Figure: PC-9 DTC-luc cells mixed with Matrigel were micro-injected into brain of mice. Treatments were started at day 4 after inoculation. (A) Bioluminescent imaging of mice over 27 days (n=10). (B) Intracranial tumor growth curve as measured by average photon intensity in the experiments. (C) The average body weight curve of mice was used in the experiment. (D) Survival curve till day 47.

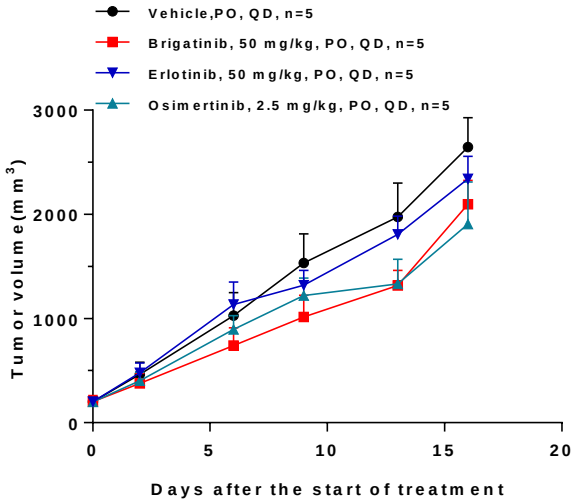
EGFR TKIs in NCI-H1975 vs. genetically engineered NCI-H1975 subcutaneous model

