CDK4/6 inhibitor resistant models

WuXi AppTec, WuXi Biology, Oncology & Immunology Unit

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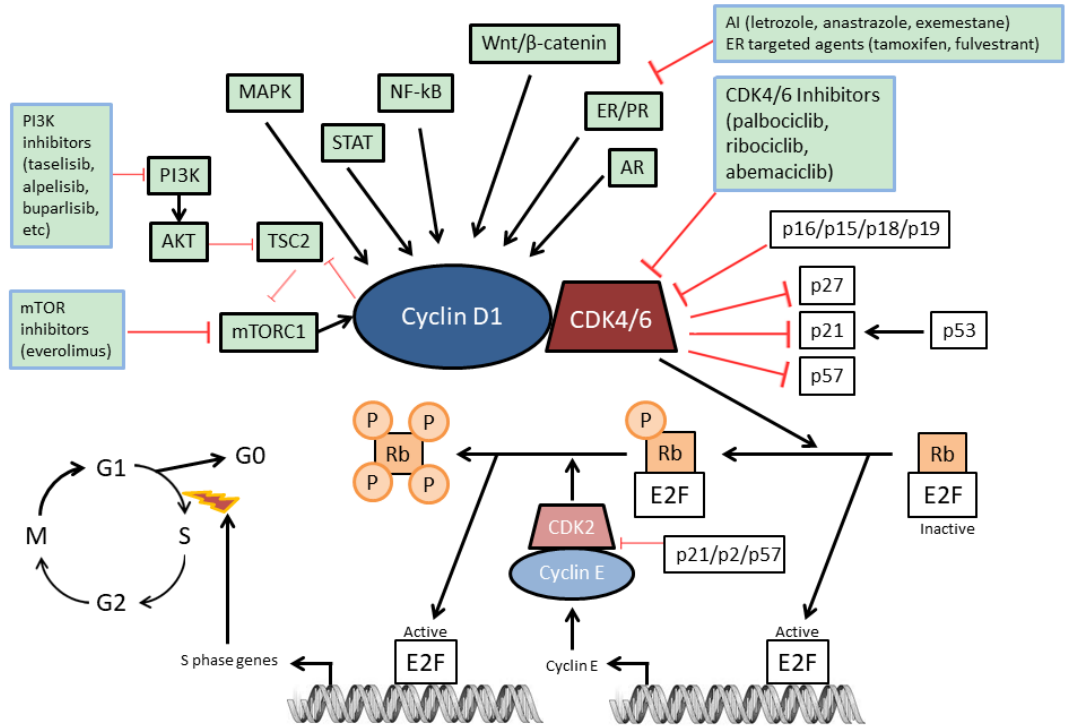
Outline

- **Background**
  - Mechanism of action of CDK4/6 inhibitors
  - Intrinsic and acquired resistance to CDK4/6 inhibitors
  - Overcoming resistance to CDK4/6 inhibitor through combinatorial therapies
- **CDK4/6 inhibitors-induced resistant cell lines/models**
  - *in vitro* induced Palbociclib resistant MCF-7 cell line
  - *in vivo* induced Palbociclib resistant MCF-7 model
  - *in vivo* induced Palbociclib resistant BR-05-0380 model
  - *in vivo* induced Abemaciclib resistant MCF-7 model
  - *in vivo* induced Ribociclib resistant MCF-7 model

Mechanism of action of CDK4/6 inhibitors



Cells 2019, 8, 321; doi:10.3390/cells8040321

- CDK4/6 activity links the cell cycle to several different extracellular signalling pathways.
- CDK4/6 inhibitors "switch-off" the kinase activity, triggering the dephosphorylation of Rb, blocking the cell cycle at the G1-to-S transition phase. Consequently, they lead to an arrest of the cell cycle and prevent the further proliferation of cancer cells.
- In recent years, three CDK4/6 inhibitors (Palbociclib, Ribociclib, and Abemaciclib) have been approved in combination with hormonal therapy as a front-line treatment for advanced or metastatic HR+, HER2- breast cancer.

Table 1. CDK4/6 Inhibitors in Active Clinical Trials

Drug Name	Developer	Targets (IC_{50})	Stage and Indications[a]
Palbociclib, PD-0332991, Ibrance	Pfizer	CDK4 (11 nM); CDK6 (16 nM)	approved for $ER^+/HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumors
Ribociclib, LEE-011, Kisqali	Novartis International	CDK4 (10 nM); CDK6 (39 nM)	approved for $ER^+/HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumors
Abemaciclib, LY2835219, Verzenios	Eli Lilly	CDK4 (2 nM); CDK6 (10 nM); CDK9 (57 nM)	approved for $ER^+/HER2^-$ metastatic breast cancer; clinical trials for multiple solid tumors
SHR6390	Jiangsu Hengrui Medicine	CDK4 (12|nM); CDK6 (10 nM)	phase III for $ER^+/HER2^-$ metastatic breast cancer; phase I/II for other tumor types
Trilaciclib, G1T28	G1 Therapeutics	CDK4 (1 nM); CDK6 (4 nM); CDK9 (50 nM)	phase II for SCLC and TNBC to reduce chemo-induced myelosuppression
Lerociclib, G1T38	G1 Therapeutics	CDK4 (1 nM); CDK6 (2 nM); CDK9 (28 nM)	phase I/II for $ER^+/HER2^-$ metastatic breast cancer and $EGFR^+$ metastatic NSCLC
PF-06873600	Pfizer	CDK2 (0.09 nM Ki); CDK4 (0.13 nM Ki); CDK6 (0.16 nM Ki)	phase I/IIa for $ER^+/HER2^-$ metastatic breast cancer, ovarian cancer and TNBC
FCN-437c	Fochon Pharmaceuticals	CDK4; CDK6	phase I for advanced solid tumors
BPI-16350	Betta Pharmaceuticals	CDK4; CDK6	phase I for advanced solid tumors
XZP-3287, Birociclib	Jilin Sihuan Pharmaceutical/ XuanZhu Pharma	CDK4; CDK6	phase I for advanced solid tumors
HS-10342	Jiangsu Hansoh Pharmaceutical Group	CDK4; CDK6	phase I for advanced solid tumors
CS3002	CStone Pharmaceuticals	CDK4; CDK6	phase I for advanced solid tumors

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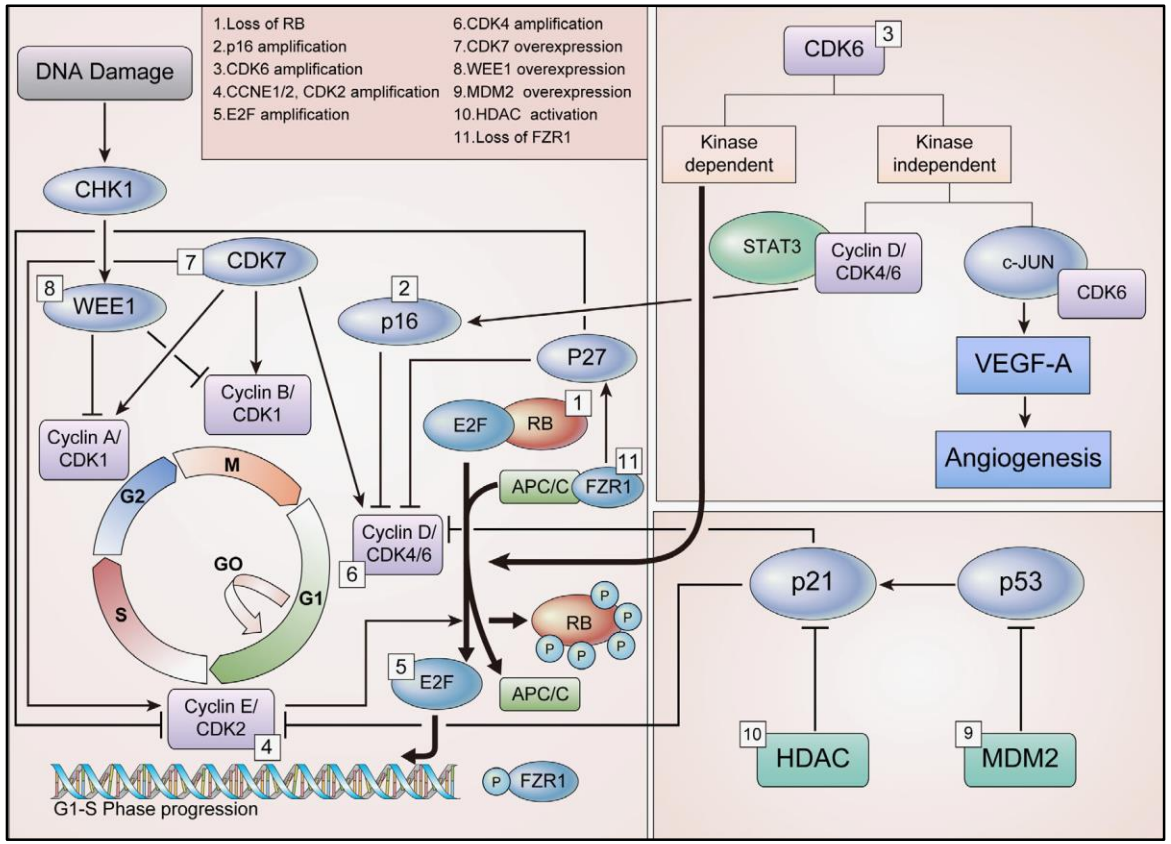
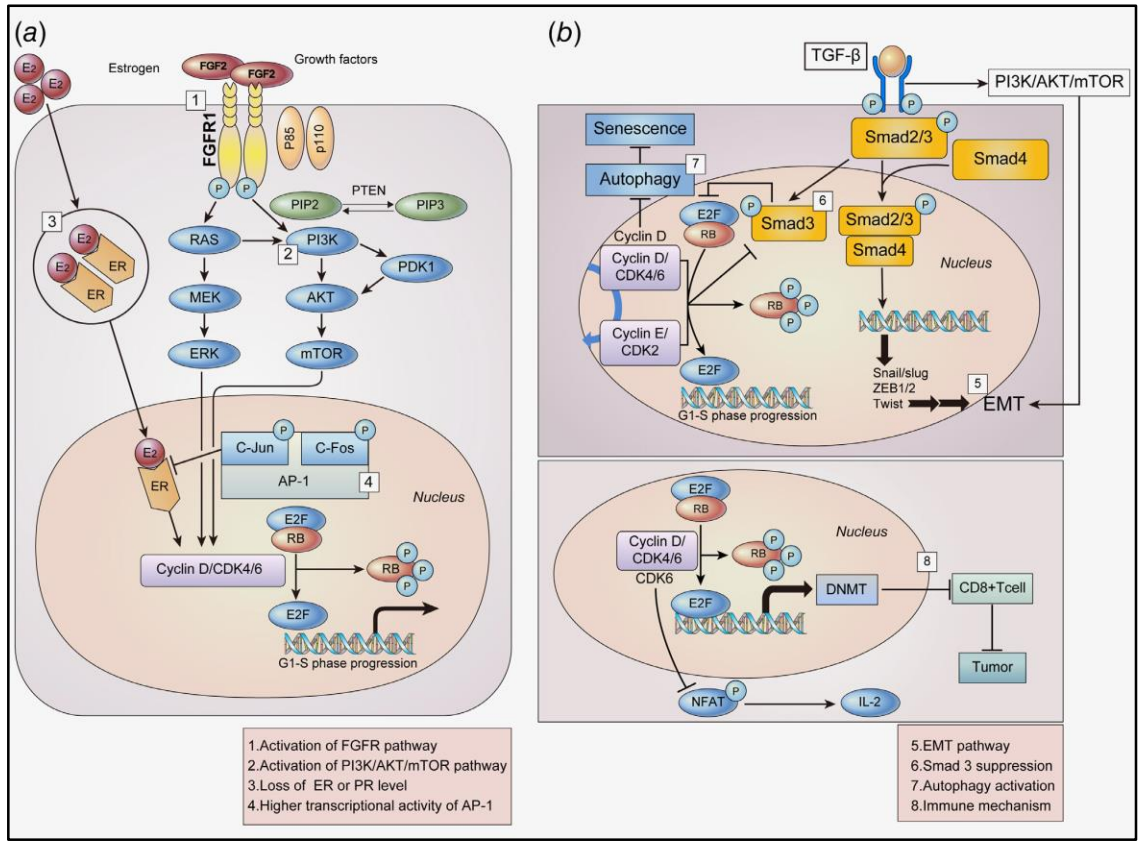
Intrinsic and acquired resistance to CDK4/6 inhibitors

