HER2 Targeted *In Vivo* Models

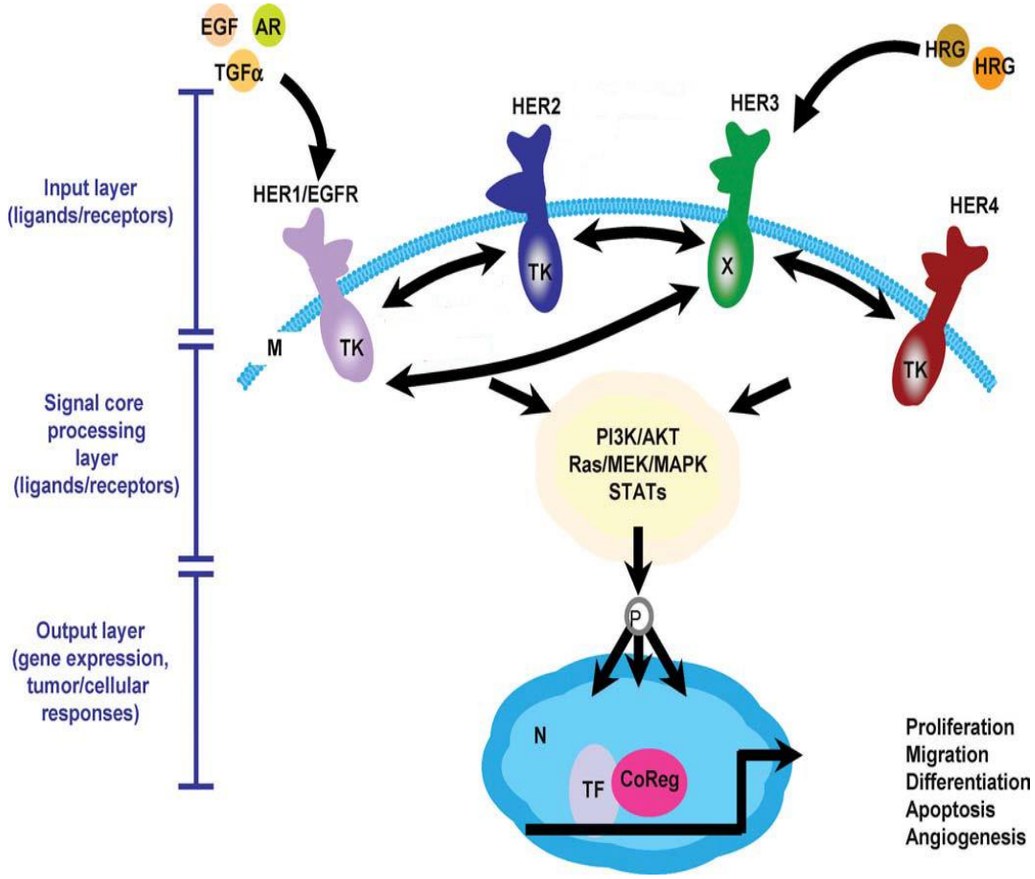
WuXi AppTec Research Service Division, Oncology & Immunology Unit

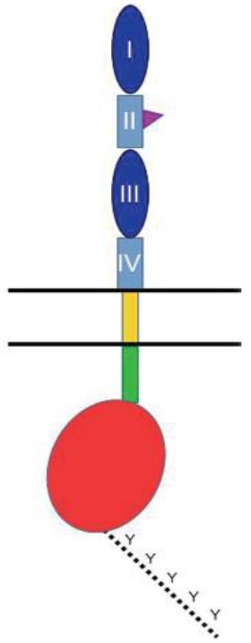
2021.05

Outline

- **HER2 background as a drug target**
  - HER2 biology
  - HER2 targeted drugs
- **HER2 related CDX models**
  - HER2 related cancer cell lines and characterization by *in vitro* assays
  - HER2 related CDX models and SOC data
- **HER2 related PDX models**
  - Summary RNAseq, IHC and FISH data of HER2-overexpressing PDX models
  - HER2 IHC results in PDX models
  - HER2 FISH results in PDX models
  - HER2 positive gastric and breast cancer PDX models
  - PDX characterization and SOC data

HER Family: structure and its ligands





Extracellular	
Domain I	*Ligand-binding domain*
Domain II (CR1)	*Dimerizationar domain*
Domain III	*Ligand-binding domain*
Domain IV (CR2)	*Cysteine-rich domain*
Transmembrane	*Transmembrane domain*
Intracellular	
Juxtamembrane	*Juxtamembrane domain*
Tyrosine kinase	*Tyrosine kinase domain*
C-terminal tail	*Tyrosines contained C-chain*

HER Family of Membrane Receptors

Receptor	Tyrosine Kinase Activity	Major Ligands
HER1 (EGFR)	Yes	EGF, amphiregulin, TGF-α
HER2	Yes	None
HER3	No	Heregulin
HER4	Yes	Heregulin

Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; TGF-α, transforming growth factor α.

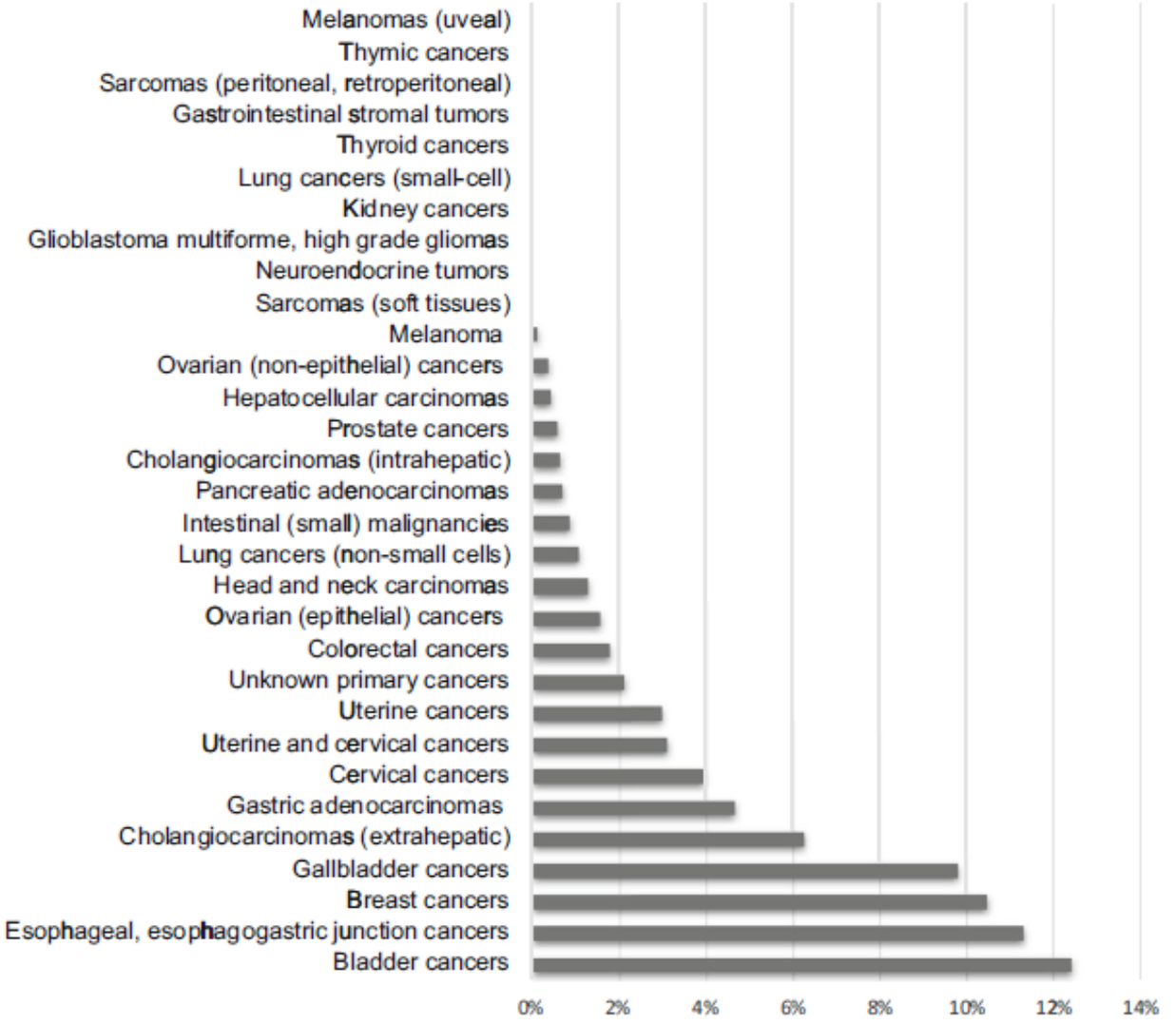
- Human Epithelial Receptor (HER) family consists of HER1 (epidermal growth factor receptor (EGFR), HER2, HER3 and HER4, each with an input layer, a signal core processing layer and an output layer.
- Major ligands: EGF, amphiregulin and TGF-α for HER1; Heregulin for HER3 and HER4; no ligands for HER2.
- The receptors facilitate the homo- or hetero- dimerization after the ligand binding.

Front Pharmacol. 2015, 24z(6):283. doi: 10.3389/fphar.2015.00283.

Arch Pathol Lab Med. 2011;135:55–62

HER2 role in cancer

- **HER2 gene amplification** is the most common mechanism leading to increased HER2 protein expression. Overexpression of the receptor disrupts normal control mechanisms, potentially leading to the formation of aggressive tumor cells.
- HER2 amplification was first noted in **human breast cancer** (in approximately 20 to 30% of breast cancers) and was subsequently identified in ovarian, lung, gastric and oral cancers with high frequencies.

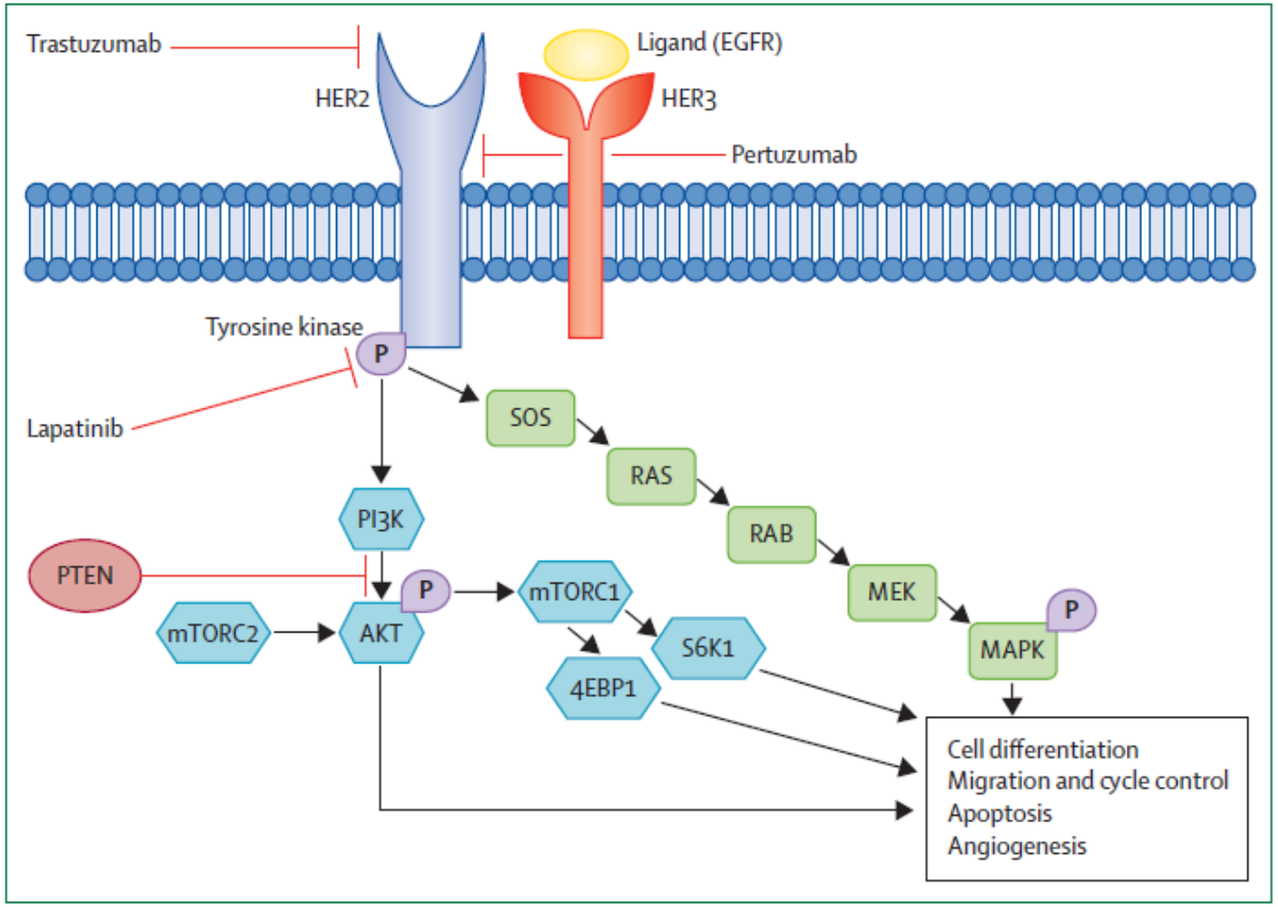


HER2 positivity across cancers: analysis of 37,992 samples by IHC. IHC 3+ was considered HER2 IHC positive

Cancer Metastasis Rev (2015) 34:157–164

Cancer Treat Rev. 2014 Jul;40(6):770-80. Mol Biol Int. 2014;2014:852748.

The HER signaling network



- Signal core processing layer involving a series of phosphorylation (eg, activation of the PI3K/AKT, RAS/MEK/MAPK, and STATs kinase cascades) to alter expression of genes regulating tumor cell proliferation, survival, and other characteristics of the malignant phenotype.

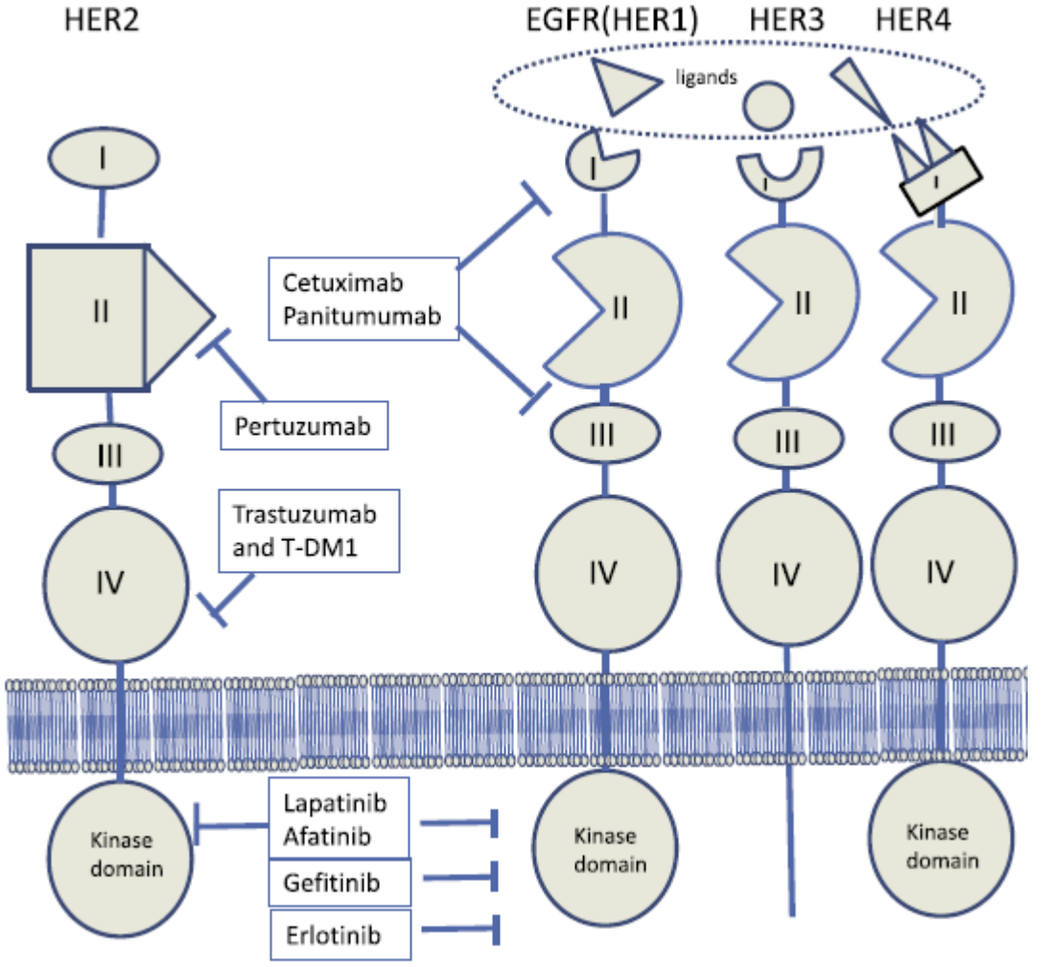
www. thelancet.com. 2016 Jun.