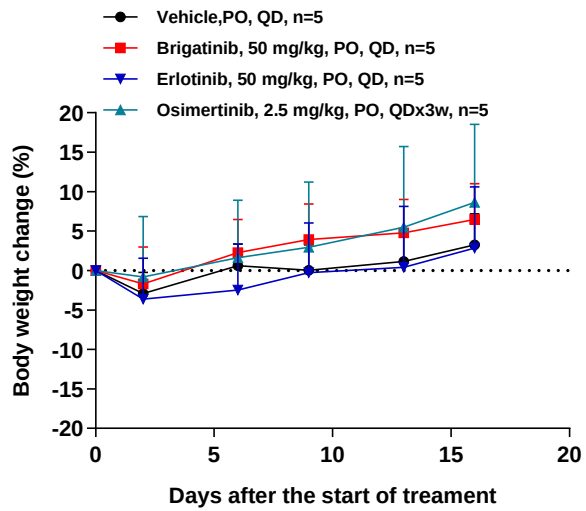
NCI-H1975



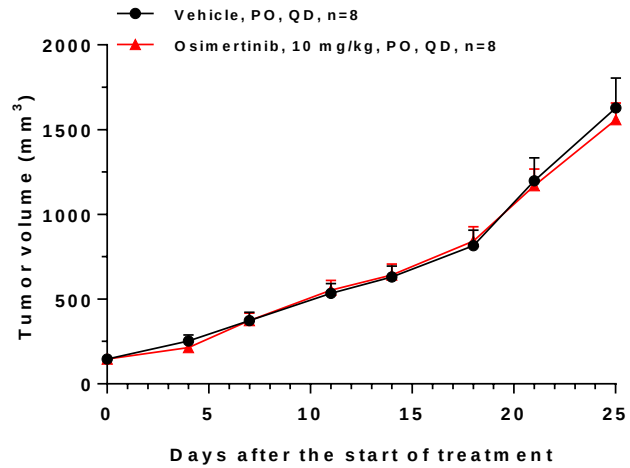


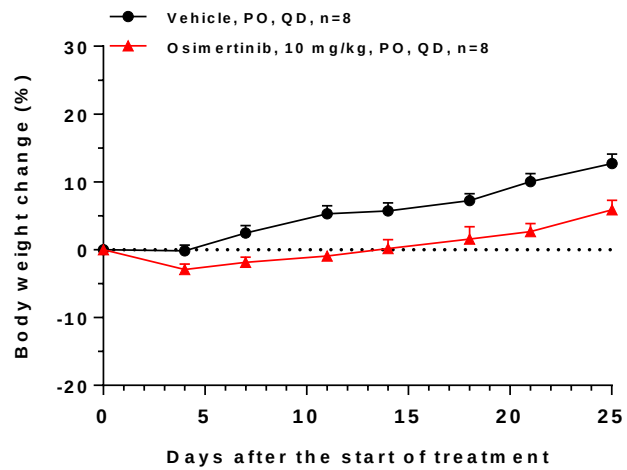
NCI-H1975 EGFR-Del19/T790M/C797S





NCI-H1975 EGFR-L858R/T790M/C797S





Genetically engineered Osimertinib-resistant Ba/F3 cell lines

IC50 of EGFR TKIs in genetically engineered Ba/F3 cell lines

Cell line	Reference compound IC50 (μM)						
	Erlotinib	Gefitinib	Afatinib	Dacomitinib	Brigatinib	Osimertinib	BI-4020
Ba/F3 EGFR-C797S	0.011	/	0.0043	/	/	1.8040	0.0686
Ba/F3 EGFR L858R/C797S	0.013±0.004	0.011±0.004	0.011±0.002	0.008±0.003	0.437±0.048	>1	0.0073
Ba/F3 EGFR L858R/T790M/C797S	>10	11.480±2.464	2.214±0.827	2.066±0.719	1.044±0.008	1.566±0.276	0.0012
Ba/F3 EGFR exon 19 deletion /C797S	0.004±0.0001	0.003±0.001	0.003±0.0001	<0.0030, 0.0019	0.169±0.090	1.147±0.116	0.0018
Ba/F3 EGFR exon 19 deletion /T790M/C797S	>10	4.17±0.30	1.39±0.014	1.45±0.17	0.20±0.13	1.75±0.015	0.0011

Genetically engineered Osimertinib-resistant Ba/F3 models

In vivo data summary of EGFR TKIs efficacy in genetically engineered Ba/F3 models

Cell type	Model ID	Drugs tested	Dosage (mg/kg)	TGI (%)	Model Genetics
Murine Pro-B	Ba/F3 EGFR C	Erlotinib Afatinib Osimertinib Brigatinib	50 15 5 75	111 92 -23 75	EGFR C797S
	Ba/F3 EGFR LC	Erlotinib Afatinib Osimertinib Brigatinib	50 15 5 75	103 35 -7 96	EGFR L858R/C797S
	Ba/F3 EGFR LTC	Erlotinib Afatinib Osimertinib Brigatinib	50 15 5 75	0 -4 4 42	EGFR L858R/T790M/C797S
	Ba/F3 EGFR DC	Erlotinib Osimertinib	50 5	115 18	EGFR exon 19 deletion /C797S
	Ba/F3 EGFR DTC	Erlotinib Afatinib Osimertinib Brigatinib	50 15 5 75	8 20 -7 91	EGFR exon 19 deletion /T790M/C797S

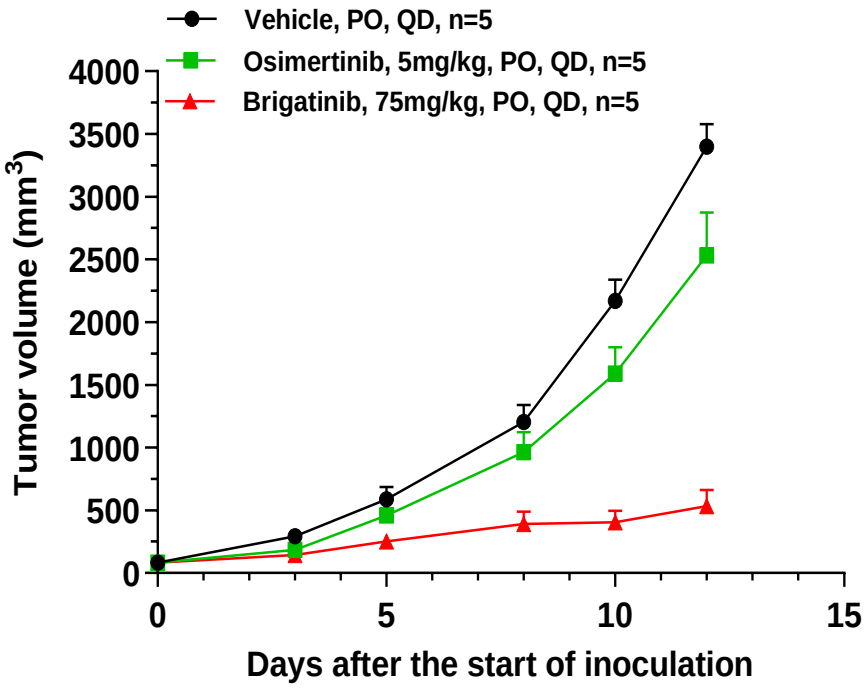
Genetically engineered Osimertinib-resistant Ba/F3 models