- Despite improved disease control that CDK4/6 inhibitors offer to patients with HR+ breast cancer, not all patients respond to these drugs and most patients whose tumors respond to CDK4/6 inhibitors eventually develop acquired resistance.
- Many preclinical studies have suggested that activation of other mitogenic signaling pathways, amplification of specific genes, alteration of the tumor environment and modification of tumor immunity result in the emergence of drug resistance.

Cell cycle-specific mechanisms



Cell cycle-non specific mechanisms



Int. J. Cancer: 145, 1179–1188 (2019), https://doi.org/10.1002/ijc.32020

Intrinsic and acquired resistance to CDK4/6 inhibitors

Table 2. a Overview of the dysregulated expression and functions of the major cell cycle proteins contribute to the intrinsic resistance to CDK4/6 inhibitors. b Overview of other mitogenic signaling activation to CDK4/6 inhibition and combination strategies.

Gene	Expression	Cancer type	Functions	References
RB1	Rb1 loss	Breast cancer	Resistance to palbociclib	[9]
RB1	Rb1 loss	Ovarian cancer	Resistance to palbociclib	[15]
RB1	Rb1degradation	HPV-positive cervical cancer and head and neck cancer	Resistance to CDK4/6 inhibitors	[16, 17]
CDK4	CDK4-amplification	Liposarcoma and Neuroblastoma	Sensitive to CDK4/6 inhibitors	[26, 27]
CDK4	Activation T172 phosphorylation	Breast cancer	Enhance the sensitivity to palbociclib	[28]
CDK4	CDK4-amplification	Rhabdomyosarcoma	Reduce the sensitivity to ribociclib	[25]
CCND1	Cyclin D1-overexpression	Endometrial cancer	Enhance the sensitivity to abemaciclib	[32]
SMARCA4	SMARCA4 loss	Small-cell carcinoma of the ovary, hypercalcemic type (SCCOHT) cells	Cause cyclin D1 deficiency and enhance the sensitivity to CDK4/6 inhibitors	[33]
]SMARCA2/4	SMARCA2/4 loss	Non-small cancer cell	Cause cyclin D1 deficiency and increase the sensitivity to CDK4/6 inhibitors	[34]
CDKN2A	$p16^{INK4A}$ loss	Melanoma	Good for palbociclib sensitivity	[40]
CDKN2A	$p16^{INK4A}$ loss	Pancreatic ductal adenocarcinoma	Resistance to CDK4/6 inhibitors	[41]
CDKN2A	$p16^{INK4A}$ deficiency	Glioblastoma	More susceptible to palbociclib	[42]
CCNE1	Cyclin E1 overexpression	Gastric cancer	Resistance to palbociclib	[37]
CCNE1	High cyclin E1 expression	HR^+ $HER2^-$ breast cancer	Resistance to palbociclib	[38]
E2F4	E2F4 activation	ER^+ breast cancer	Resistance to palbociclib	[21]
E2F	E2F activation	Mutant BRAF and NRAS melanoma	Resistance to palbociclib	[22]
TP53	p53 loss	Breast cancer	Resistance to abemaciclib	[45]
CDK2	High levels of CDK2 activity	Luminal androgen receptor breast cancer	Resistance to CDK4/6 inhibitors	[31]

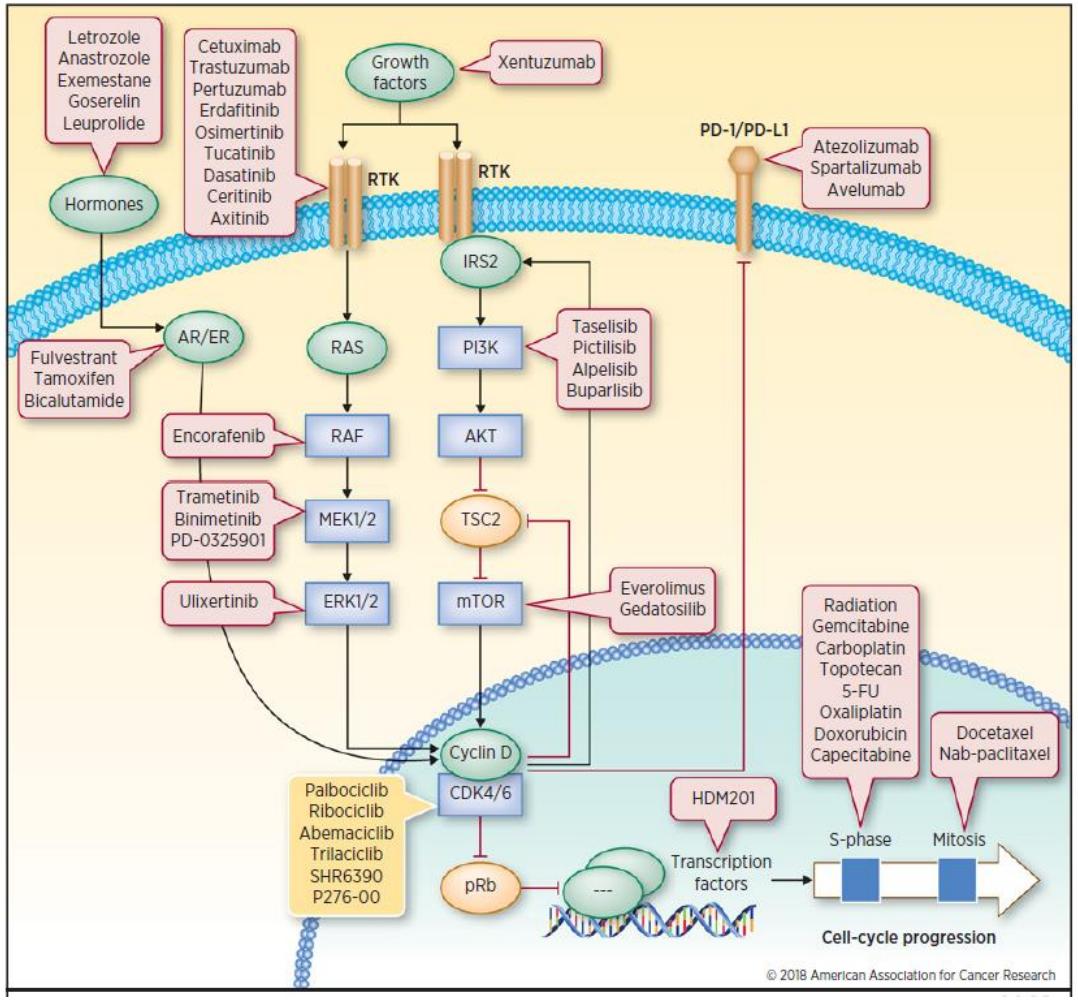
Other mitogenic signaling	Cancer type	Functions	Combination with other inhibitors	References
FGFR1 amplification	ER^+ breast cancer	Resistance to CDK4/6 inhibitors	Lucitanib (FGFR inhibitors)	[54]
FGFR1 signaling pathway activation	KRAS-mutant non-small lung cancer	Resistance to CDK4/6 inhibitors	MEK inhibitors	[55]
MAPK signaling pathway activation	prostate cancer	Resistance to CDK4/6 inhibitors	MEK inhibitors	[56]
PIK3CA E545K mutation	NRAS-mutant melanoma	Resistance to CDK4/6 inhibitors	mTOR inhibitors and S6K1 inhibitors	[57]
mTOR activation	Pancreatic cancer	Resistance to CDK4/6 inhibitors	mTOR inhibitors	[60]
PDK1 activation	ER^+ breast cancer	Resistance to ribociclib	PDK1 inhibitors or CDK2 inhibitors	[61]
NF-κB-HGF pathway	Glioblastoma	Resistance to ribociclib	Altiratinib (c-Met/Trk inhibitor)	[62]
Androgen Receptor activation	Breast cancer	Resistance to palbociclib	Androgen receptor inhibitors	[63]
FAT1 loss (Hippo pathway suppression)	ER^+ breast cancer	CDK6 overexpression		[64, 65]
MYC-driven (mTOR activation)	Colorectal carcinoma	Resistance to CDK4/6 inhibitors		[66]
Fbxo4 loss (Gln-addition)	Esophageal squamous cell carcinoma	Resistance to CDK4/6 inhibitors	Glutaminase1plus metformin or phenformin	[67]
IL6/STAT3 activation	ER^+ breast cancer	Resistance to CDK4/6 inhibitors	STAT3 inhibitor plus PARP inhibitor	[68]

Acta Pharmacologica Sinica (2020) 0:1–8; https://doi.org/10.1038/s41401-020-0416-4

Overcoming resistance to CDK4/6 inhibitor through combinatorial therapies

Combination strategies testing CDK4/6 inhibitors in clinical trials

- Combining with targeted therapies
- Combining with cytotoxic chemotherapeutics
- Combining with immunotherapies



Clin Cancer Res. 2019 Jan 1;25(1):6-8. doi: 10.1158/1078-0432.CCR-18-2177

Acta Pharmacologica Sinica (2020) 0:1–8; https://doi.org/10.1038/s41401-020-0416-4

Table 1. CDK4/6 inhibitors in combination with endocrine therapy in advanced HR^+ $HER2^-$ breast cancer in various clinical settings.