HER2 targeted drugs

HER family receptors and anti-HER agents approved in clinical practice

Two classes of drugs thus far have shown clinical efficacy and much of the ongoing pharmaceutical efforts continue to be in the realm of these two classes:

- Monoclonal antibodies that can target extracellular epitopes;
- Small molecule cell permeable inhibitors of catalytic kinase function.



Cancer Treat Rev. 2014 Jul;40(6):770-80. Mol Biol Int. 2014;2014:852748.

HER2 targeted drugs

Drug	Inventor	Major mechanism	Indications	Status
Trastuzumab	Roche	Binding to juxtamembrane domain of HER2	HER2-positive breast, gastric and gastroesophageal junction cancer	Approved (1998)
Lapatinib	GSK	Reversibly blocking ATP binding site on kinase domain of EGFR and HER2	HER2-positive metastatic breast cancer with prior exposure to trastuzumab	Approved (2007)
Pertuzumab	Genentech	Binding to domain II of HER2, blocking ligand-dependent dimerization of HER2 with other HER members	HER2-positive breast cancer in metastatic or neoadjuvant setting	Approved (2012)
Ado-trastuzumab emtansine (T-DM1)	Roche	Anti-tumor properties of trastuzumab combined with Cytotoxic microtubule depolymerizing DM1	HER2-positive metastatic breast cancer with prior exposure to trastuzumab	Approved (2013)
Afatinib	Boehringer-Ingelheim	Irreversibly blocking kinase domain of EGFR/HER2, including erlotinib-resistant EGFR T790M variant	Advanced NSCLC with EGFR mutation (Breast cancer)	Approved (2013)
Dacomitinib (PF-00299804)	Pfizer	Irreversible inhibitor of EGFR and HER2; effective (preclinical) against EGFR T790M and gefitinib-resistant HER2 mutation	Advanced NSCLC	Approved (2018)

HER2 targeted drugs

Drug	Inventor	Major mechanism	Indications	Status
Neratinib (HKI-272)	Puma	Irreversible inhibitor of EGFR and HER2; effective (preclinical) against T790M EGFR and gefitinib-resistant HER2 mutation	HER2-positive breast cancer	Approved (2017)
Enhertu (DS-8201, fam-trastuzumab deruxtecan-nxki)	Daiichi Sankyo Inc.	Humanized anti-HER2 IgG1 antibody, the small molecule portion of this drug, deruxtecan (DXd), is a topoisomerase I inhibitor	HER2-positive breast cancer	Approved (2019)
Tucatinib (ARRY-380; ONT-380)	Seattle Genetics, Inc.	ONT-380 selectively inhibits HER2 without significant inhibition of EGFR	HER2-positive breast cancer	Approved (2020)
E75 (NeuVax)	Galena	Intradermal injection of HLA A2/A3–restricted HER2 peptide as adjuvant therapy to prevent disease recurrence	Adjuvant therapy in HER2 low expressing breast cancer	III
MM-302	Merrimack Pharmaceuticals	An antibody-drug conjugate composed of a HER2-targeted antibody linked to the cytotoxic chemotherapy liposomal doxorubicin.	HER2-positive breast cancer	II/III
biosimilar trastuzumab (PF-05280014)	Pfizer	Similar as Trastuzumab	Early breast cancer	III
biosimilar trastuzumab (ABP 980)	Amgen|Actavis Inc.	Similar as Trastuzumab	Breast cancer	III

HER2 targeted drugs

Drug	Inventor	Major mechanism	Indications	Status
AZD8931 (Sapitinib)	AstraZeneca	A reversible inhibitor of signaling EGFR, HER2, and HER3	EGFR and HER2-positive cancer	II
ADXS31-164	Advaxis, Inc.	Activate Immunotherapy. ADXS31-164 caused a reduction in regulatory T cells (Treg) accompanied by an increase in the CD8 (+)/Treg ratio	Solid Tumors	I/II
AEE788	Novartis	Potent inhibitor of EGFR and HER2	Glioblastoma Multiforme	I/II
XL647	Kadmon Corporation, LLC	Multitargeted tyrosine kinase inhibitor, including EGFR, VEGFR2, HER2, and EphB4	Solid Tumors	II
HER2-specific CAR-expressing T cells	Baylor College of Medicine	Genetically modified T cells expressing a HER2-specific CAR	Sarcoma	I
AVX901	AlphaVax	A virally based vaccine that triggers immune response against HER2-positive tumor cells	HER2-positive cancer	II

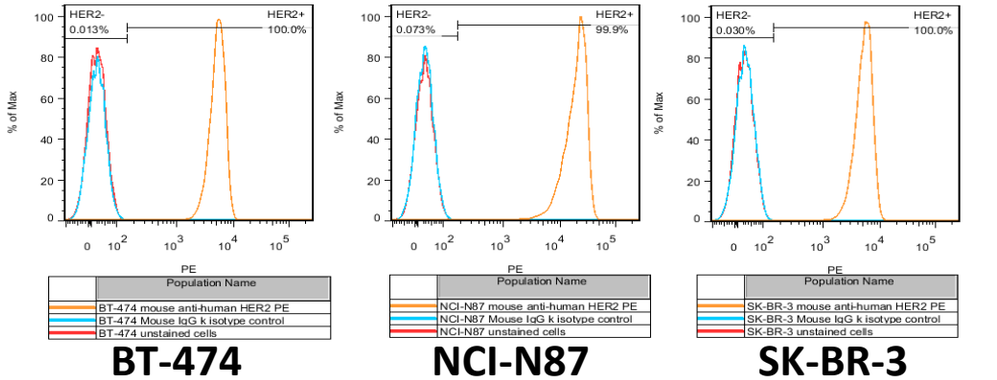
HER2 high expression cell lines

Cell name	Cancer Type	mRNA level (RNAseq)
OE19	Esophagus	11.16
NCI-N87*	Gastric	10.92
HCC1954*	Breast	10.86
UACC893	Breast	10.84
HCC1419*	Breast	10.72
EFM192A*	Breast	10.71
AU565*	Breast	10.69
ZR7530*	Breast	10.57
NCIH2170*	Lung	10.48
UACC812*	Breast	10.37
HCC2218*	Breast	10.30
HCC202*	Breast	10.22
TE4	Esophagus	10.19
SKBR3*	Breast	10.10
HCC1569*	Breast	10.08
SKOV3*	Ovary	10.04
KYSE410	Esophagus	9.80
BT474*	Breast	9.76
CALU3*	Lung	9.75
MKN7	Gastric	9.30
LN229	Glioblastoma	L755S mutation

Note: High expression: RNAseq value>9; * Cells in Onco;

Data on CCLE (http://www.broadinstitute.org/ccle)

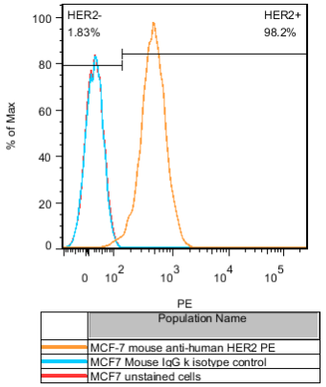
HER2 positive cell lines *in vitro* validation_FACS



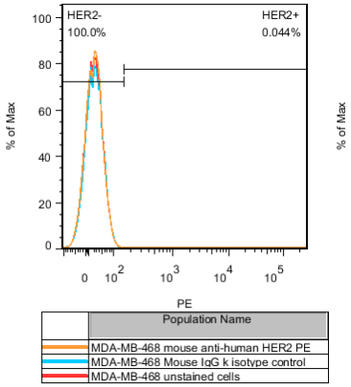
BT-474

NCI-N87

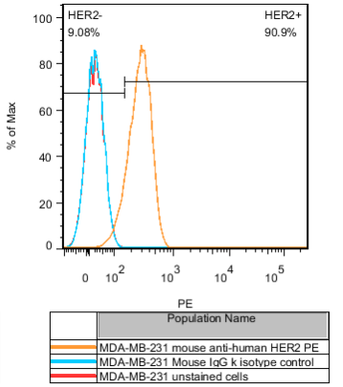
SK-BR-3



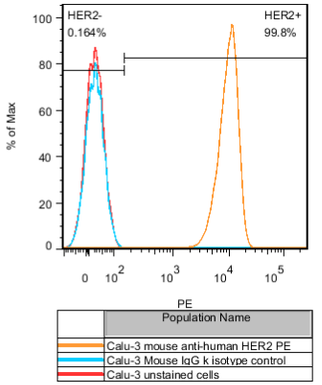
MCF-7



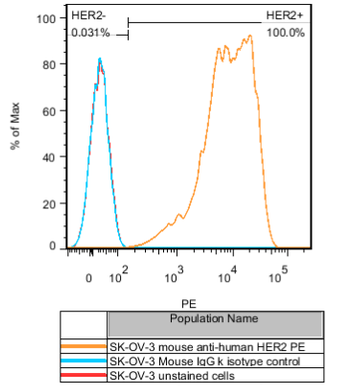
MDA-MB-468



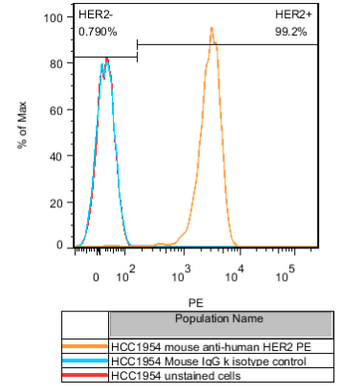
MDA-MB-231



Calu-3

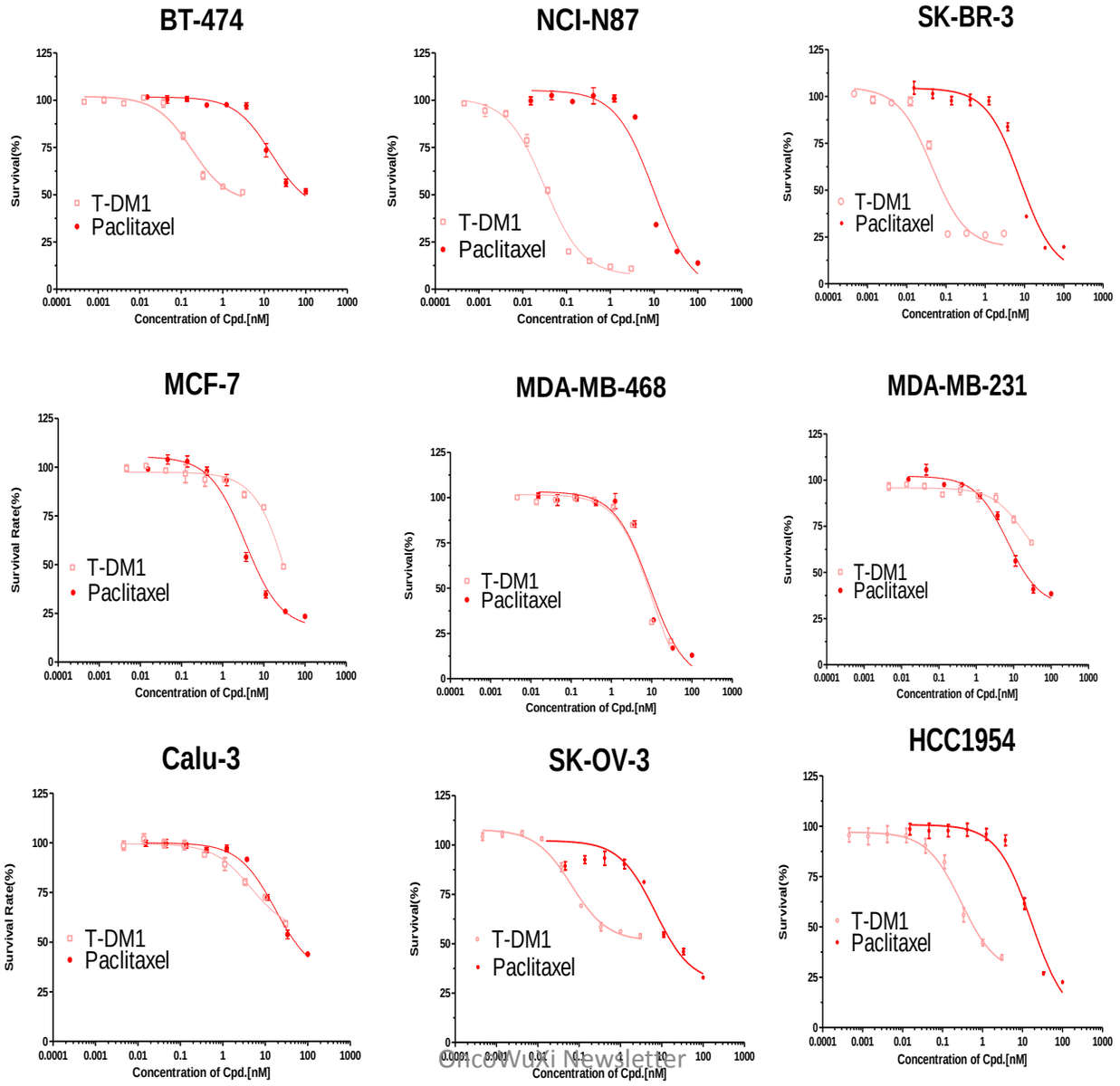


SK-OV-3



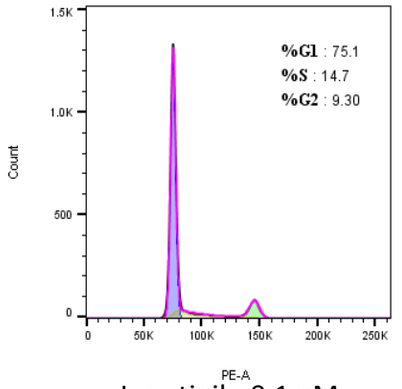
HCC1954

In vitro anti-proliferation effects of Paclitaxel and T-DM1 on cells

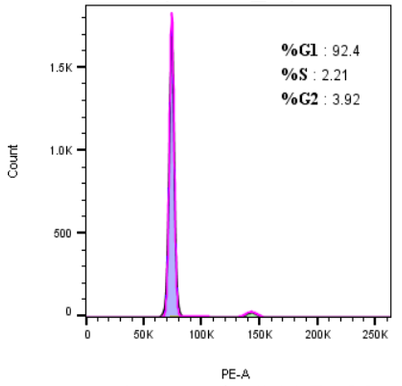


In vitro cell cycle arrest effects of Lapatinib on BT474 cell line

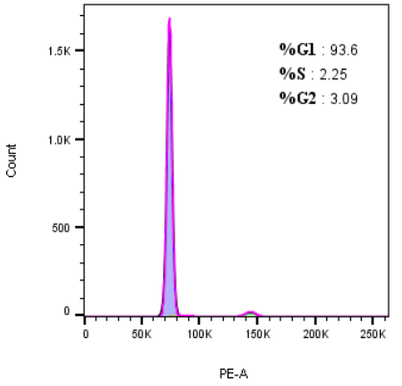
DMSO:



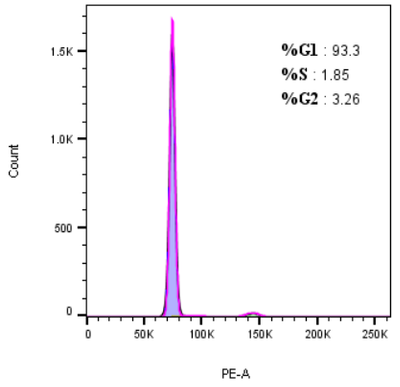
Lapatinib_1 μM:



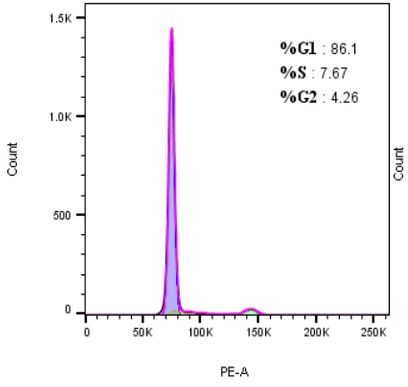
Lapatinib_0.3 μM:



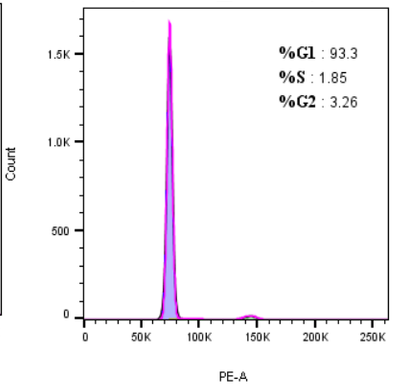
Lapatinib_0.1 μM:



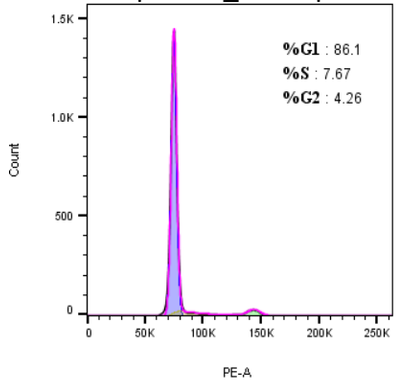
Lapatinib_0.037 μM:



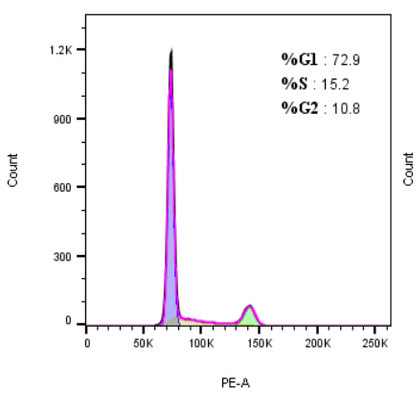
Lapatinib_0.012 μM:



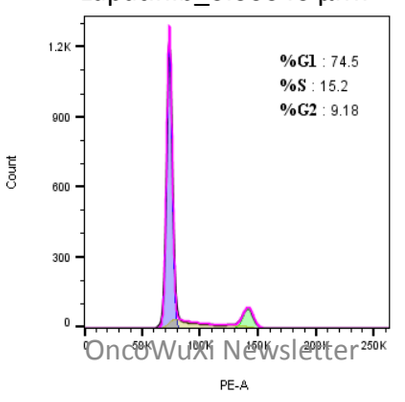
Lapatinib_0.004 μM:

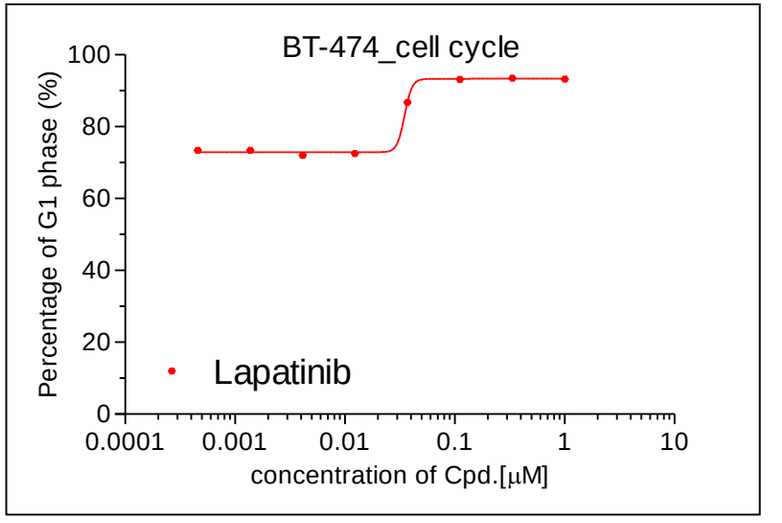


Lapatinib_0.0014 μM:

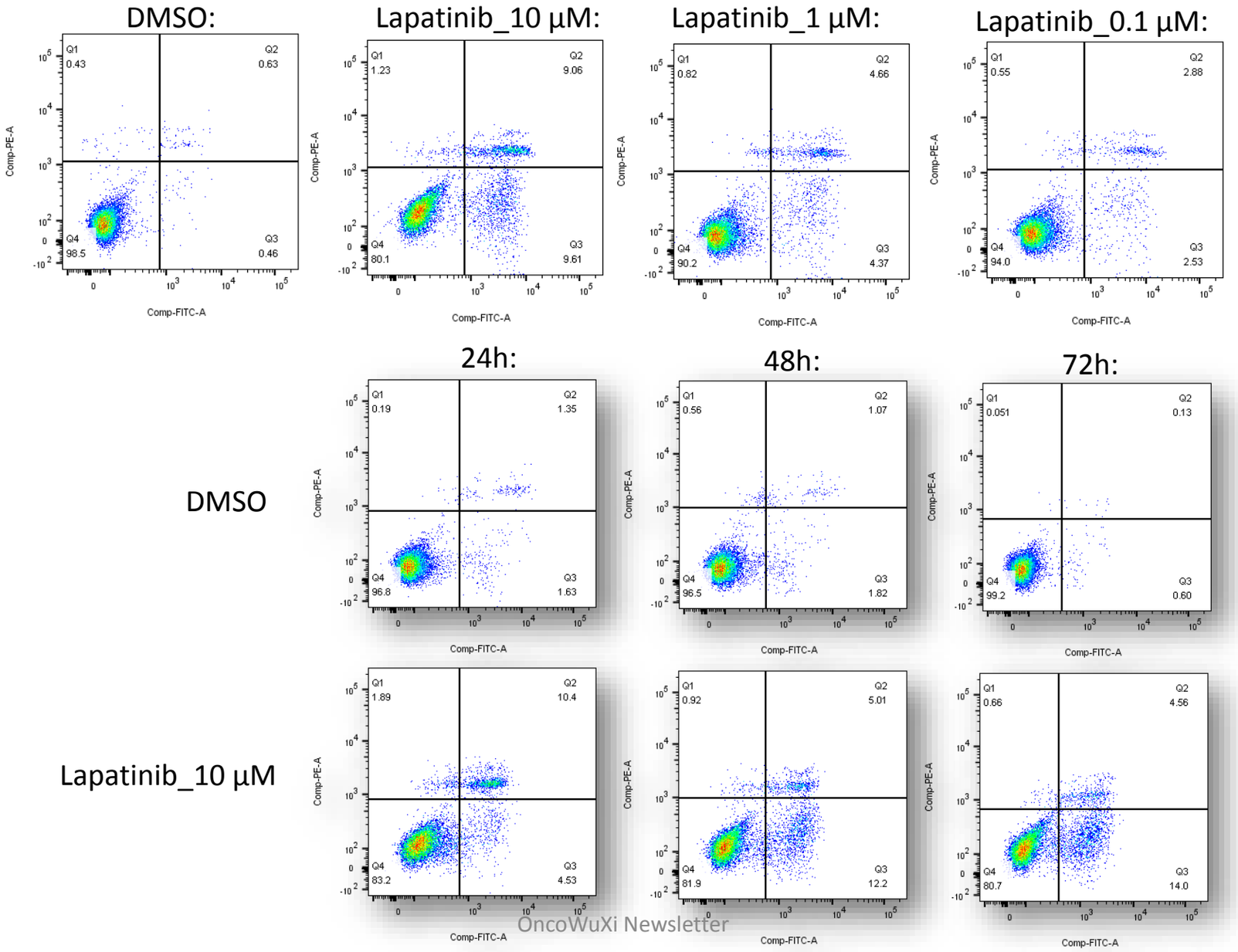


Lapatinib_0.00046 μM:





In vitro apoptosis induction effects of Lapatinib on BT474 cell line



HER2 targeted CDX models-1

CDX models treated with Paclitaxel, Trastuzumab and T-DM1

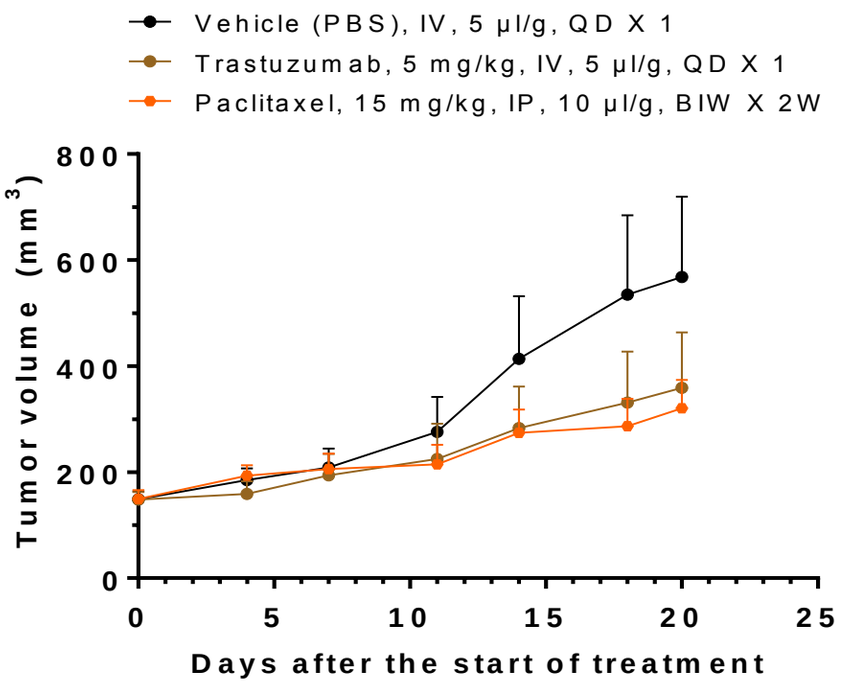
Cancer type	Model ID	Drug tested	Dosage	TGI (%)
Breast cancer	BT-474	Trastuzumab	5 mg/kg, IV, single dose	49.7
		Paclitaxel	15 mg/kg, IP, BIW x 2W	59.2
	HCC1954	Trastuzumab	5 mg/kg, IV, single dose	66.2
		Paclitaxel	15 mg/kg, IP, BIW x 2W	85
		T-DM1	0.55 mg/kg, IV, single dose	56.8
			1.67 mg/kg, IV, single dose	106.3
			5 mg/kg, IV, single dose	107.1
	MDA-MB-468	Trastuzumab	5 mg/kg, IV, single dose	-76.9
		Paclitaxel	15 mg/kg, IP, BIW x 2W	105.5
		T-DM1	0.55 mg/kg, IV, single dose	-42.5
			1.67 mg/kg, IV, single dose	-17.8
			5 mg/kg, IV, single dose	-35.1

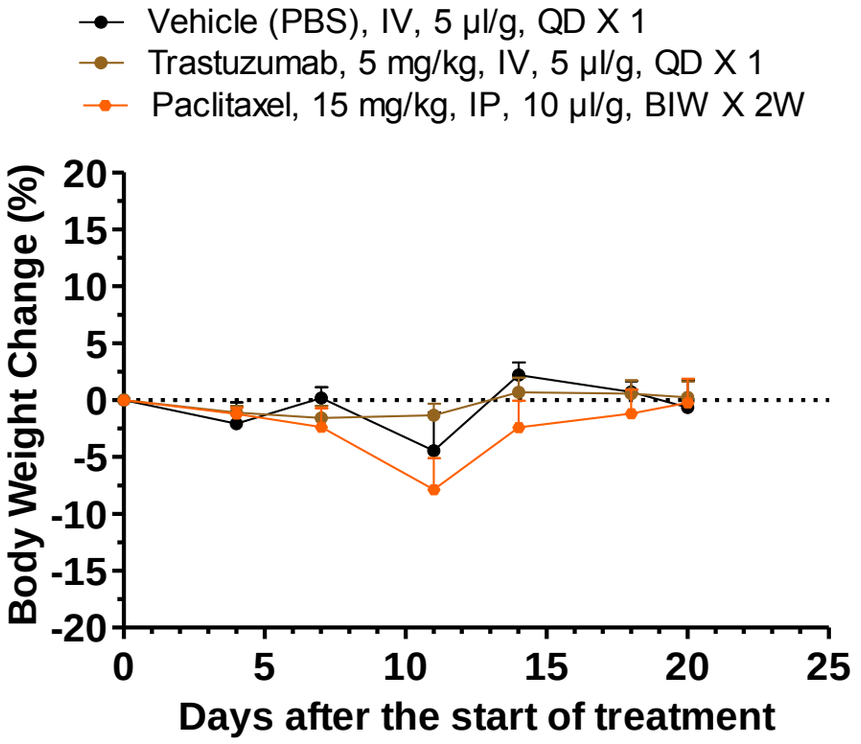
HER2 targeted CDX models-2

CDX models treated with Paclitaxel, Trastuzumab and T-DM1

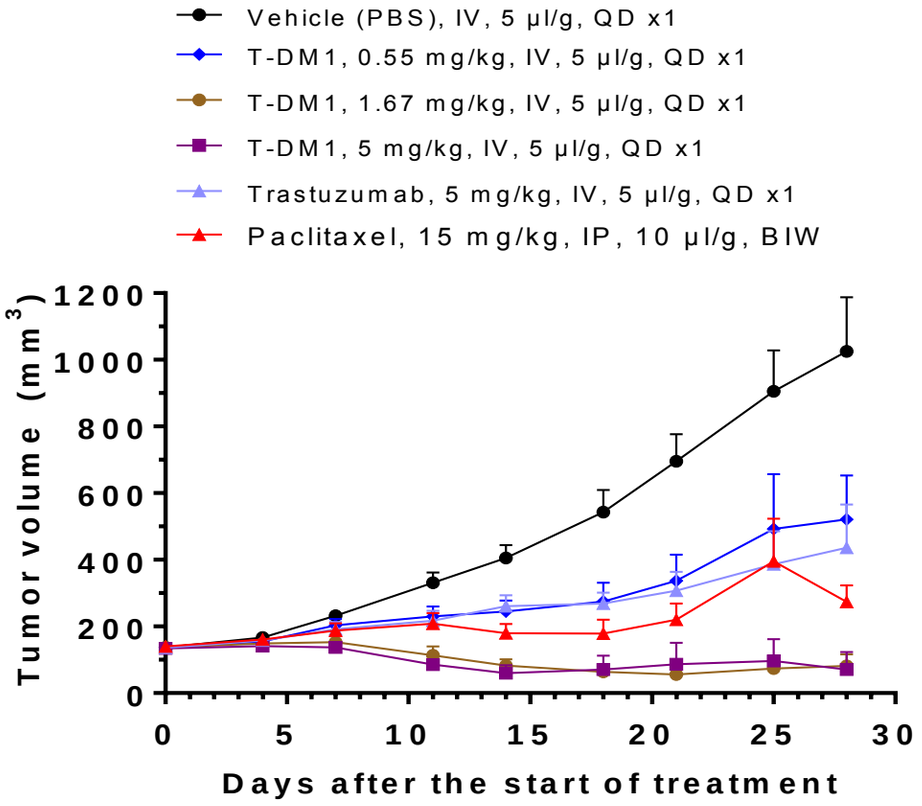
Cancer type	Model ID	Drug tested	Dosage	TGI (%)
Gastric cancer	NCI-N87	Trastuzumab	5 mg/kg, IV, single dose	30.5
		Paclitaxel	15 mg/kg, IP, BIW x 2W	54.6
		T-DM1	0.55 mg/kg, IV, single dose	16.3
			1.67 mg/kg, IV, single dose	45.2
			5 mg/kg, IV, single dose	58.5
Ovarian cancer	SK-OV-3	Trastuzumab	5 mg/kg, IV, single dose	31
		Paclitaxel	15 mg/kg, IP, BIW x 2W	32.1
		T-DM1	0.55 mg/kg, IV, single dose	4.5
			1.67 mg/kg, IV, single dose	38.4
			5 mg/kg, IV, single dose	86.2