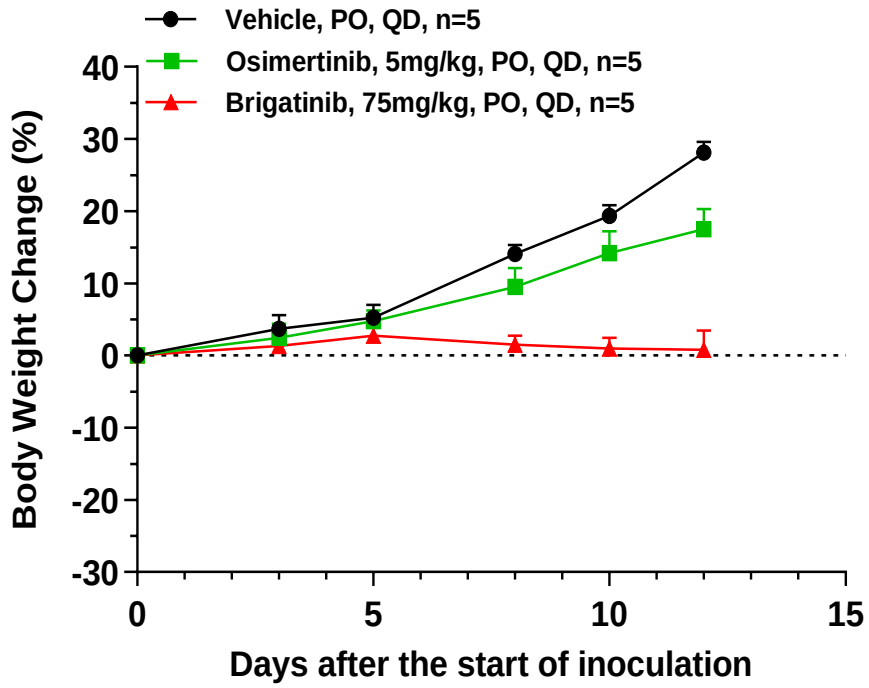
In vivo data summary of EGFR TKIs efficacy in genetically engineered Ba/F3 models

Cell type	Model ID	Drugs tested	Dosage (mg/kg)	TGI (%)	Model Genetics
Murine Pro-B	Ba/F3 EGFR LTL	Osimertinib Brigatinib	5 75	26 87	EGFR L858R/T790M/L792H
	Ba/F3 EGFR LTG	Osimertinib Brigatinib	5 75	-3 69	EGFR L858R/T790M/G796R
	Ba/F3 EGFR exon20ins ASV	Erlotinib Afatinib Osimertinib Poziotinib	50 15 5/25 5/1/0.5	13 11 11 47	EGFR exon20ins ASV
	Ba/F3 EGFR exon20ins SVD	Erlotinib Afatinib Osimertinib Poziotinib	50 15 5/25 5/1/0.5	4 -4 12 61	EGFR exon20ins SVD
	Ba/F3 EGFR exon20ins NPH	Erlotinib Afatinib Osimertinib Poziotinib	50 15 5 0.5	13 5 12 40	EGFR exon20ins NPH