Drug	Combination	Line	Menopausal status	Clinical settings
Palbociclib	Letrozole	1st	Post	PALOMA-2
	Fulvestrant	2nd	Pre and Post	PALOMA-3
Ribociclib	Letrozole	1st	Pre	MONALEESA-7
	Letrozole	1st	Post	MONALEESA-2
	Fulvestrant	1st and 2nd	Post	MONALEESA-3
Abemaciclib	Letrozole	1st	Post	MONARCH 3
	Fulvestrant	2nd	Pre and Post	MONARCH 2
	–	2+	Pre	MONARCH 1

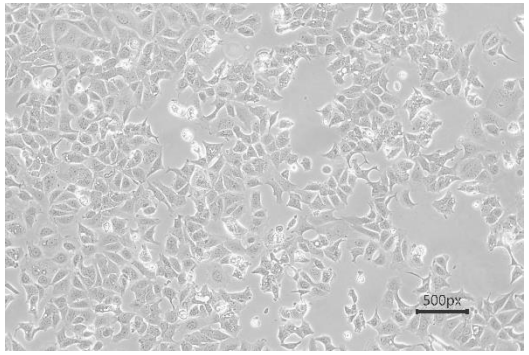
Table 3. Clinical trials studying on CDK4/6 inhibitors in combination with other therapies in other cancer treatment.

Disease	Combination	Phase	Trial
Palbociclib			
KRAS mutant non-small-cell lung cancer, solid tumors	PD-0325901(MEK inhibitor)	I/II	NCT02022982
Squamous cell carcinoma of the head and neck (SCCHN)	Cetuximab	II	NCT02499120
Squamous cell carcinoma of the head and neck	Carboplatin	II	NCT03194373
Recurrent mantle cell lymphoma	Ibrutinib (BTK Inhibitor)	I	NCT02159755
Advanced solid tumors, breast cancer	Taselisib or pictilisib (PI3K inhibitor)	I	NCT02389842
Endometrial cancer	Letrozole	II	NCT02730429
Ovarian epithelial carcinoma		II	NCT01536743
Advanced solid tumor malignancies	5-FU and oxaliplatin	I	NCT01522989
Palbociclib			
Advanced hepatocellular carcinoma,		II	NCT01356628
HCC, liver cancer			
Non-small-cell lung cancer	PF-06747775 and avelumab	II	NCT02349633
Ribociclib			
Glioblastoma glioma		I	NCT02345824
Acute lymphoblastic leukemia ALL	Dexamethasone and everolimus	I	NCT03740334
High grade glioma,		I/II	NCT02607124
Diffuse intrinsic pontine glioma,			
Bithalamic high grade glioma			
Gastrointestinal cancer		II	NCT02420691
Metastatic pancreatic	Everolimus	II	NCT02985125
Ribociclib			
Adenocarcinoma			
Liposarcoma	HDM201	I/II	NCT02343172
Solid tumors harboring	LGX818 and MEK162	I/II	NCT01543698
Abemaciclib			
Glioblastoma		II	NCT02981940
Non-small-cell lung cancer stage IV	Docetaxel	II	NCT02450539
Non-small-cell lung cancer	Erlotinib	III	NCT02152631
Non-small-cell lung cancer	Pembrolizumab and anastrozole	I	NCT02779751
Breast cancer, mantle cell lymphoma		II	NCT01739309

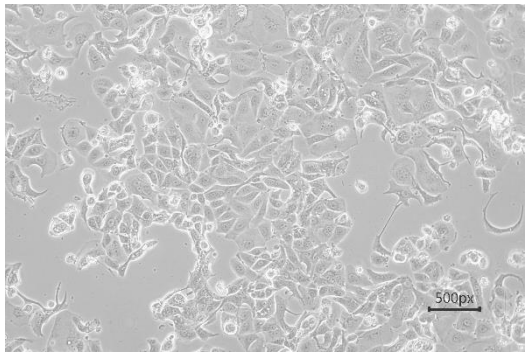
Palbociclib induced resistant MCF-7 cell line (MCF-7/Palbo-R)

in vitro & *in vivo* validation of MCF-7/Palbo-R

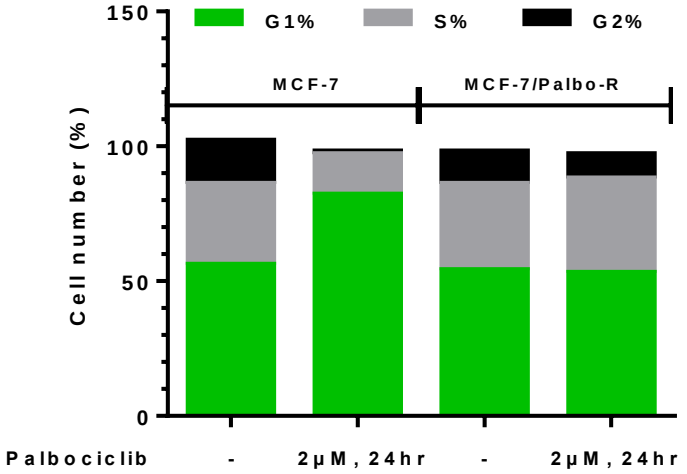
MCF-7 cell line

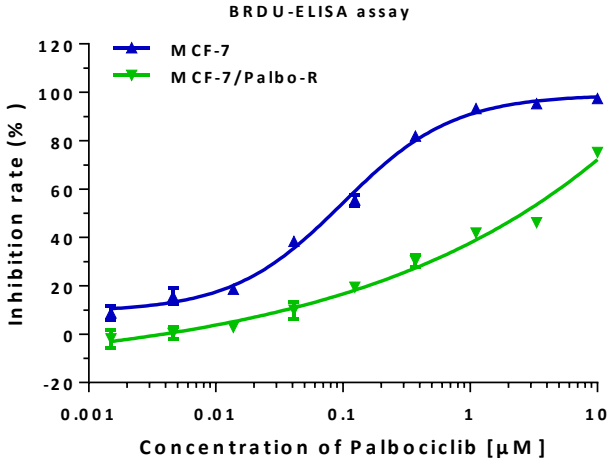


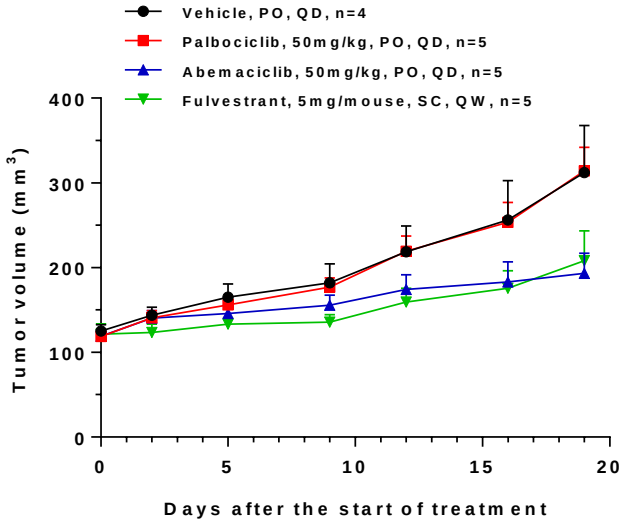
MCF-7/Palbo-R cell line



- MCF-7/Palbo-R cell line was established through chronic exposure to increased concentrations of Palbociclib (up to 4 μM) for ~7 months.







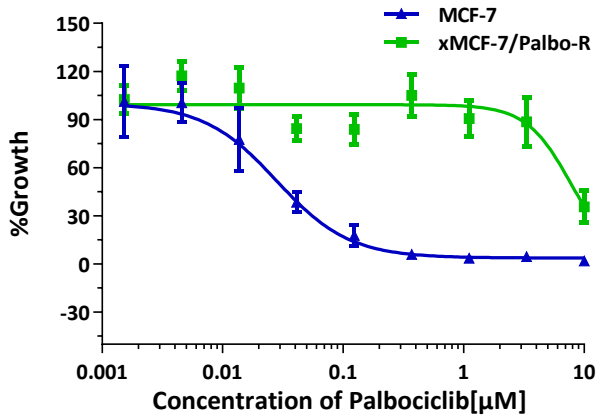
Compound	Cell line	AbsIC50 (μM)	RelC50 (μM)	Bottom (%)	Top (%)
Palbociclib	MCF-7	0.083	0.099	8.70	97.47
	MCF-7/Palbo-R	2.603	NA	-1.90	75.09

- The established MCF-7/Palbo-R model is still resistant to Palbociclib. MCF-7/Palbo-R tumors grow very slowly *in vivo*, establish tumor derived cell line **xMCF-7/Palbo-R**.