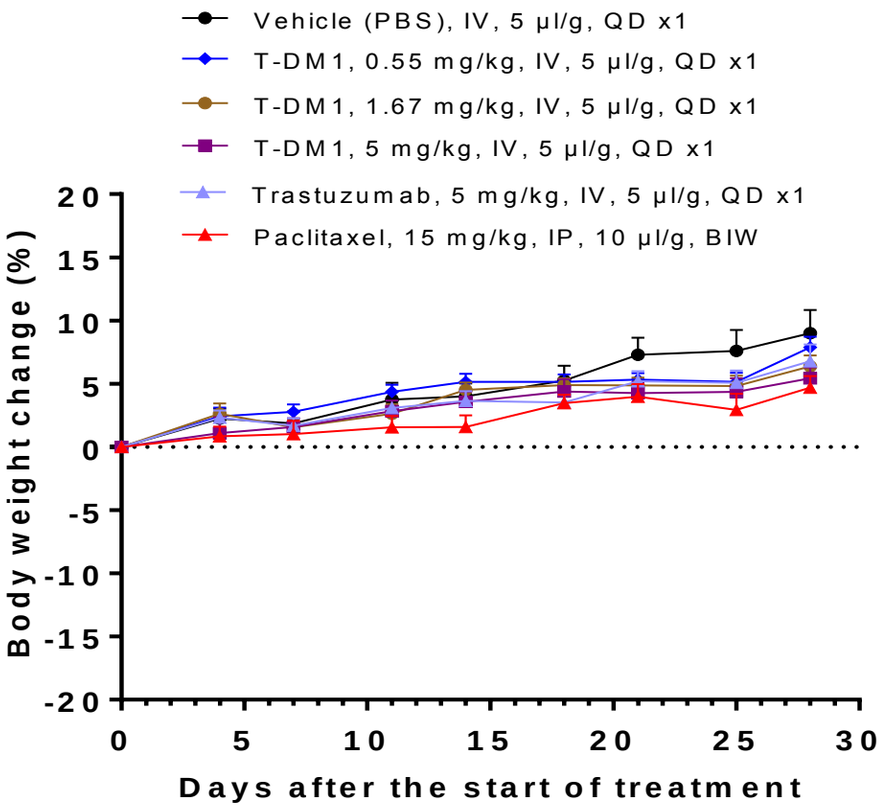
Trastuzumab and Paclitaxel in BT-474



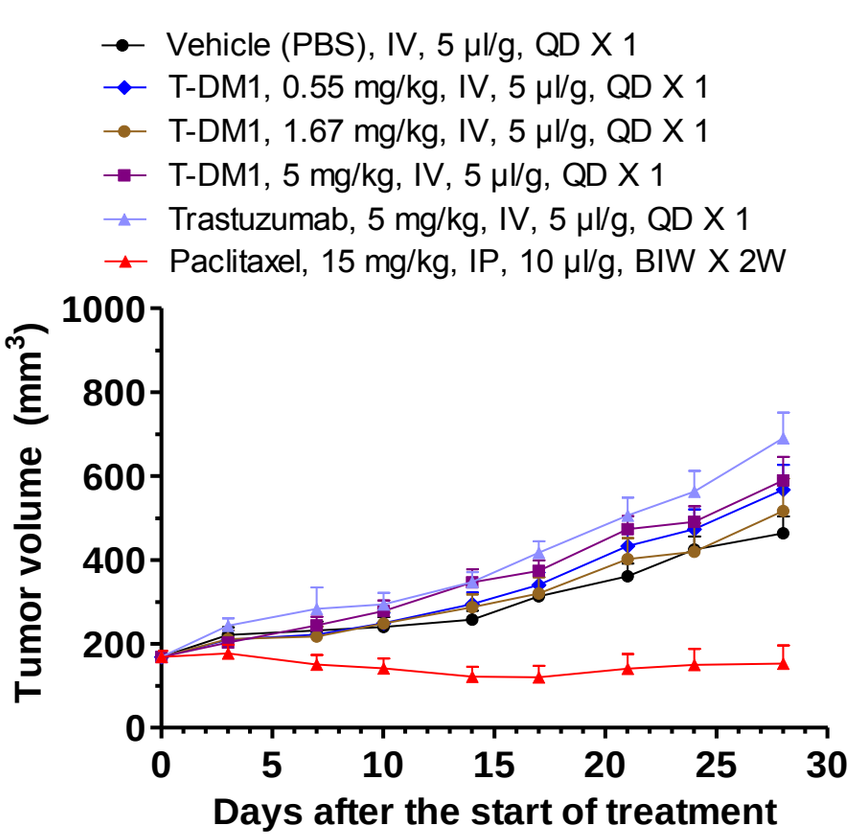


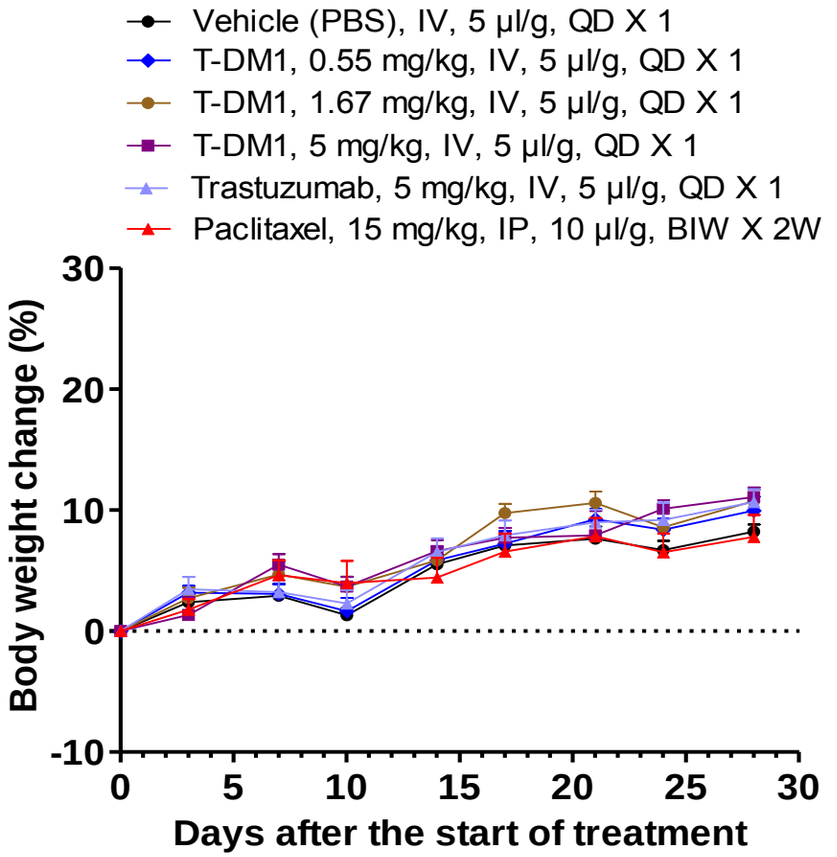
T-DM1, Trastuzumab and Paclitaxel in HCC1954



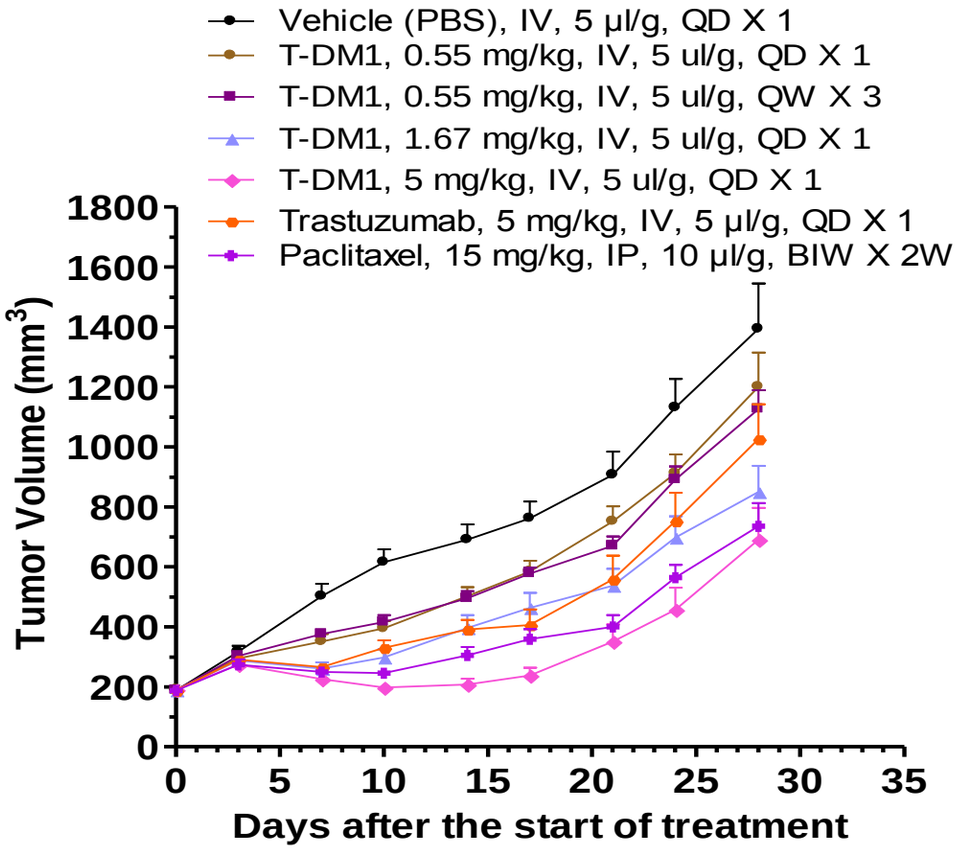


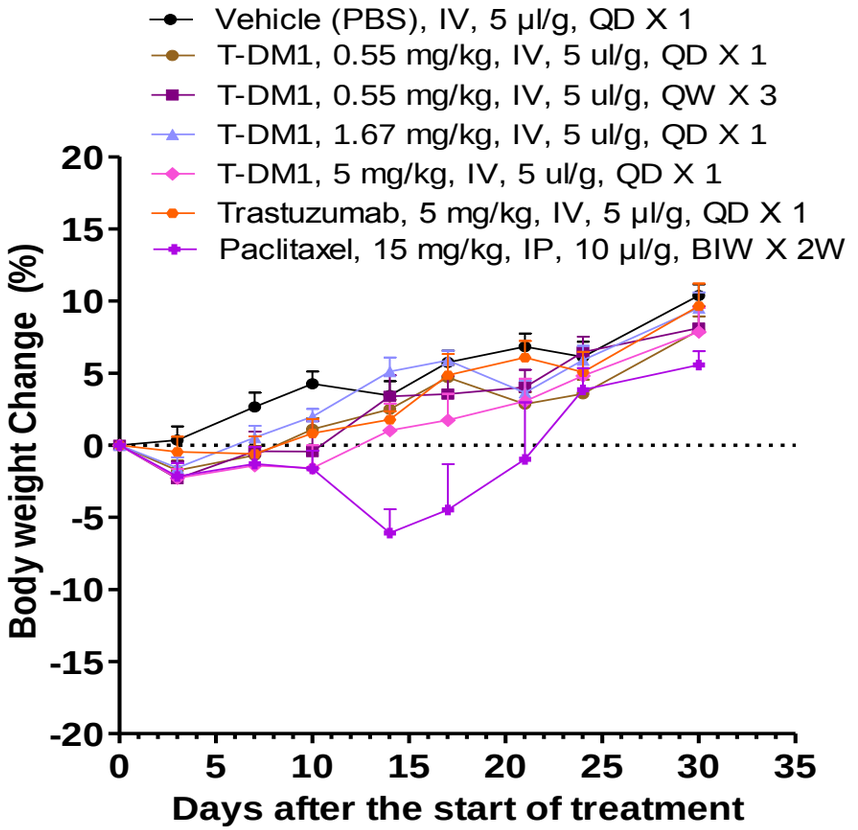
T-DM1, Trastuzumab and Paclitaxel in MDA-MB-468



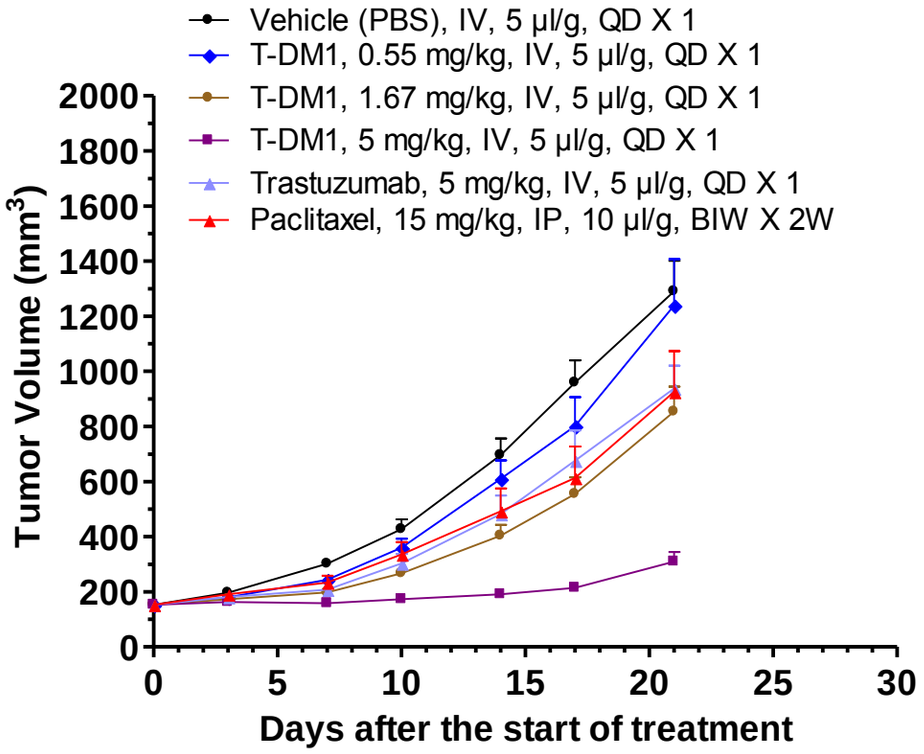


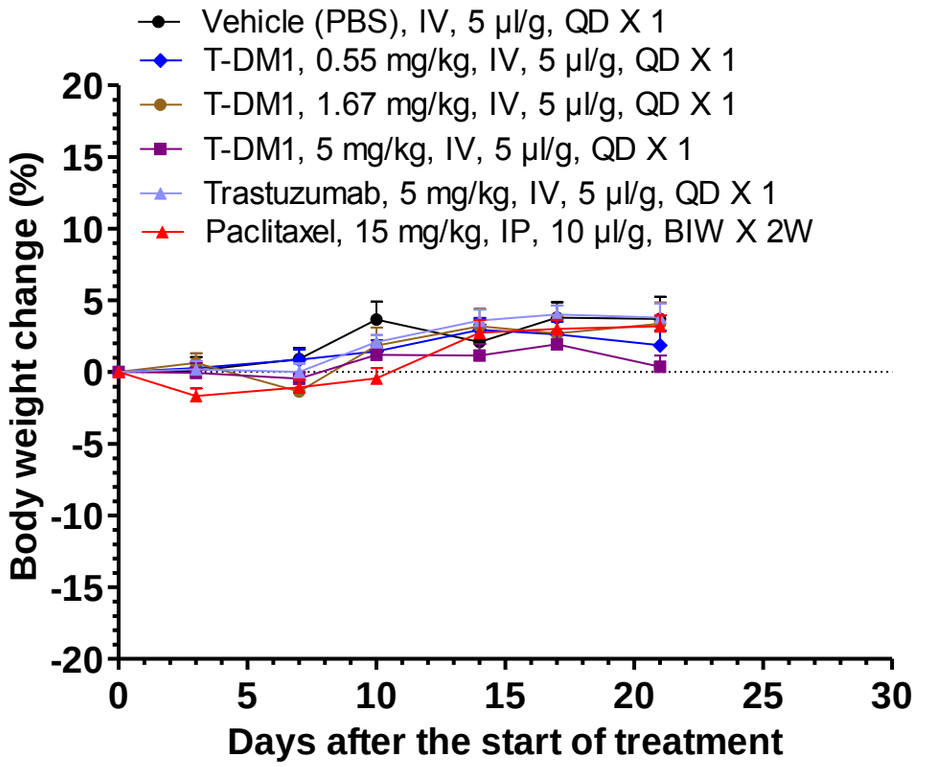
T-DM1, Trastuzumab and Paclitaxel in NCI-N87