Showcase: EGFR TKIs in Ba/F3 EGFR LTL model

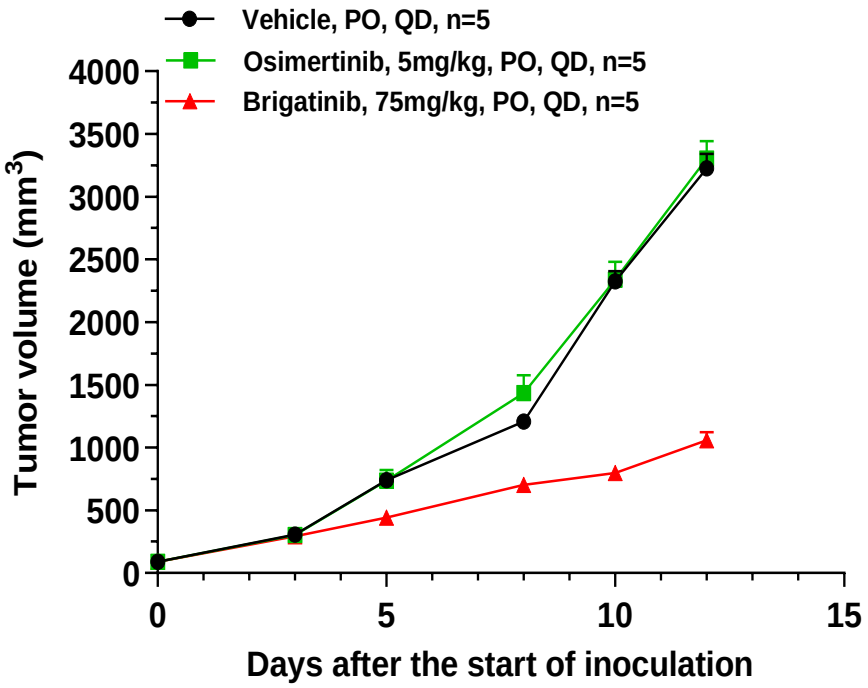
Cell type	Model ID	Drugs tested	Dosage (mg/kg)	TGI (%)	Model Genetics
Murine Pro-B	Ba/F3 EGFR LTL	Osimertinib Brigatinib	5 75	26 87	EGFR L858R/T790M/L792H

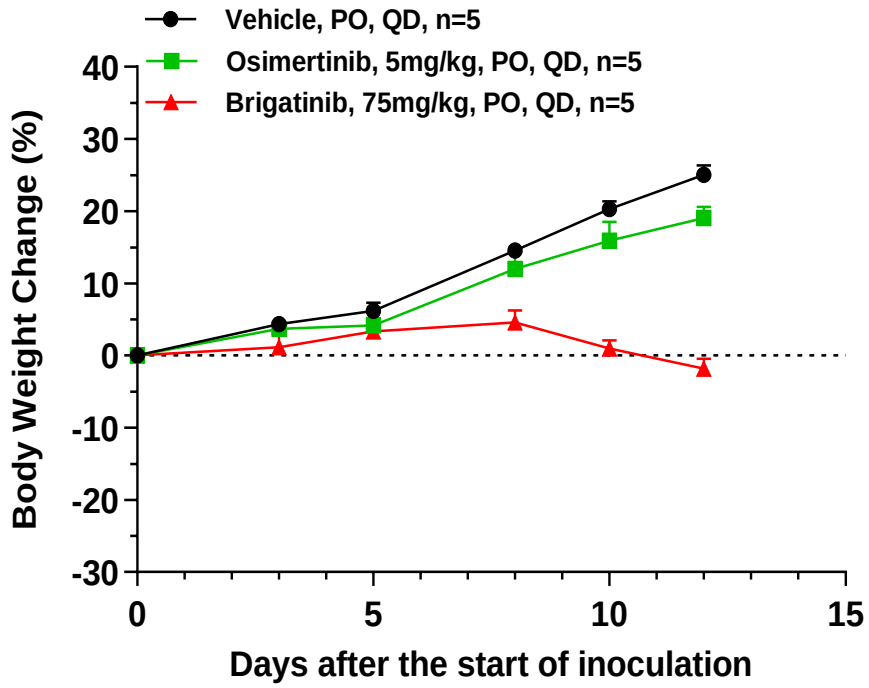




Showcase: EGFR TKIs in Ba/F3 EGFR LTG model

Cell type	Model ID	Drugs tested	Dosage (mg/kg)	TGI (%)	Model Genetics
Murine Pro-B	Ba/F3 EGFR LTG	Osimertinib Brigatinib	5 75	-3 69	EGFR L858R/T790M/G796R





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