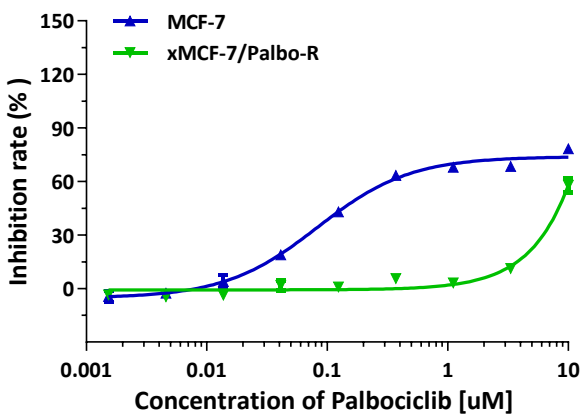
Palbociclib induced resistant MCF-7 cell line (xMCF-7/Palbo-R)

In vitro validation of xMCF-7/Palbo-R

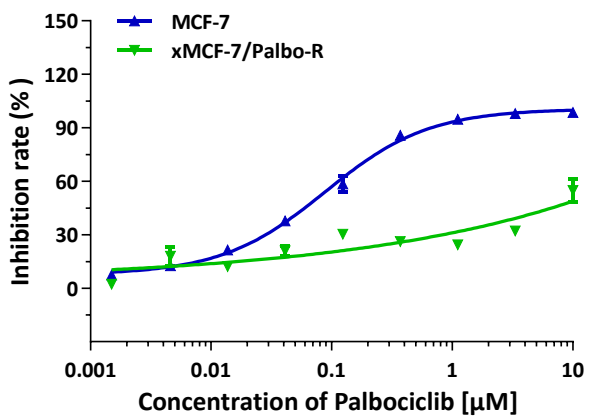
CyQuant assay



7 days CTG assay



BRDU-ELISA assay



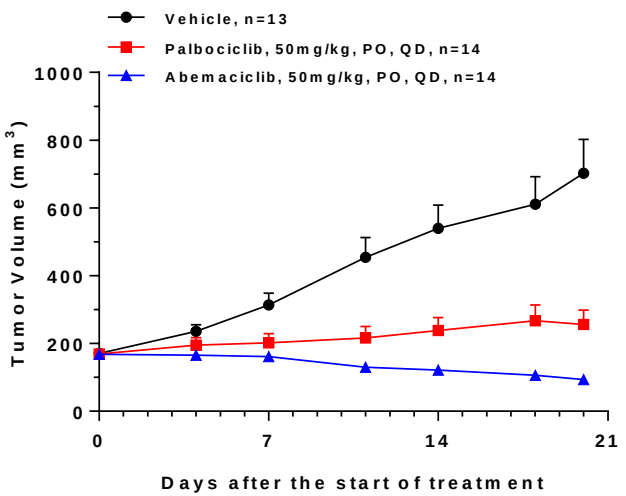
Cell line	ReIC50 (μM)		
	CyQuant	BRDU-ELISA	CTG
MCF-7	0.030	0.088	0.083
xMCF-7/Palbo-R	7.842	NA	NA

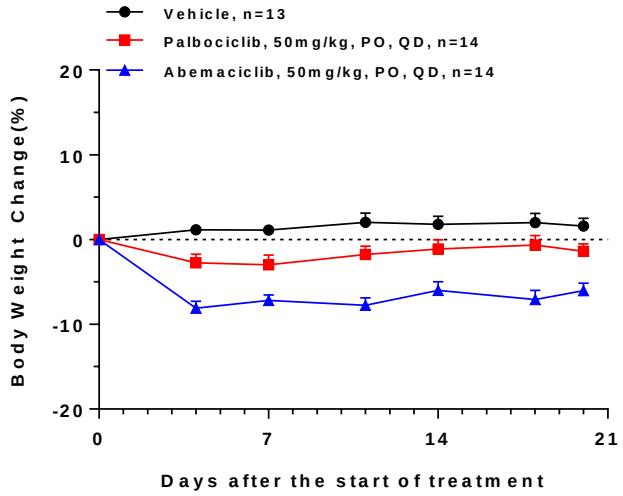
- xMCF-7/Palbo-R cell line was derived from MCF-7/Palbo-R tumor and still resistant to Palbociclib *in vitro.*

Palbociclib induced resistant MCF-7 cell line (xMCF-7/Palbo-R)

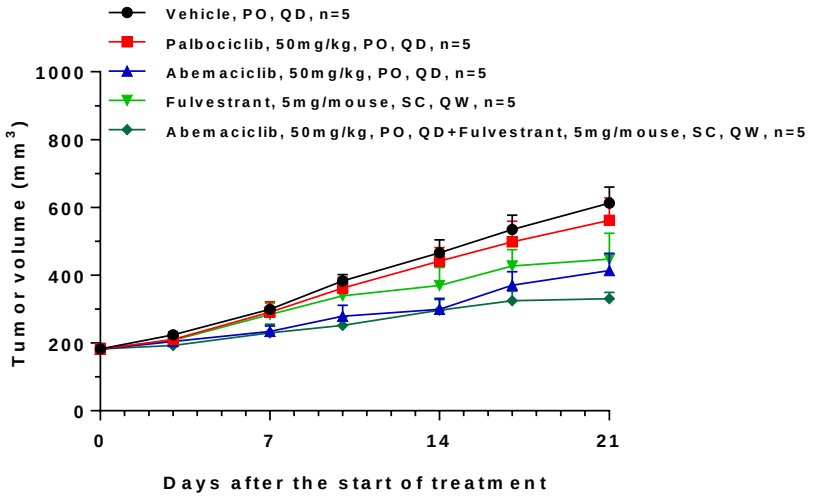
In vitro validation of xMCF-7/Palbo-R

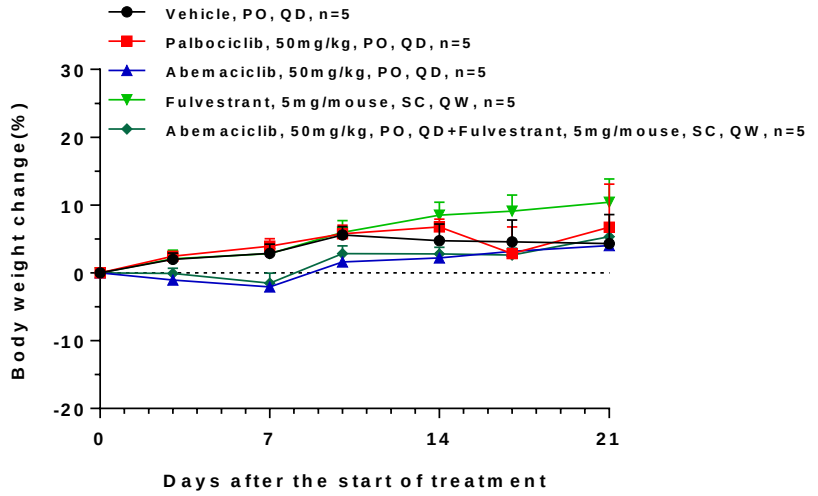
Parental MCF-7





xMCF-7/Palbo-R



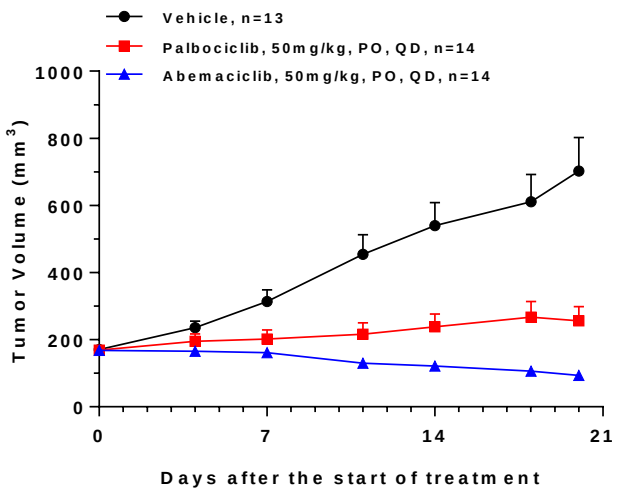


- Data shows that the established xMCF-7/Palbo-R model is still resistant to Palbociclib.

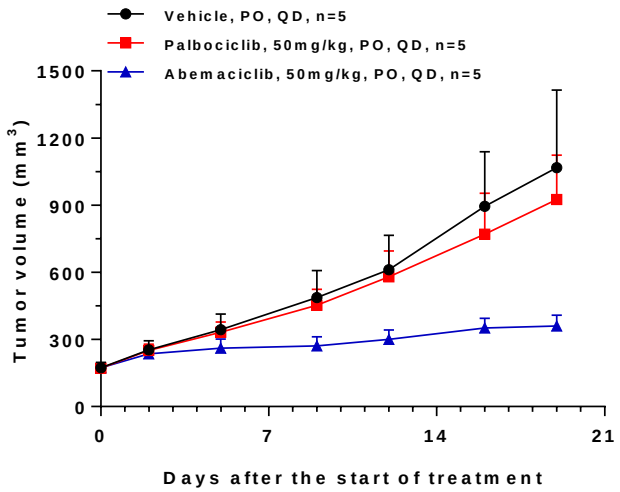
Palbociclib induced resistant MCF-7 model (Palbociclib-R-MCF-7)

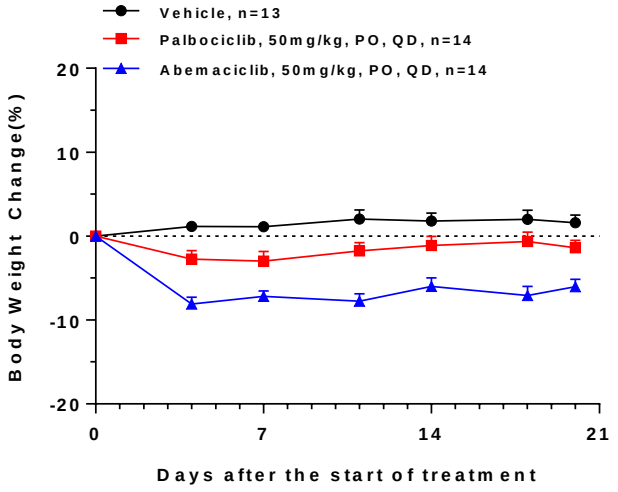
CDK4/6 inhibitors in Parental MCF-7 model vs. Palbociclib-R-MCF-7