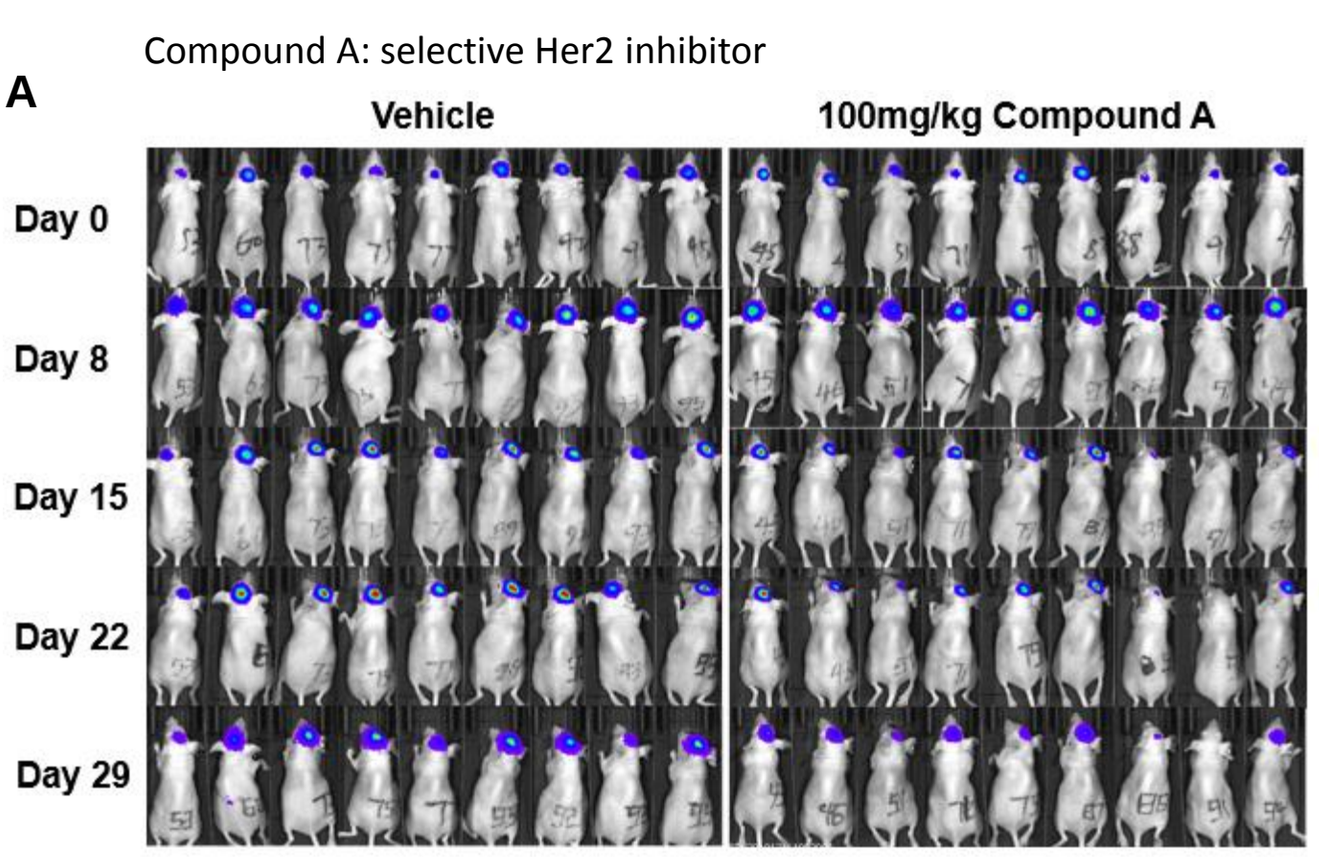
T-DM1, Trastuzumab and Paclitaxel in SK-OV-3





Anti-tumor efficacy of Compound A in the NCI-N87luc intracranial model

Compound A: selective Her2 inhibitor



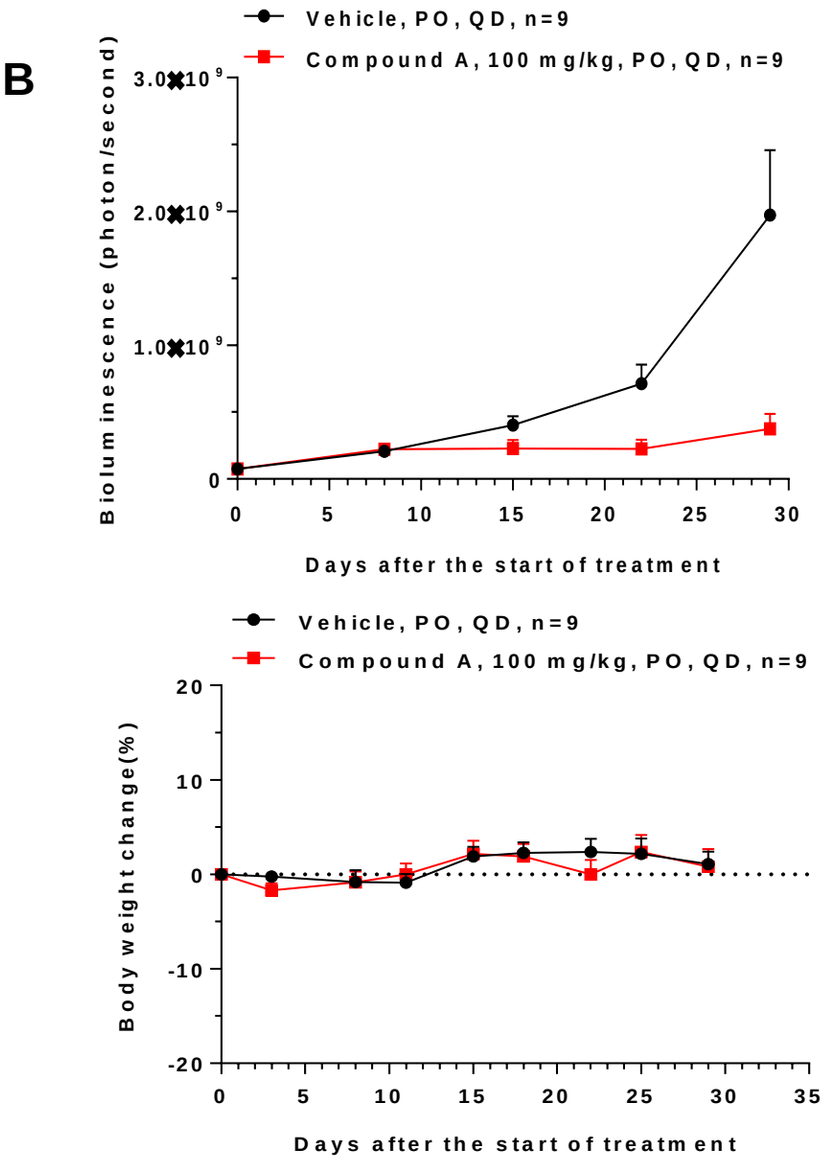


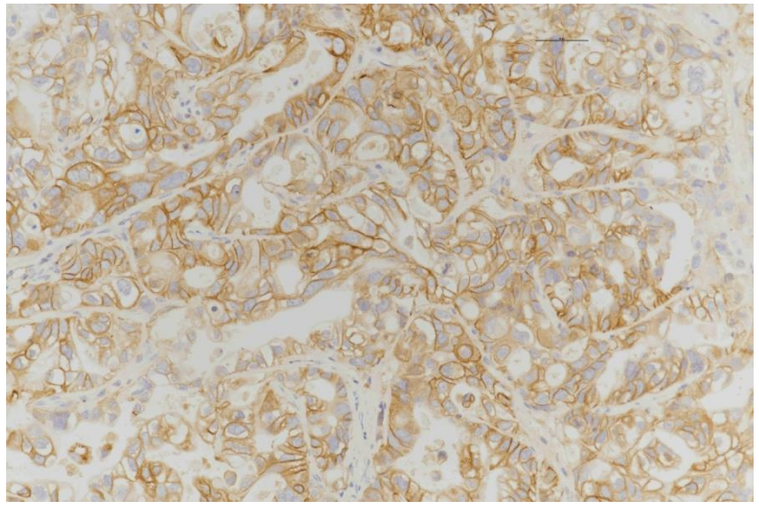
Figure: 4.5x10^5 NCI-N87luc cells were inoculated into the 6-8 week BALB/c nude mice by Orthotopic brain surgery. Mice were exposed to Xenogen machine for bioluminescence measurement at various days. Treatment by vehicle or Compound A was started at day 8 after inoculation. (**A**) Bioluminescent imaging of mice (n=9). (**B**) Bioluminescence and Body weight change of mice injected with NCI-N87luc after treatment by vehicle or Compound A.

HER2 targeted PDX models: RNAseq, IHC and FISH data analysis

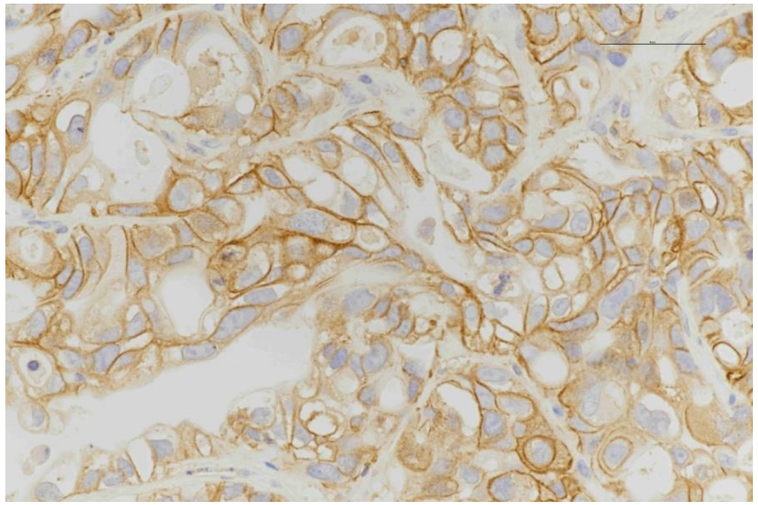
Model ID	RNAseq	IHC	IHC (repeat)	FISH
ST-02-0001	1337.04	3+	3+	Positive
ST-02-0002	129.74	0	/	/
ST-02-0074	/	1+	2+	Positive
ST-02-0077	1336.05	3+	3+	Positive
ST-02-0079	691.82	1+	2+	Positive
ST-02-0103	1664.43	2+	2+	Positive
ST-02-0195	280.65	0	/	/
ST-02-0216	115.48	0	/	/
ST-02-0318	2104.62	2+	2+	Positive
ST-02-0322	114.67	0	/	/
ST-02-0328	105.29	0	/	/
ST-02-0382	105.44	0	/	/