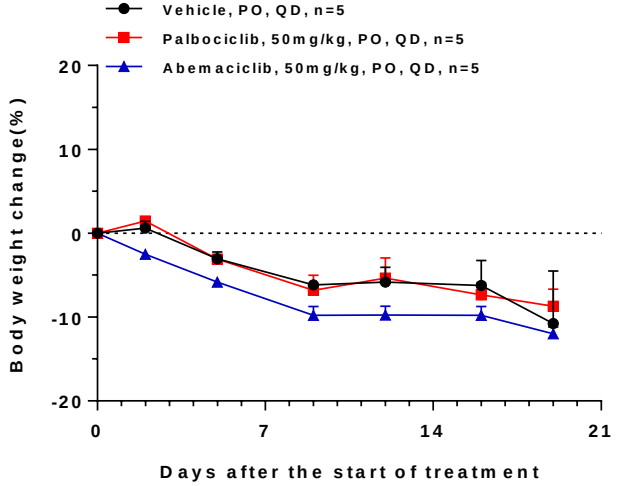
Parental MCF-7

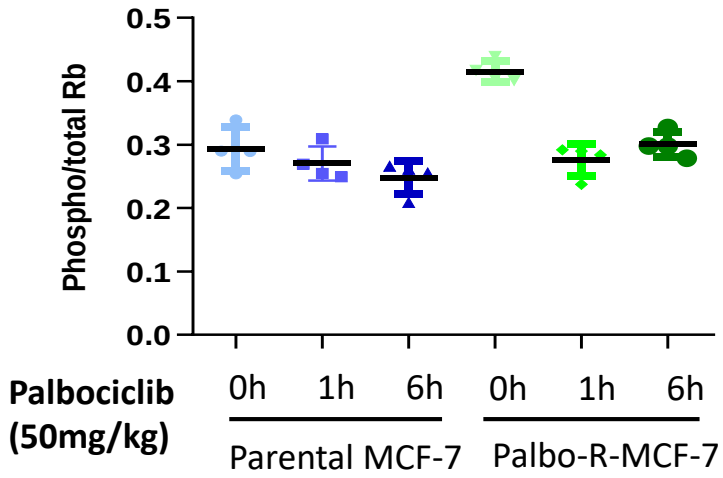


Palbociclib-R-MCF-7 (P5)







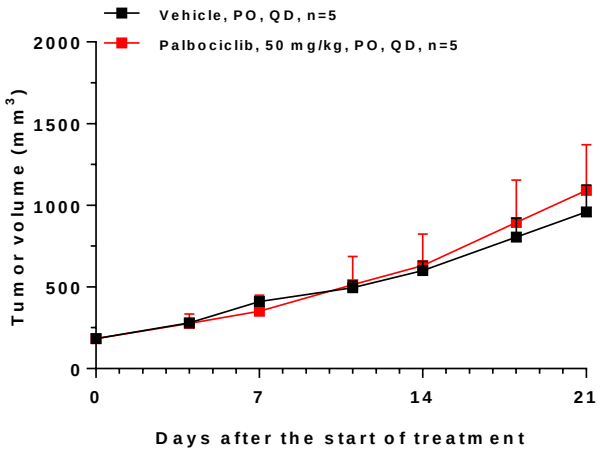


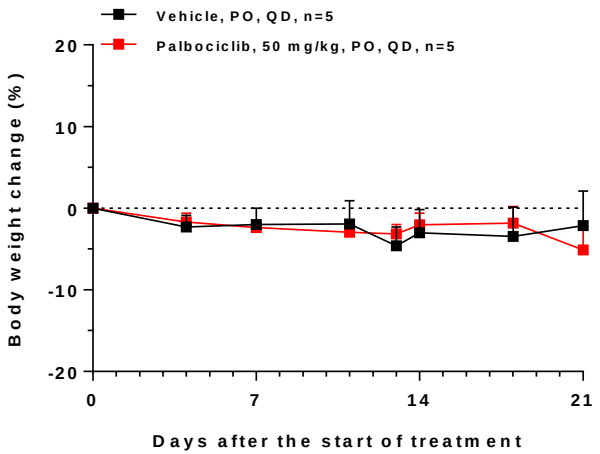
- Palbociclib-R-MCF-7 model was established by chronic treatment *in vivo*, Palbociclib treated tumors were passaged and dosed until a stable resistance phenotype occurred.
- HTRF assay shows p-Rb level of Palbo-R-MCF-7 tumors is higher than parental MCF-7 tumors'.

Palbociclib induced resistant MCF-7 model (Palbociclib-R-MCF-7)

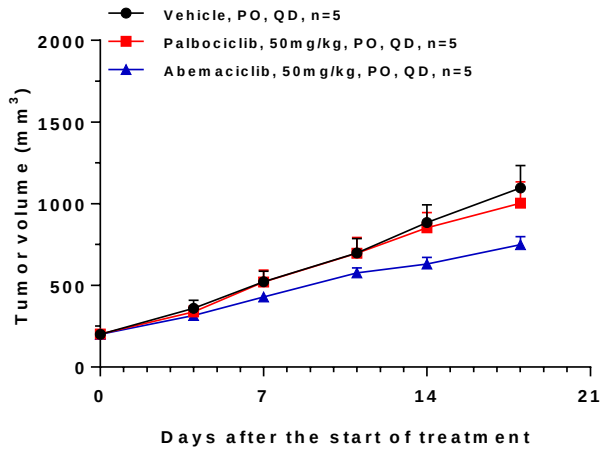
Tumor fragment revival validation test

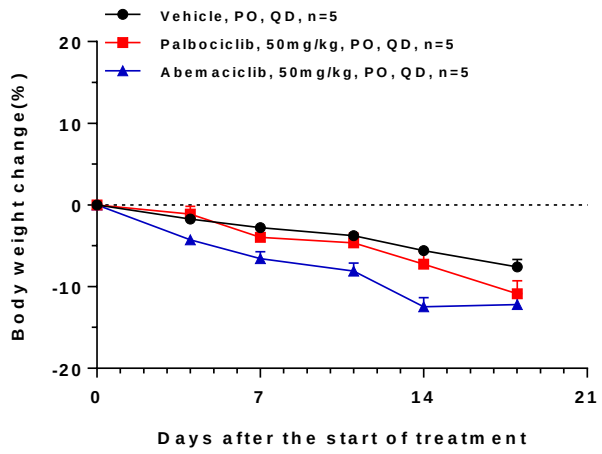
Palbociclib-R-MCF-7 (FP6)



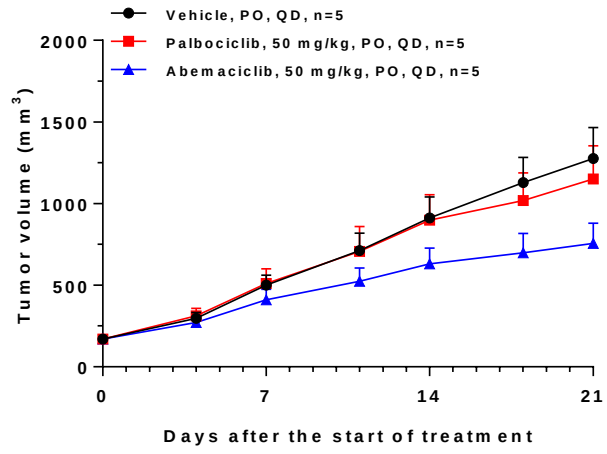


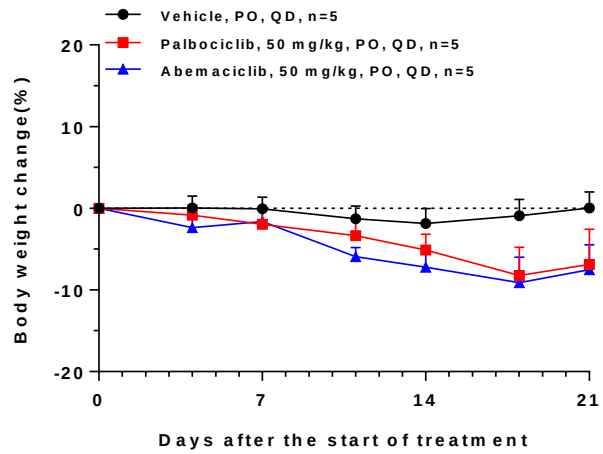
Palbociclib-R-MCF-7 (FP8)





Palbociclib-R-MCF-7 (FP12)



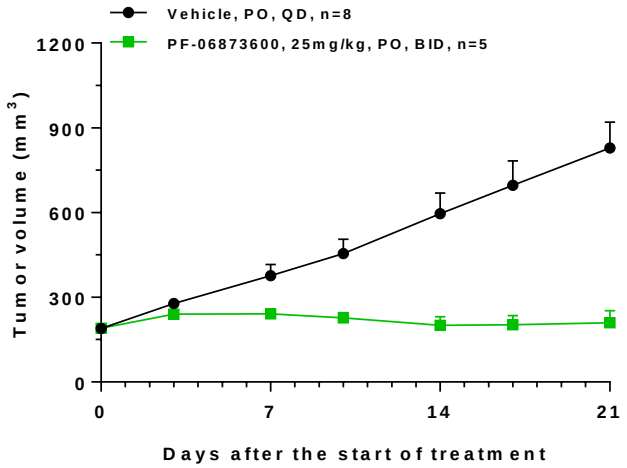


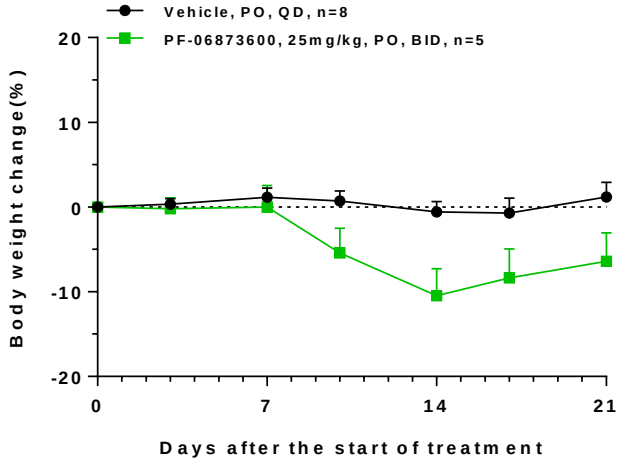
- P4 frozen tumor fragments were revived for validation.
- Data shows that the resistant phenotype of Palbociclib-R-MCF-7 model is stable.

Overcoming resistance to CDK4/6 inhibitor

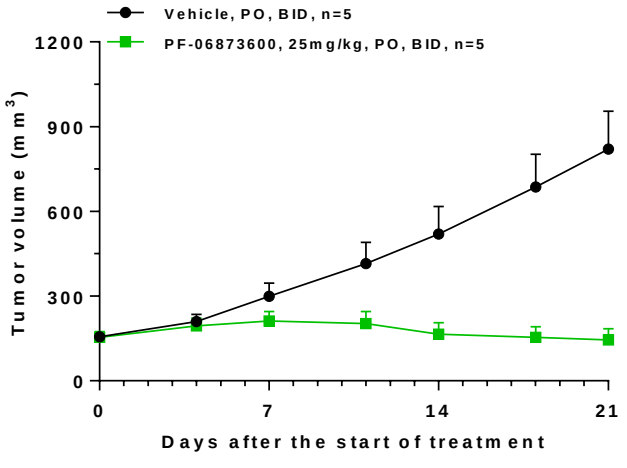
CDK2/4/6 inhibitor PF-06873600 in Palbociclib induced resistant MCF-7 models

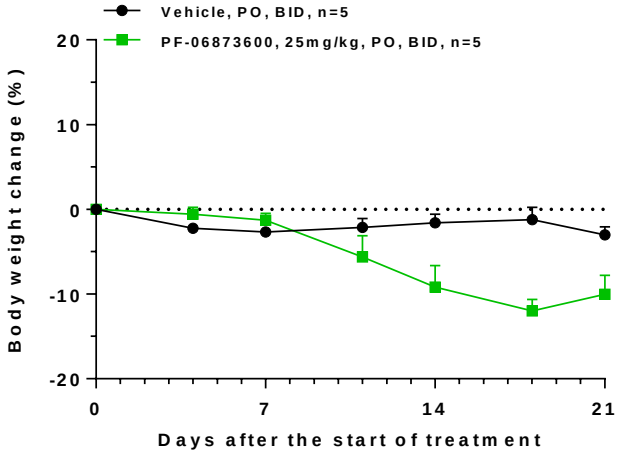
Parental MCF-7



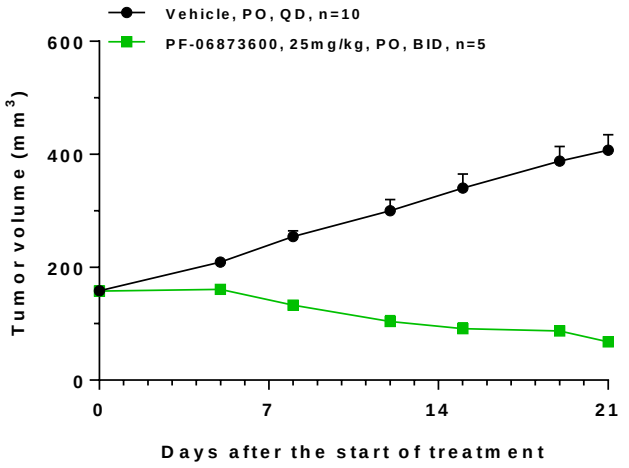


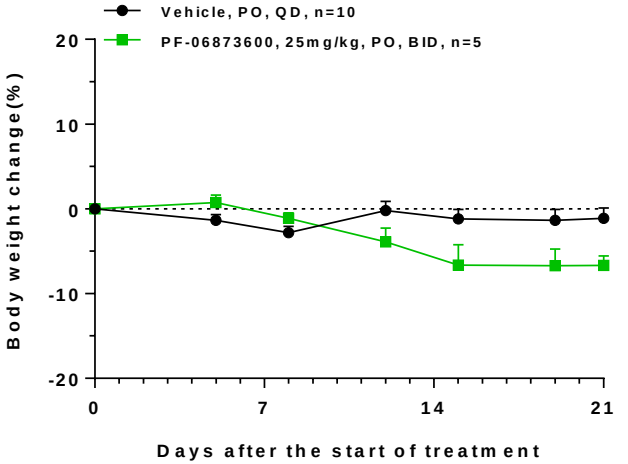
Palbociclib-R-MCF-7 (FP7)





xMCF-7/Palbo-R





Model summary

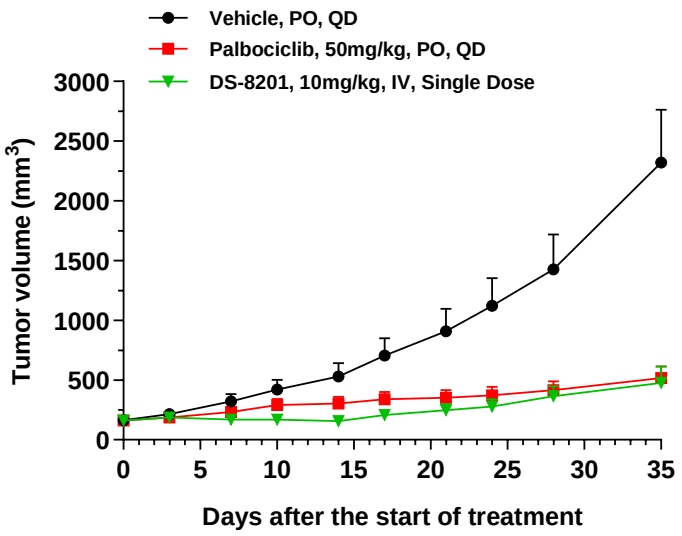
Induce method	Model ID	Cancer type	Inoculation	Drugs tested	Dosage	TGI (%)
Parental	Parental MCF-7	Breast cancer	Cell line	Palbociclib Abemaciclib PF-06873600	50 mg/kg, QD 50 mg/kg, QD 25 mg/kg, BID	83 114 97
***In vitro* induce**	MCF-7/Palbo-R	Breast cancer	Cell line	Palbociclib Abemaciclib Fulvestrant	50 mg/kg, QD 50 mg/kg, QD 5 mg/mouse, QW	-1 62 55
	xMCF-7/Palbo-R (derived from MCF-7/Palbo-R tumors)	Breast cancer	Cell line	Palbociclib Abemaciclib Fulvestrant PF-06873600	50 mg/kg, QD 50 mg/kg, QD 5 mg/mouse, QW 25 mg/kg, BID	12 46 38 136
***In vivo* induce**	Palbociclib-R-MCF-7 (P5)	Breast cancer	Tumor fragment	Palbociclib Abemaciclib	50 mg/kg, QD 50 mg/kg, QD	16, -3 79, 60
	Palbociclib-R-MCF-7 (FP6)	Breast cancer	Tumor fragment	Palbociclib	50 mg/kg, QD	0
	Palbociclib-R-MCF-7 (FP8)	Breast cancer	Tumor fragment	Palbociclib Abemaciclib	50 mg/kg, QD 50 mg/kg, QD	11 39
	Palbociclib-R-MCF-7 (FP12)	Breast cancer	Tumor fragment	Palbociclib Abemaciclib	50 mg/kg, QD 50 mg/kg, QD	11 47
	Palbociclib-R-MCF-7 (FP7)	Breast cancer	Tumor fragment	PF-06873600	25 mg/kg, BID	102

Palbociclib induced resistant BR-05-0380 model (Palbociclib-R-BR-05-0380)

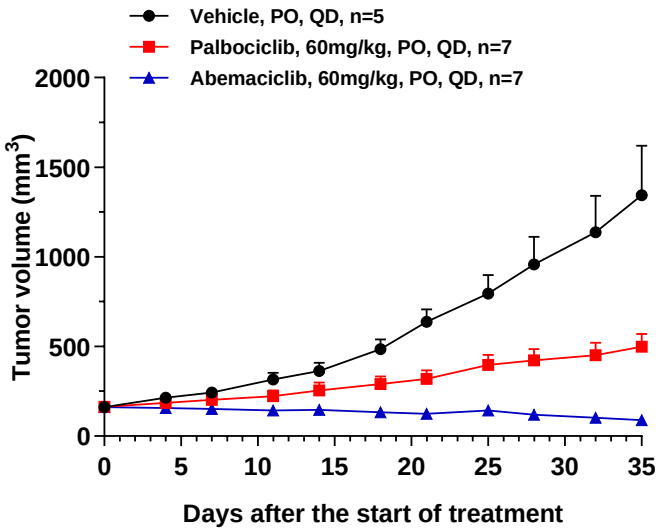
CDK4/6 inhibitors in Parental BR-05-0380 model vs. Palbociclib-R-BR-05-0380

Model ID	Cancer Type	Gender	Age	Tumor Grade	Tumor Stage	Pathological Diagnosis	Subtype
BR-05-0380	Breast cancer	Female	54	NA	NA	Infiltrating carcinoma	ER(90%+), PR(-), HER2(2+), Ki-67(30%+)

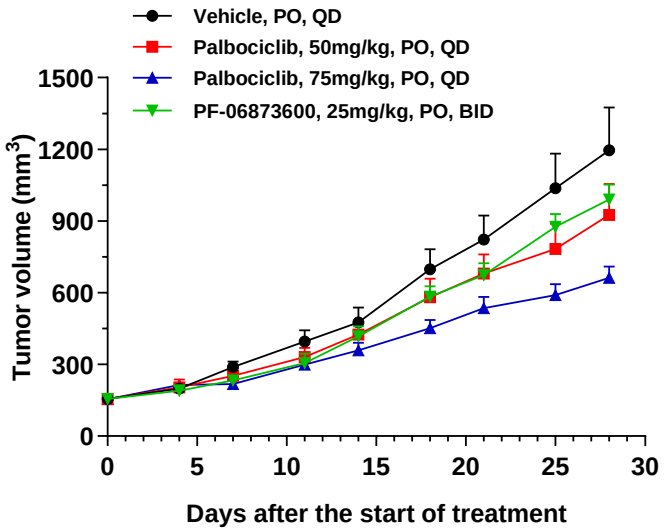
Parental BR-05-0380 (FP6)



Parental BR-05-0380 (FP7)