HER2 IHC: ST-02-0001 and ST-02-0002

Score = 3+

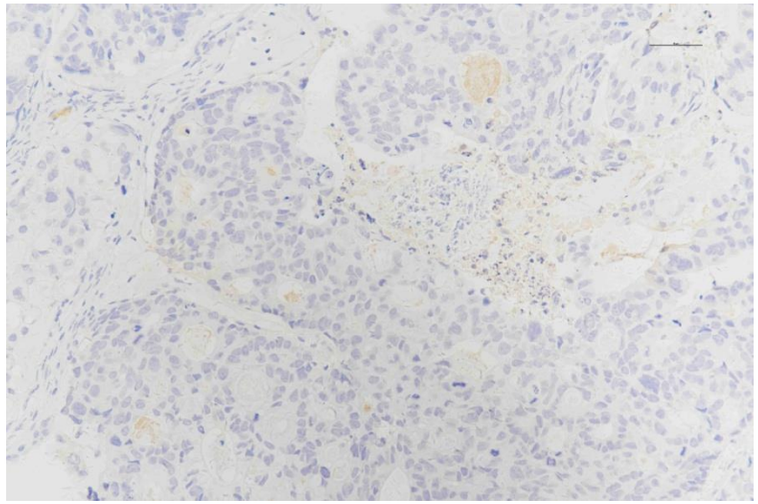


ST-02-0001 P4 20x

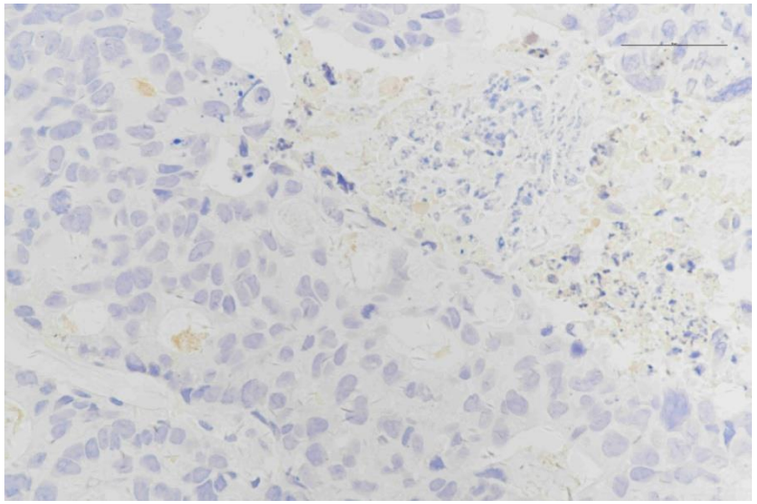


ST-02-0001 P4 40x

Score = 0



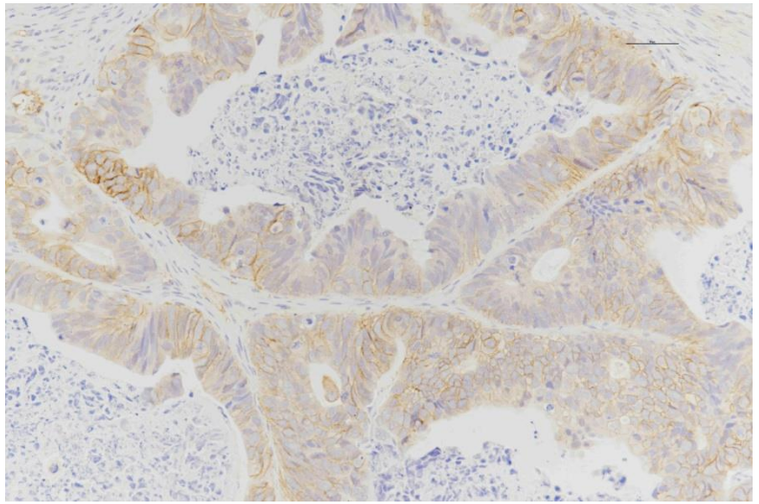
ST-02-0002 P3 20x



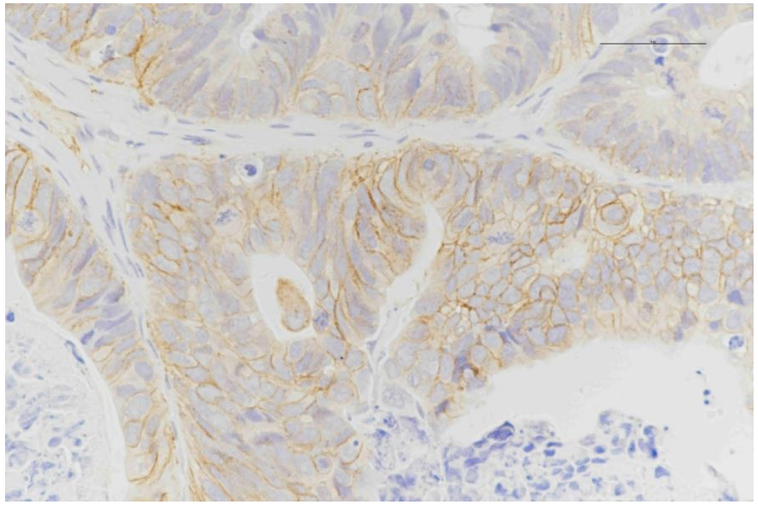
ST-02-0002 P3 40x

HER2 IHC: ST-02-0074 and ST-02-0103

Score = 2+

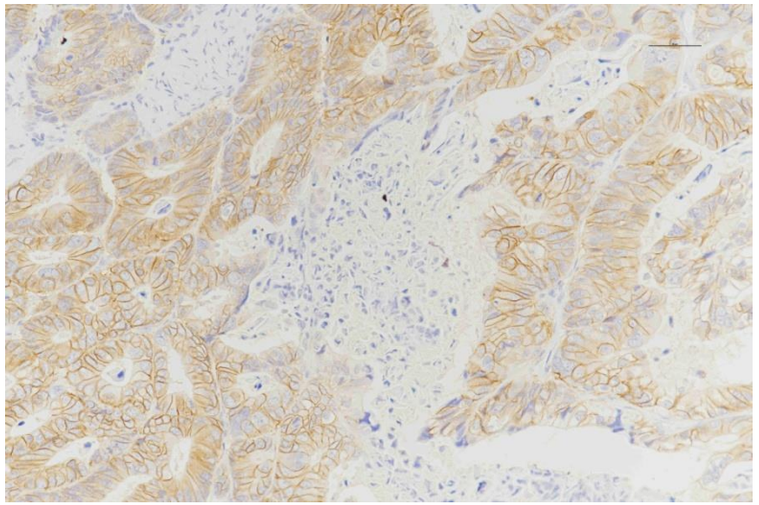


ST-02-0074 P3 20x

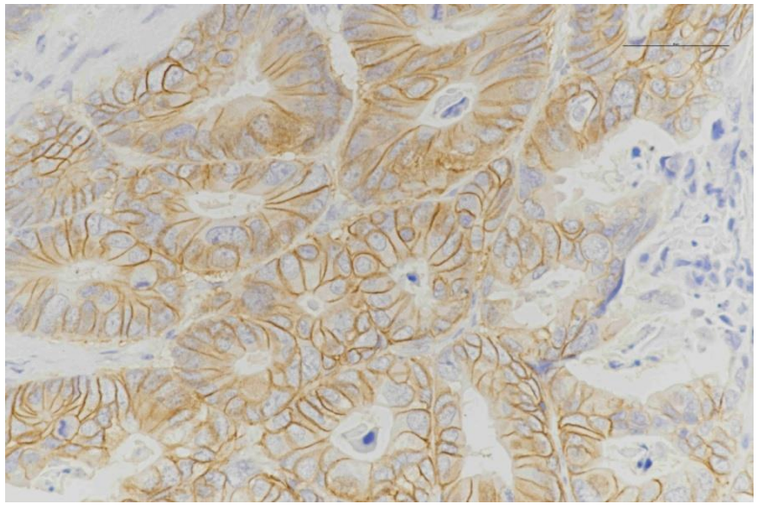


ST-02-0074 P3 40x

Score = 2+



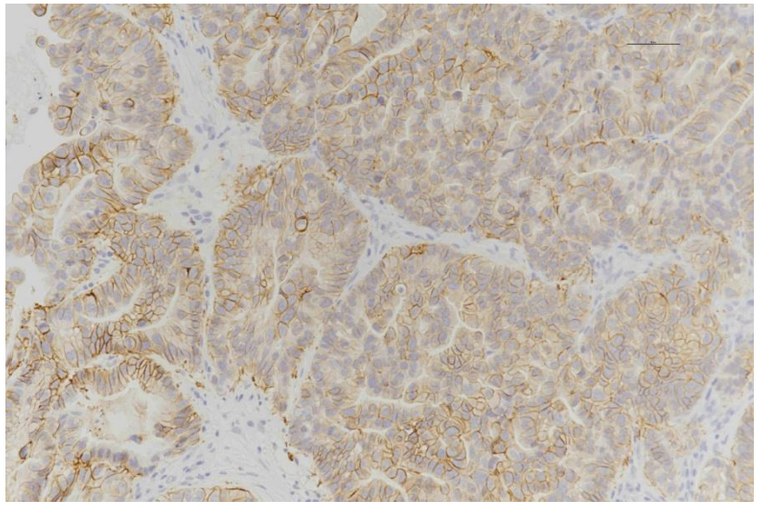
ST-02-0103 P5 20x



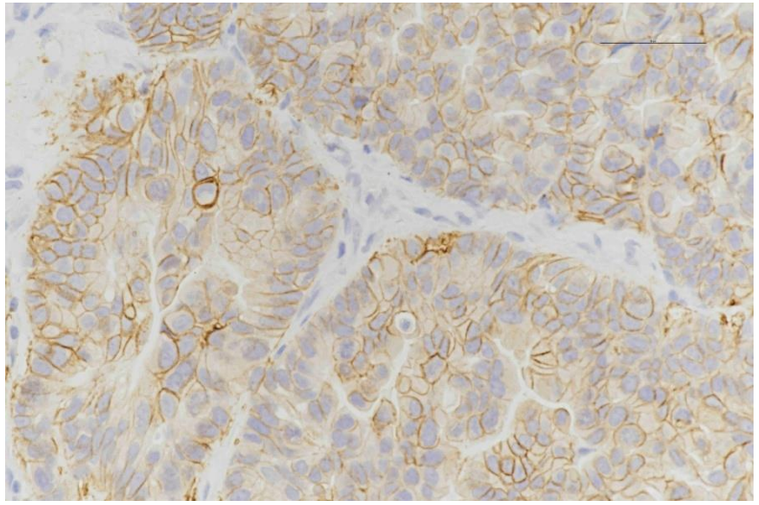
ST-02-0103 P5 40x

HER2 IHC: ST-02-0077 and ST-02-0079

Score = 3+

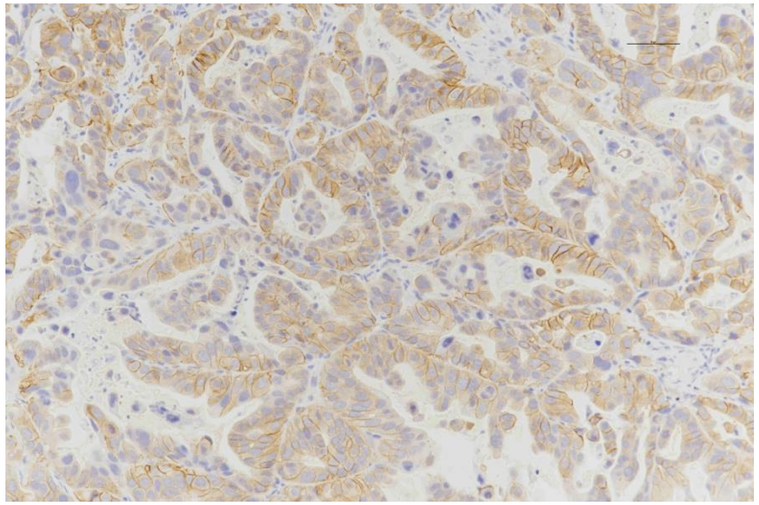


ST-02-0077 P3 20x

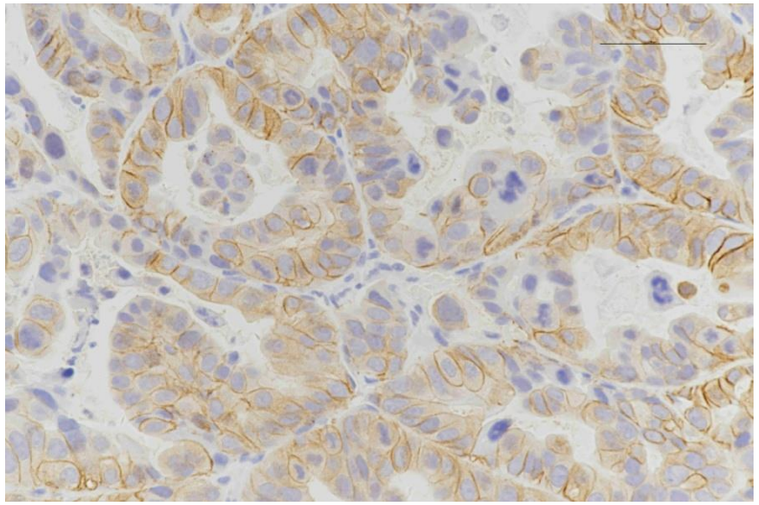


ST-02-0077 P3 40x

Score = 2+



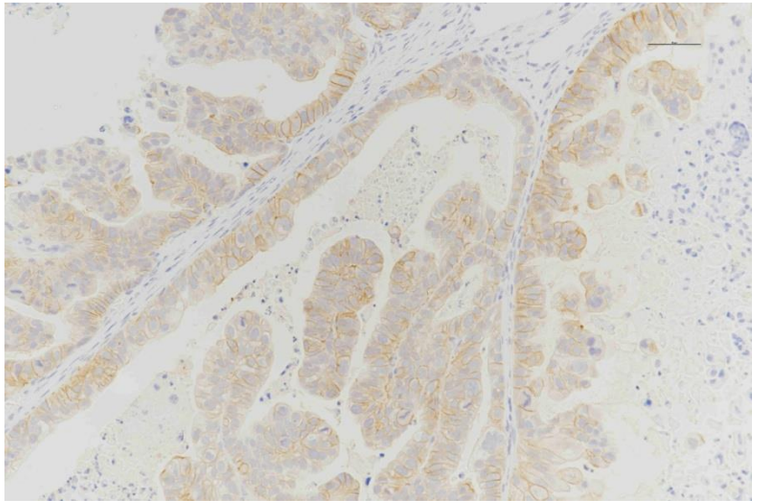
ST-02-0079 P5 20x



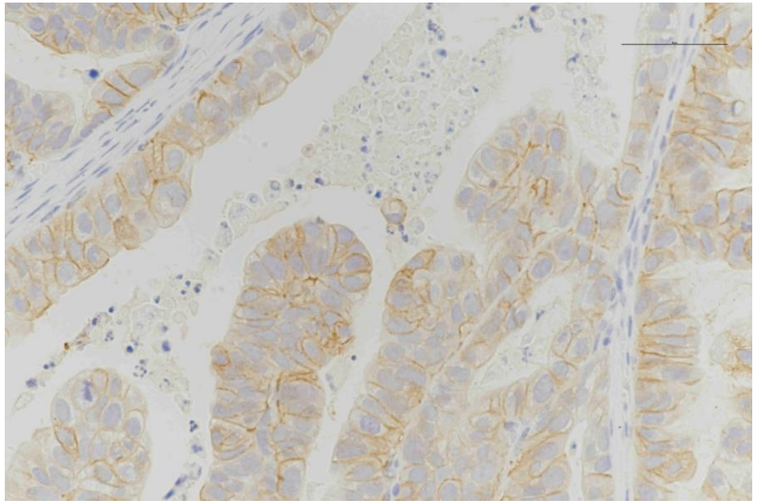
ST-02-0079 P5 40x