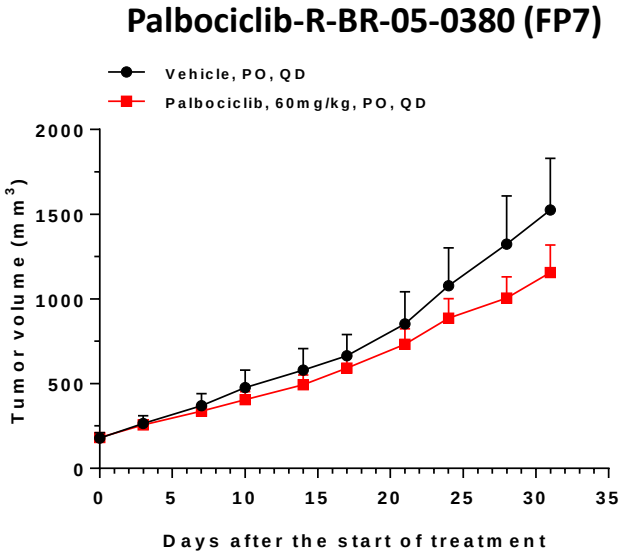
Palbociclib-R-BR-05-0380 (P6)

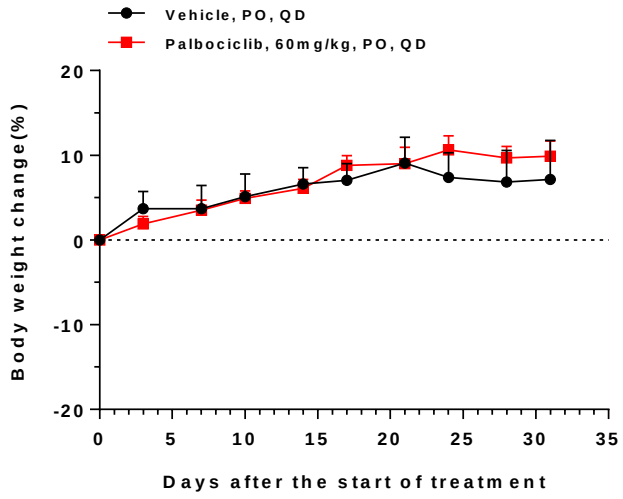


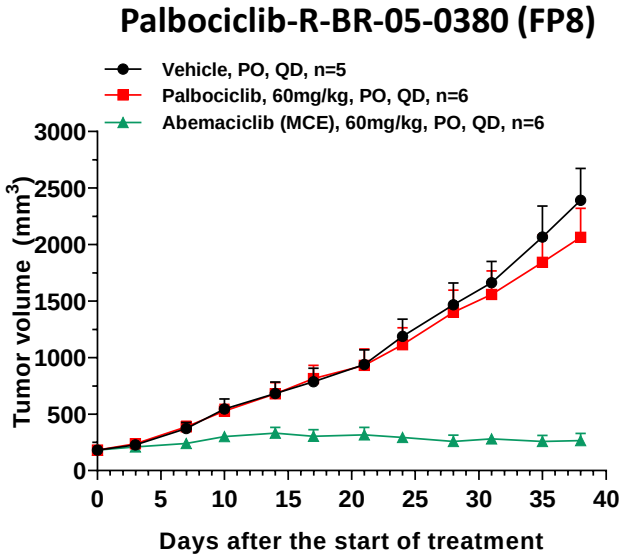
- Parental BR-05-0380 PDX model is originally derived from a breast cancer patient and is sensitive to Palbociclib.
- Palbociclib-R-BR-05-0380 model was established by chronic treatment *in vivo*, Palbociclib treated tumors were passaged and dosed until a stable resistance phenotype occurred.

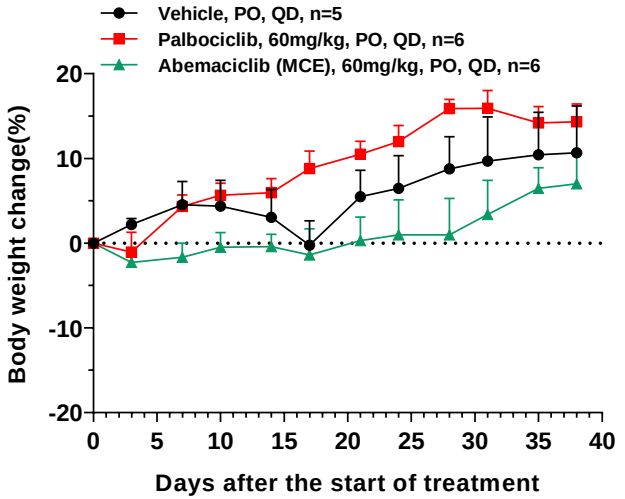
Palbociclib induced resistant BR-05-0380 model (Palbociclib-R-BR-05-0380)

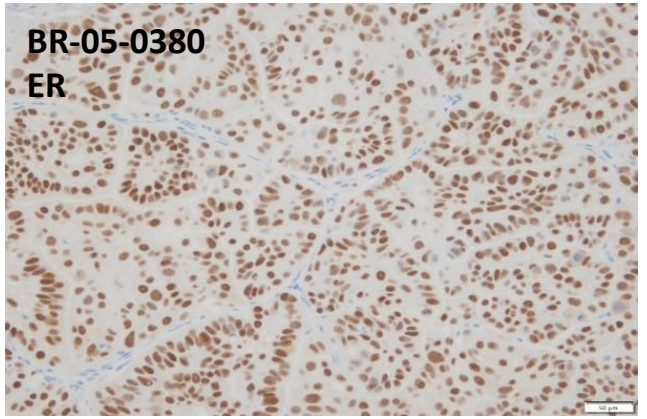
Tumor fragment revival validation test

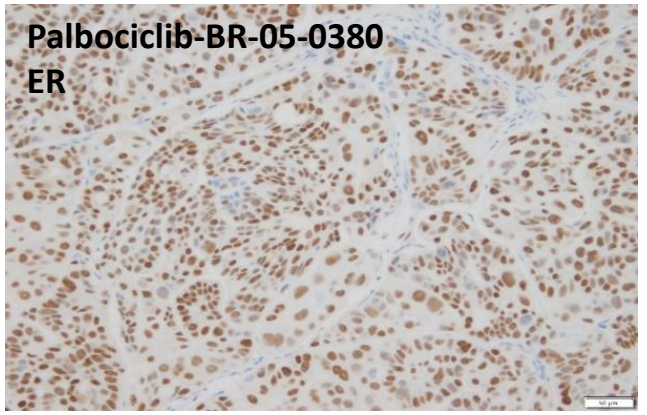












- ER expression in Palbociclib-R-BR-05-0380 is consistent with parental BR-05-0380.

- P5 frozen tumor fragments were revived and implanted into mice, the revival passage was defined as FP6. Tumors were passaged for further validation.
- Data shows that the resistant phenotype of Palbociclib-R-BR-05-0380 model is stable.

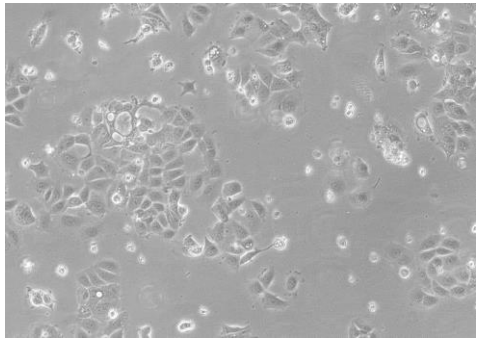
Model summary

Induce method	Model ID	Cancer type	Inoculation	Drugs tested	Dosage	TGI (%)
Parental	Parental BR-05-0380 (FP6)	Breast cancer	Tumor Fragment	Palbociclib DS-8201	50 mg/kg, QD 10 mg/kg, single dose	84 86
	Parental BR-05-0380 (FP7)	Breast cancer	Tumor Fragment	Palbociclib Abemaciclib	60 mg/kg, QD 60 mg/kg, QD	72 106
***In vivo* induce**	Palbociclib-R-BR-05-0380 (P6)	Breast cancer	Tumor fragment	Palbociclib Palbociclib PF-06873600	50 mg/kg, QD 75 mg/kg, QD 50 mg/kg, QD	26 51 20
	Palbociclib-R-BR-05-0380 (FP7)	Breast cancer	Tumor fragment	Palbociclib	60 mg/kg, QD	28
	Palbociclib-R-BR-05-0380 (FP8)	Breast cancer	Tumor fragment	Palbociclib Abemaciclib	60 mg/kg, QD 60 mg/kg, QD	15 96

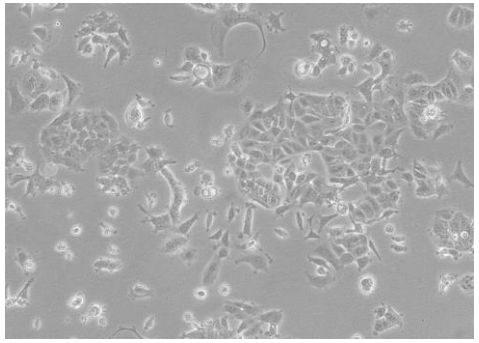
Abemaciclib induced resistant MCF-7 model (xAbemaciclib-R-MCF-7)

in vitro & in vivo validation of xAbemaciclib-R-MCF-7

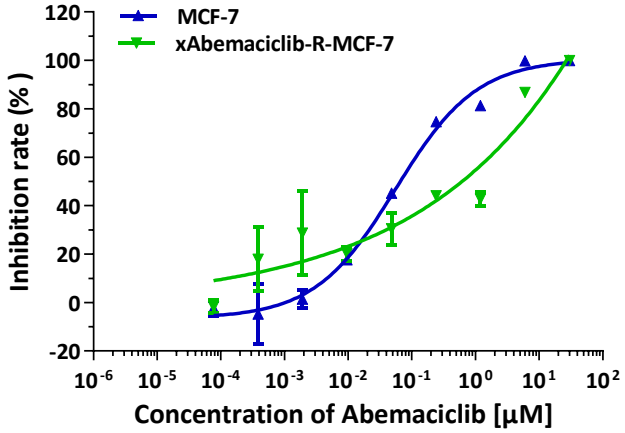
MCF-7 cell line



xAbemaciclib-R-MCF-7 cell line

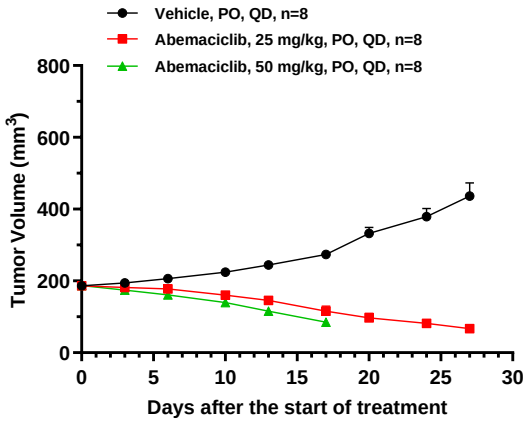


- Abemaciclib-R-MCF-7 model was established by chronic treatment *in vivo,* xAbemaciclib-R-MCF-7 cell line was derived from Abemaciclib-R-MCF-7 tumor.