HER2 IHC: ST-02-0318

Score = 2+

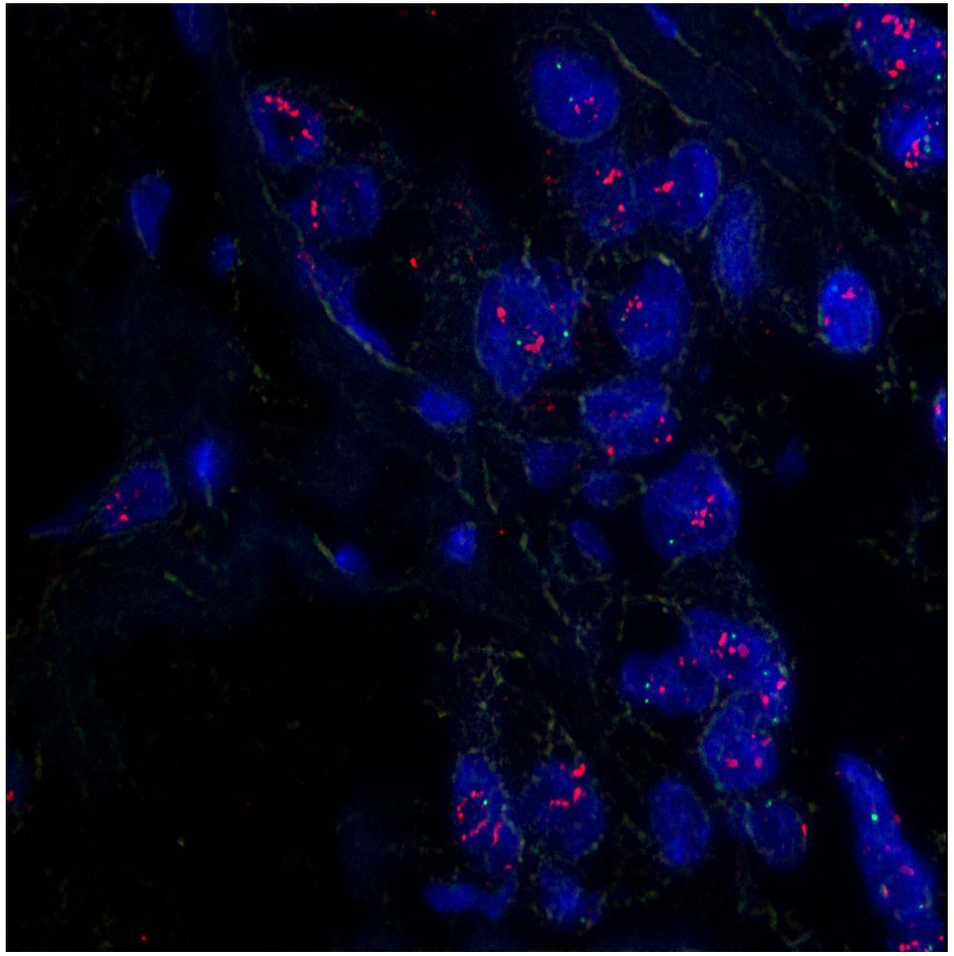


ST-02-0318 P3 20x

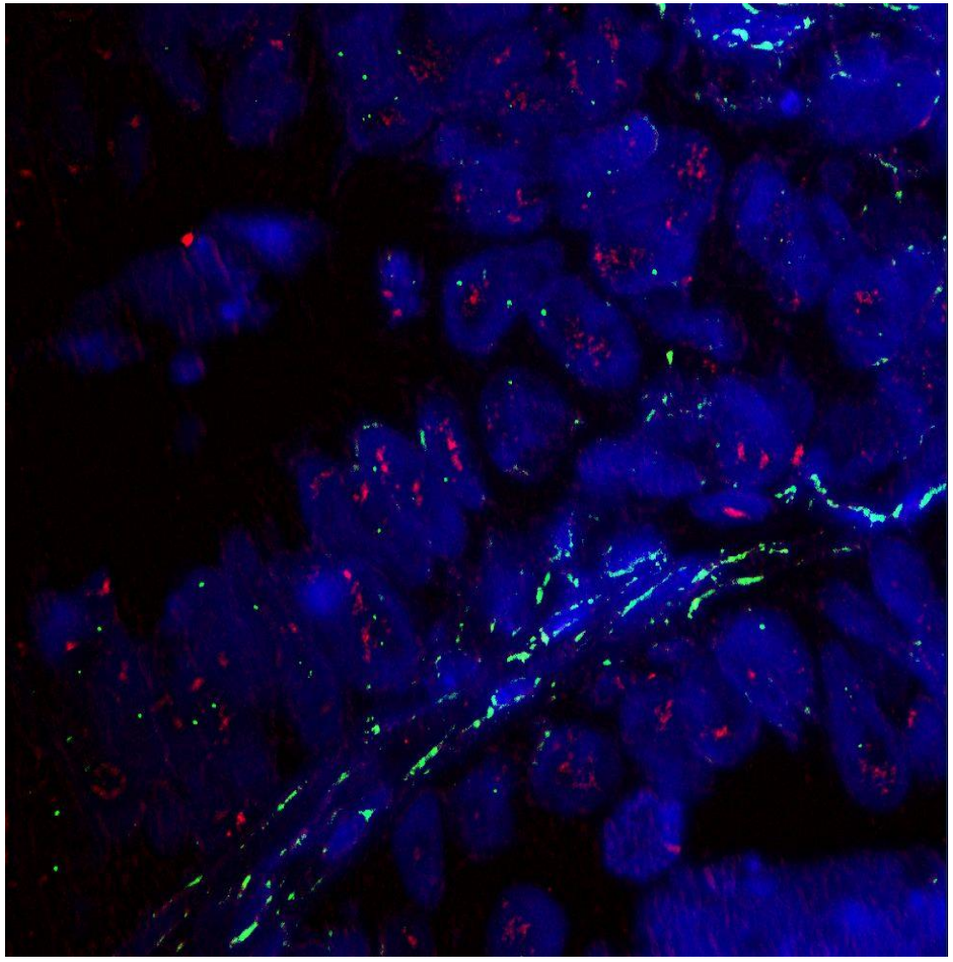


ST-02-0318 P3 40x

HER2 FISH: ST-02-0001 and ST-02-0074

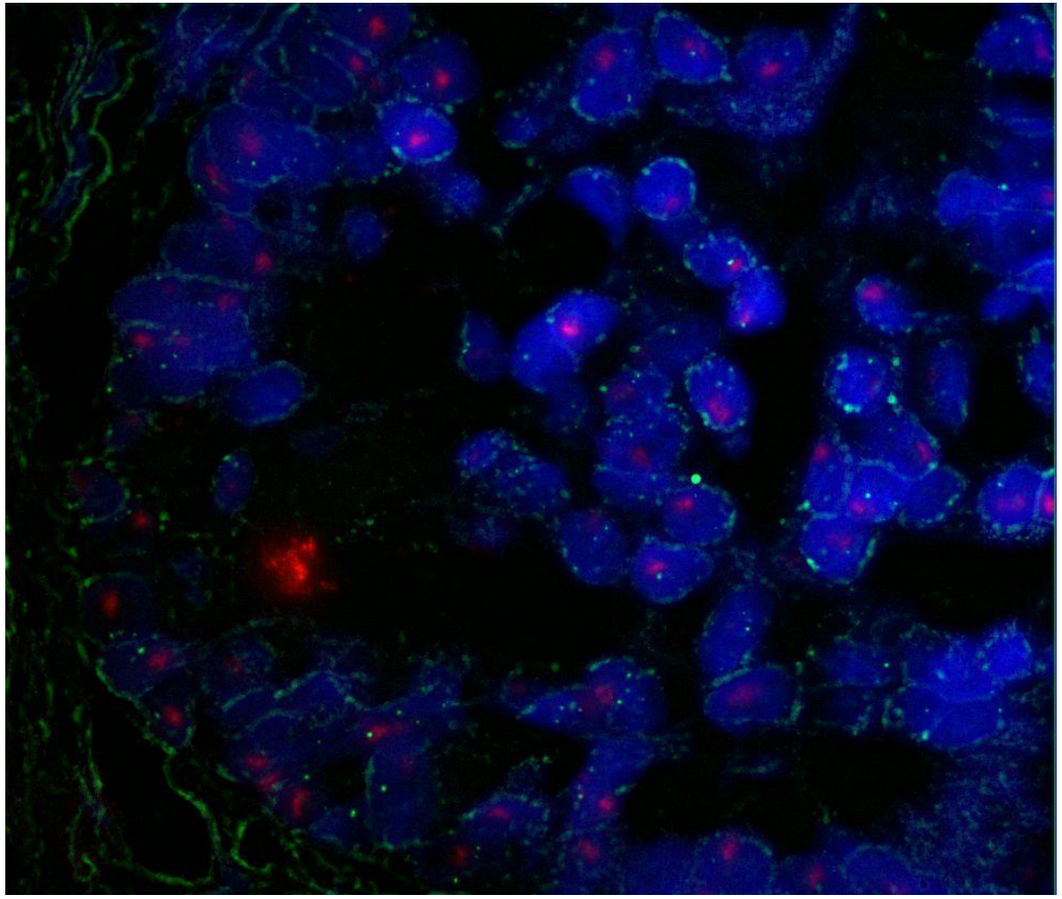


ST-02-0001
HER2 IHC: 3+
HER2 FISH: positive（HER2 cluster amplification）

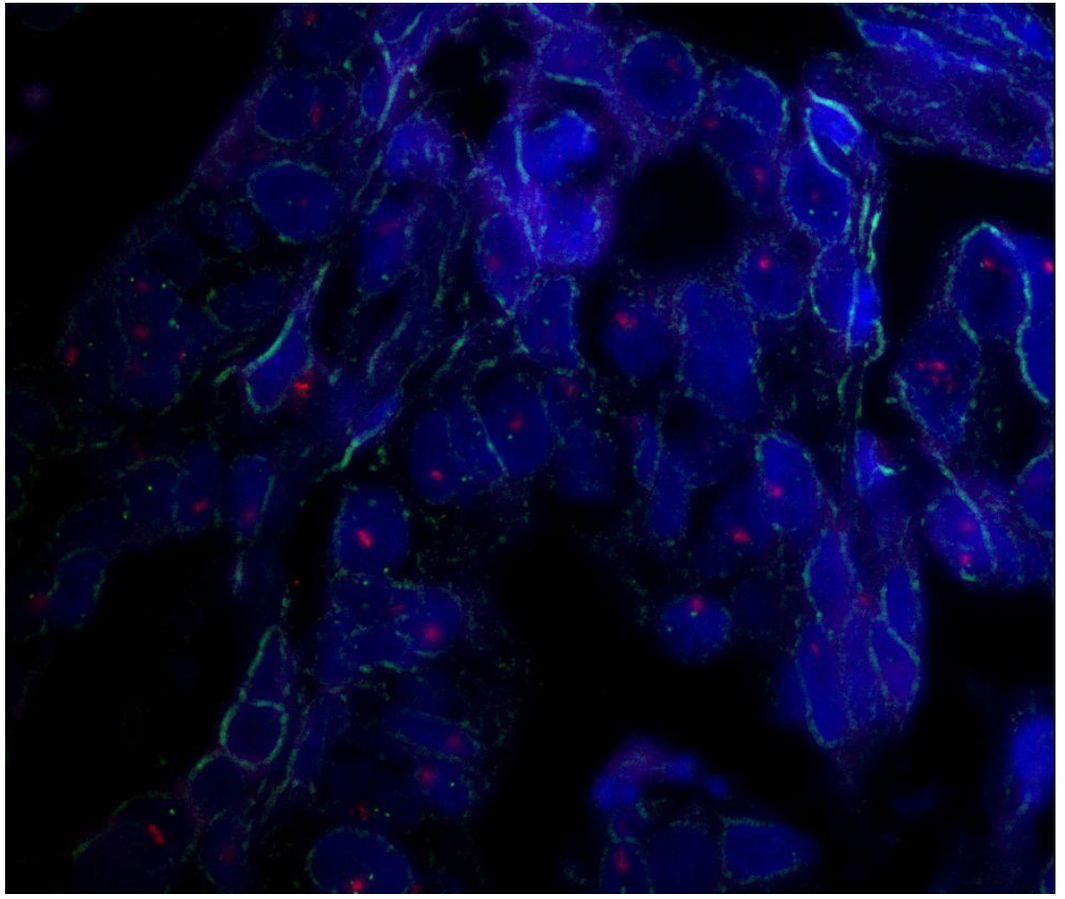


ST-02-0074
HER2 IHC: 2+
HER2 FISH: positive（HER2 cluster amplification）

HER2 FISH: ST-02-0077 and ST-02-0079

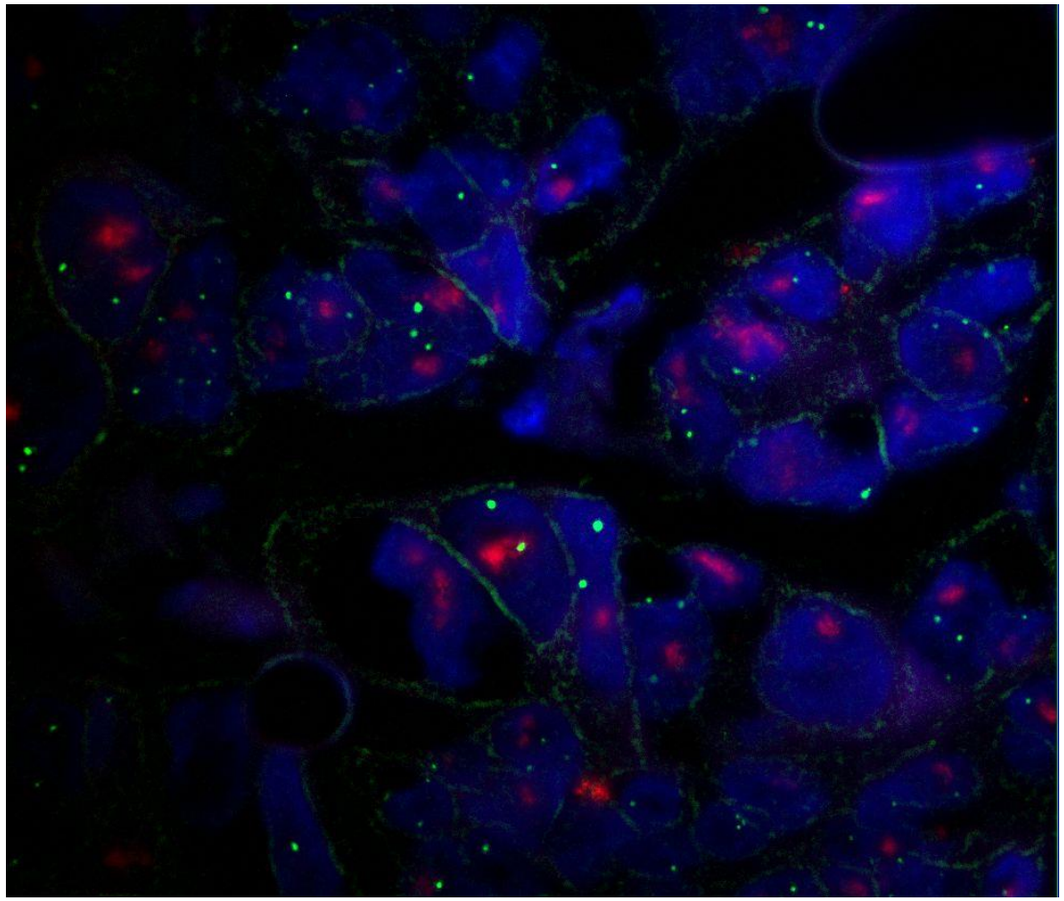


ST-02-0077
HER2 IHC: 3+
HER2 FISH: positive （HER2 cluster amplification）

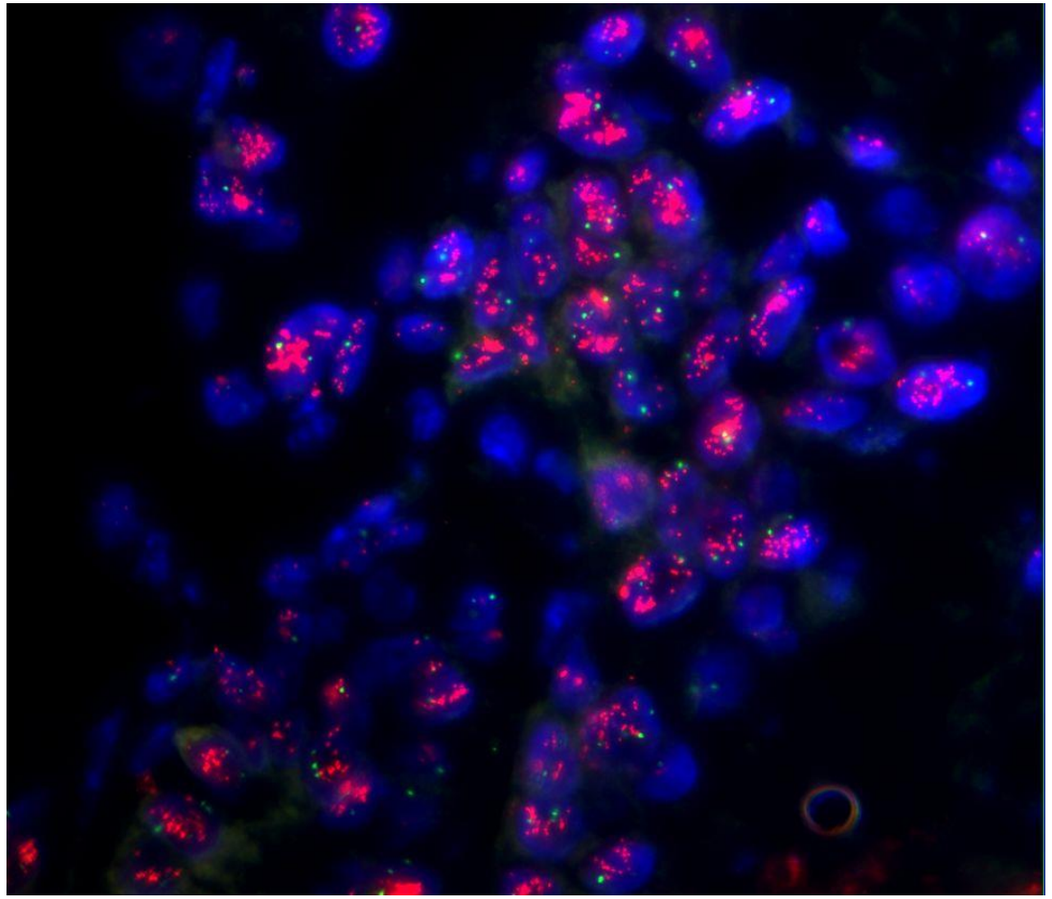


ST-02-0079
HER2 IHC: 2+
HER2 FISH: positive （HER2 cluster amplification）

HER2 FISH: ST-02-0103 and ST-02-0318



ST-02-0103
HER2 IHC: 2+
HER2 FISH: positive（HER2 cluster amplification）



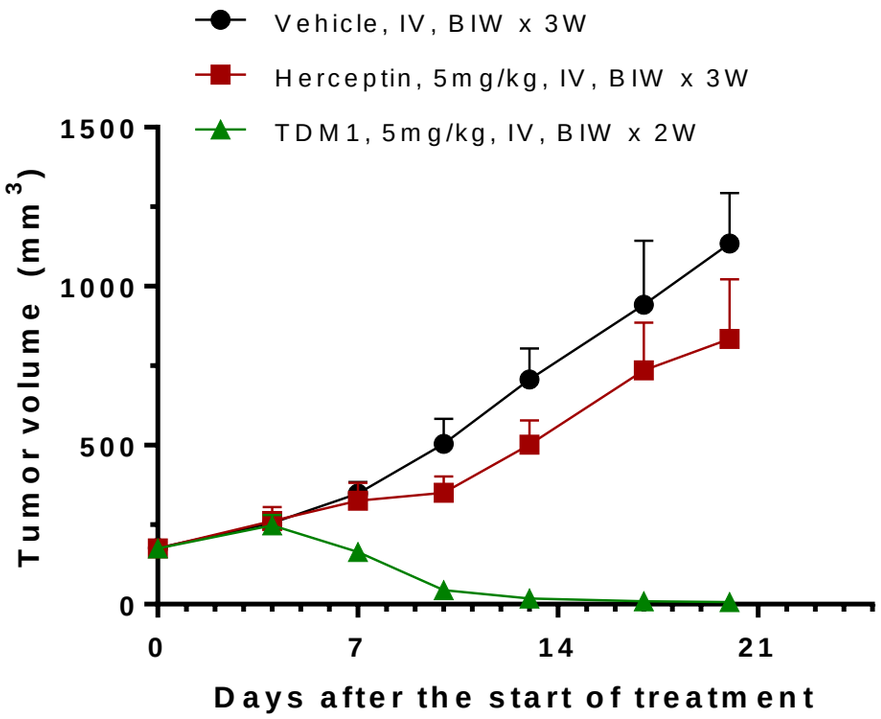
ST-02-0318
HER2 IHC: 2+
HER2 FISH: positive（HER2: CSP17=8.6）