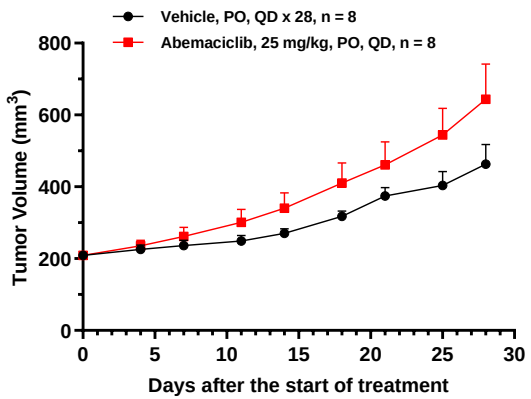


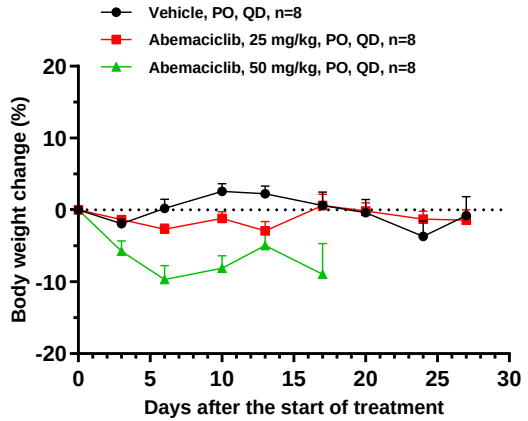
Compound	Cell line	AbsIC50 (μM)	ReIC50 (μM)	Bottom (%)	Top (%)
Abemaciclib	MCF-7	0.066	0.056	-4.77	99.96
	xAbemaciclib-R-MCF-7	0.617	1.156	-1.64	99.94

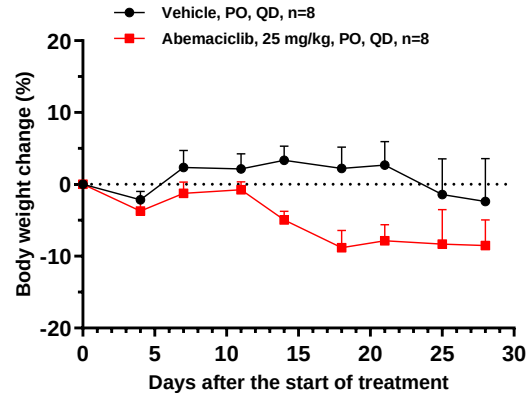
MCF-7



xAbemaciclib-R-MCF-7





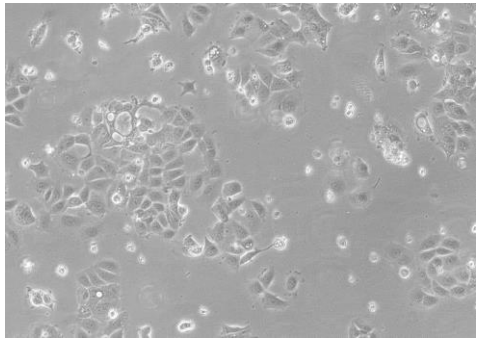


- The xAbemaciclib-R-MCF-7 cell line is resistant to Abemaciclib *in vitro* and *in vivo*.

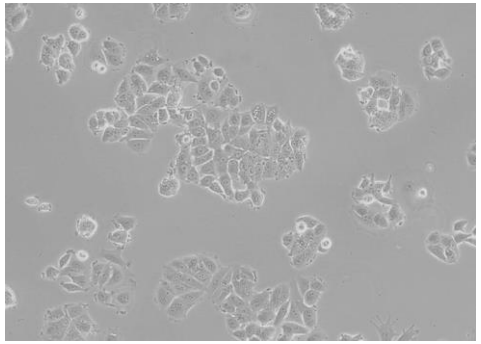
Ribociclib induced resistant MCF-7 model (xRibociclib-R-MCF-7)

in vitro & in vivo validation of xRibociclib-R-MCF-7

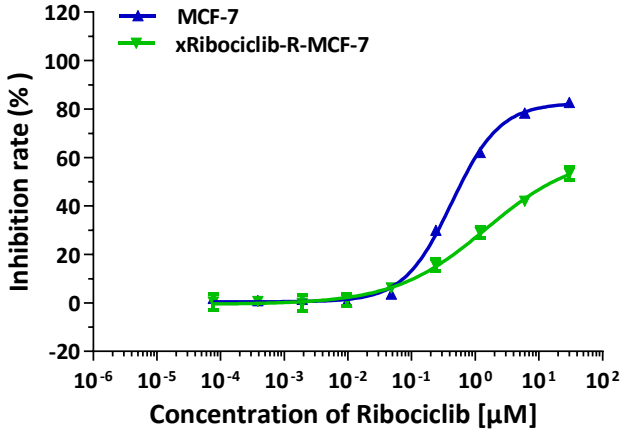
MCF-7 cell line



xRibociclib-R-MCF-7 cell line

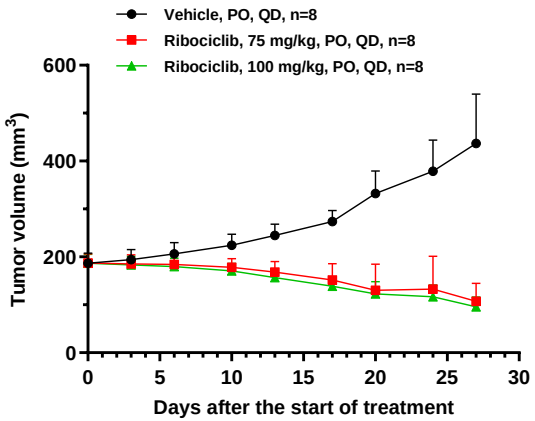


- Ribociclib-R-MCF-7 model was established by chronic treatment *in vivo,* xRibociclib-R-MCF-7 cell line was derived from Ribociclib-R-MCF-7 tumor.

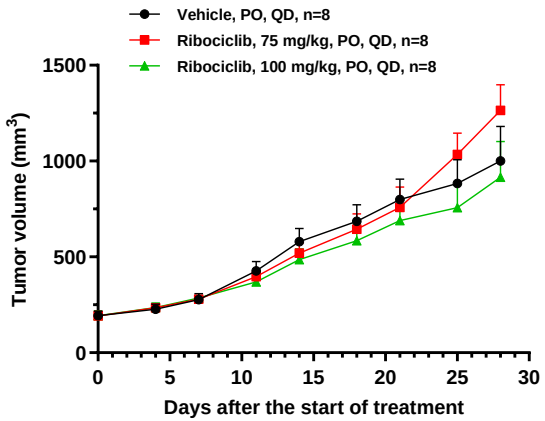


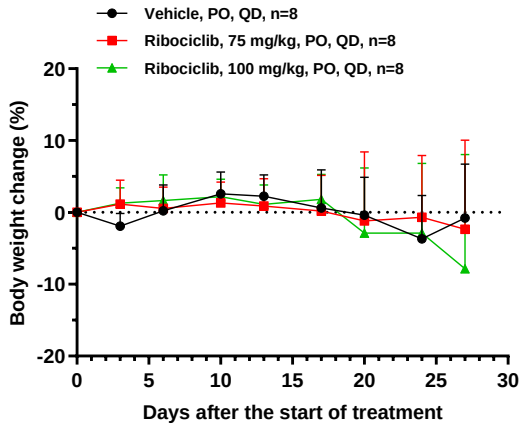
Compound	Cell line	AbsIC50 (μM)	ReIC50 (μM)	Bottom (%)	Top (%)
Ribociclib	MCF-7	0.621	0.431	0.30	82.72
	xRibociclib-R-MCF-7	16.693	1.470	-0.16	53.49

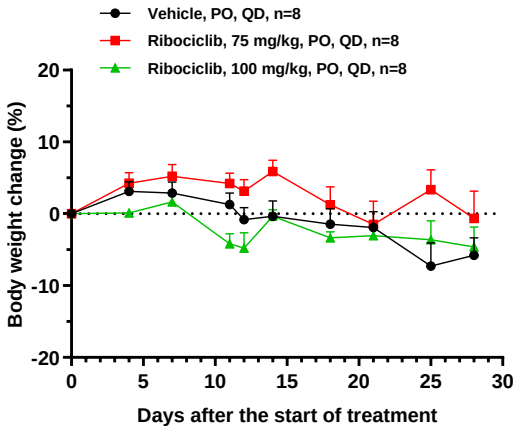
MCF-7



xRibociclib-R-MCF-7







- xRibociclib-R-MCF-7 cell line shows minor resistance to Ribociclib.
- The established xRibociclib-R-MCF-7 model is still resistant to Ribociclib *in vivo*.

Model summary

Induce method	Model ID	Cancer type	Inoculation	Drugs tested	Dosage	TGI (%)
Parental	Parental MCF-7	Breast cancer	Cell line	Abemaciclib	25 mg/kg, QD 50 mg/kg, QD	148 141
	Parental MCF-7	Breast cancer	Cell line	Ribociclib	75 mg/kg, QD 100 mg/kg, QD	132 137
***In vivo* induce**	xAbemaciclib-R-MCF-7	Breast cancer	Cell line	Abemaciclib	25 mg/kg, QD	-71
	xRibociclib-R-MCF-7	Breast cancer	Cell line	Ribociclib	75 mg/kg, QD 100 mg/kg, QD	-33 11

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