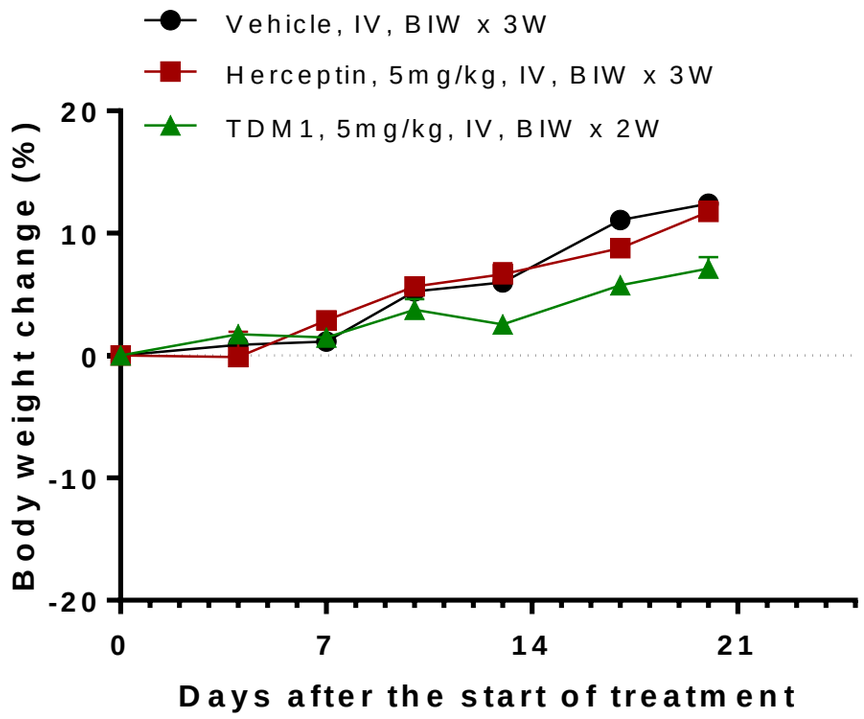
Summary - *In vivo* PDX models-SOC

No.	Cell line	Cancer type	Treatment	TGI (%)	Model genomics
1	ST-02-0077	Gastric cancer	Trastuzumab: 5 mg/kg, IV, BIW x 3W T-DM1: 5 mg/kg, IV, BIW x 2W	Trastuzumab (31) T-DM1 (118)	HER2 overexpression
2	ST-02-0079		Trastuzumab: 5 mg/kg, IV, QW x 3W T-DM1: 5 mg/kg, IV, QW x 3W	Trastuzumab (18) T-DM1 (46)	
3	ST-02-0103		Trastuzumab: 5 mg/kg, IV, BIW x 3W T-DM1: 5 mg/kg, IV, BIW x 3W	Trastuzumab (62) T-DM1 (125)	
4	ST-02-0074		Trastuzumab: 5 mg/kg, IV, D0,3,7,10,14 T-DM1: 5 mg/kg, IV, D0,3,7,10,14	Trastuzumab (88) T-DM1 (115)	
5	BR-05-0434	Breast cancer	Herceptin 5 mg/kg , i.v., BIW	10	
6	BR-05-0452E		Herceptin 5 mg/kg , i.v., BIW	76	

Trastuzumab and T-DM1 in ST-02-0077

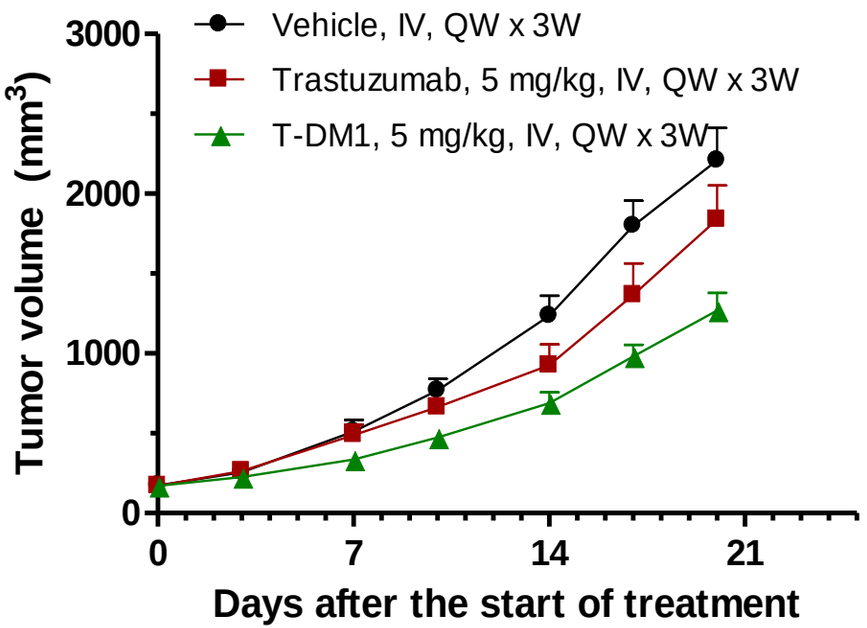
Model ID	Cancer type	Gender	Age	Tumor Grade	Tumor Stage	Target
ST-02-0077	Gastric cancer	Male	52	G2/G3	T3N3M0	HER2 overexpression

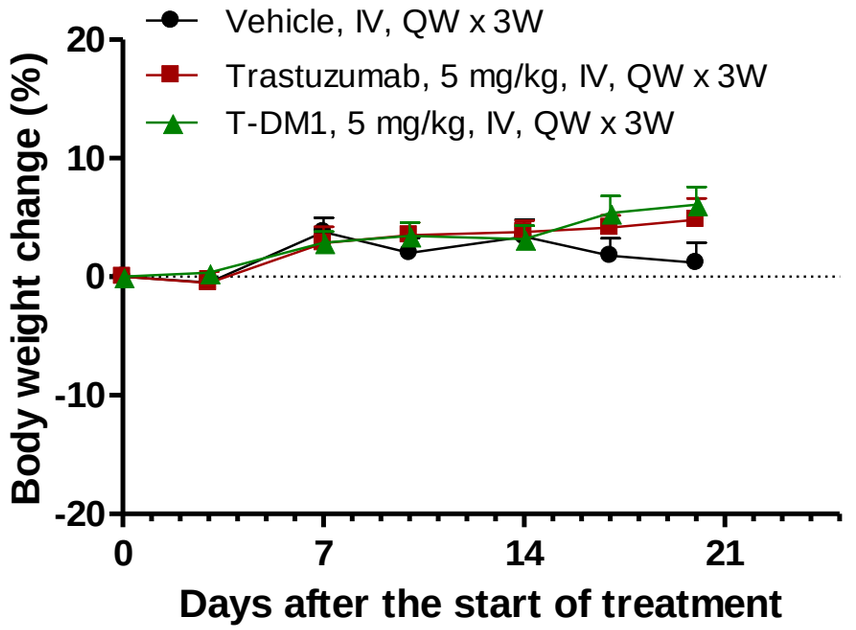




Trastuzumab and T-DM1 in ST-02-0079

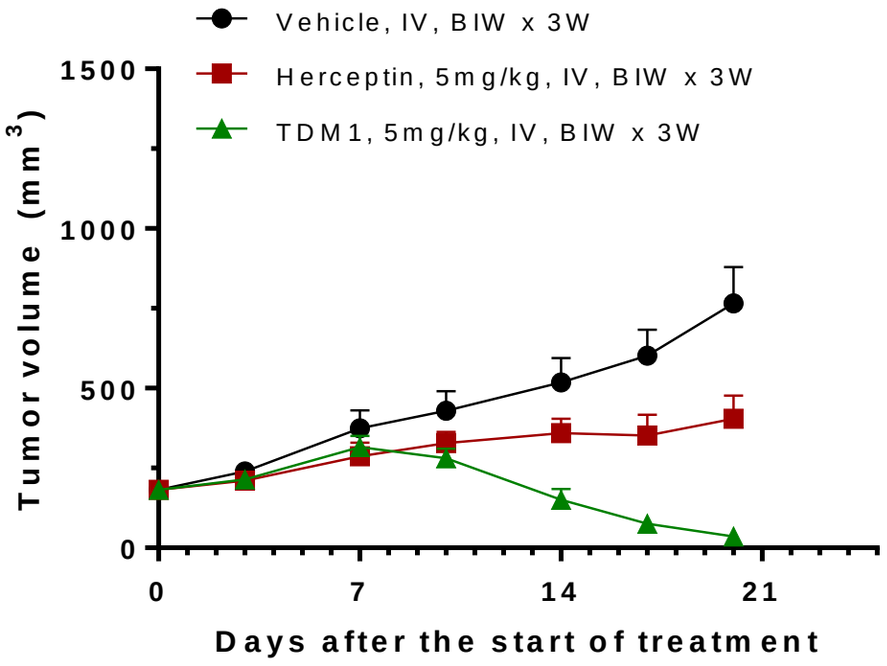
Model ID	Cancer type	Gender	Age	Tumor Grade	Tumor Stage	Target
ST-02-0079	Gastric cancer	Male	53	G2	NA	HER2 overexpression

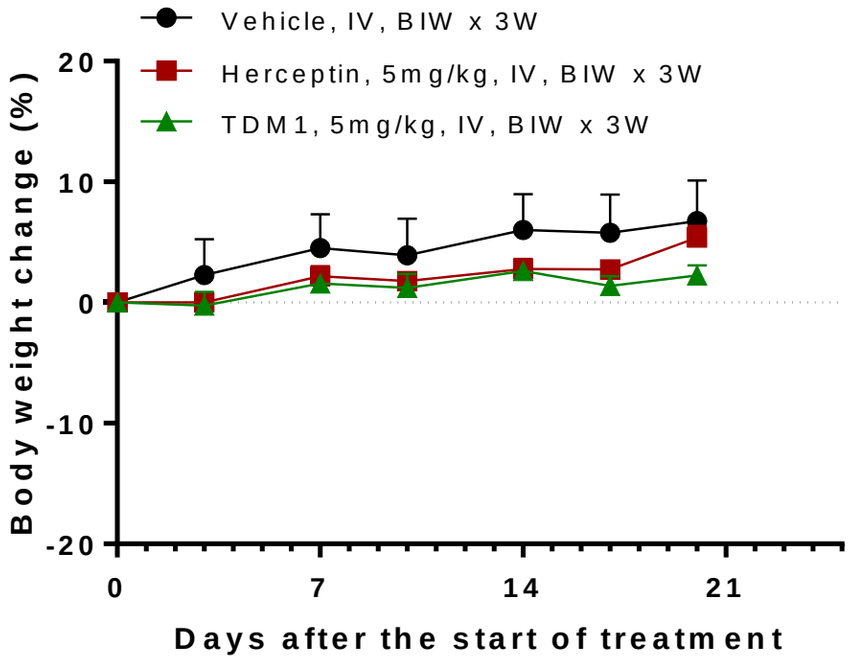




Trastuzumab and T-DM1 in ST-02-0103

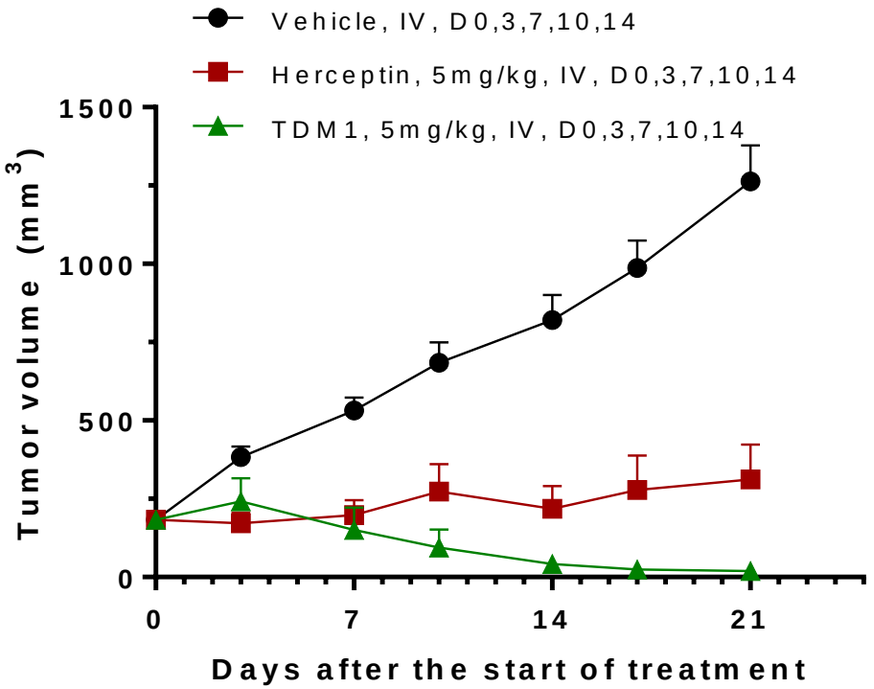
Model ID	Cancer type	Gender	Age	Tumor Grade	Tumor Stage	Target
ST-02-0103	Gastric cancer	Male	59	G2	T3N0M0	HER2 overexpression

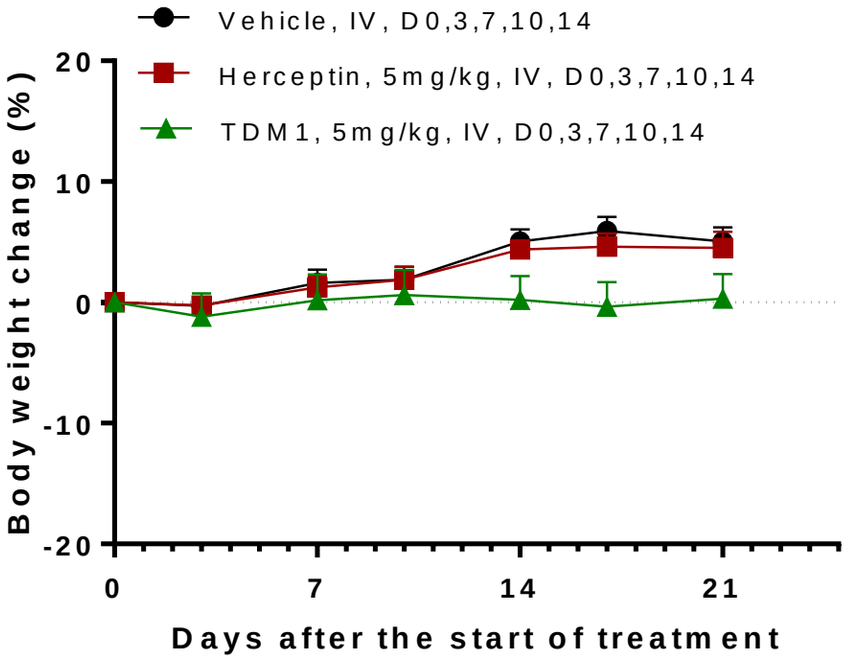




Trastuzumab and T-DM1 in ST-02-0074

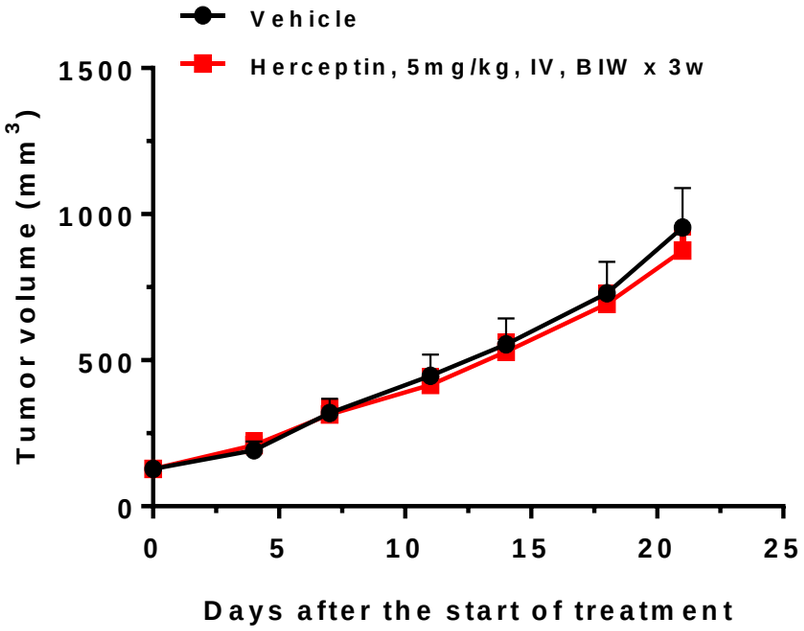
Model ID	Cancer type	Gender	Age	Tumor Grade	Tumor Stage	Target
ST-02-0074	Gastric cancer	Male	78	G2	T3N0M0	HER2 overexpression

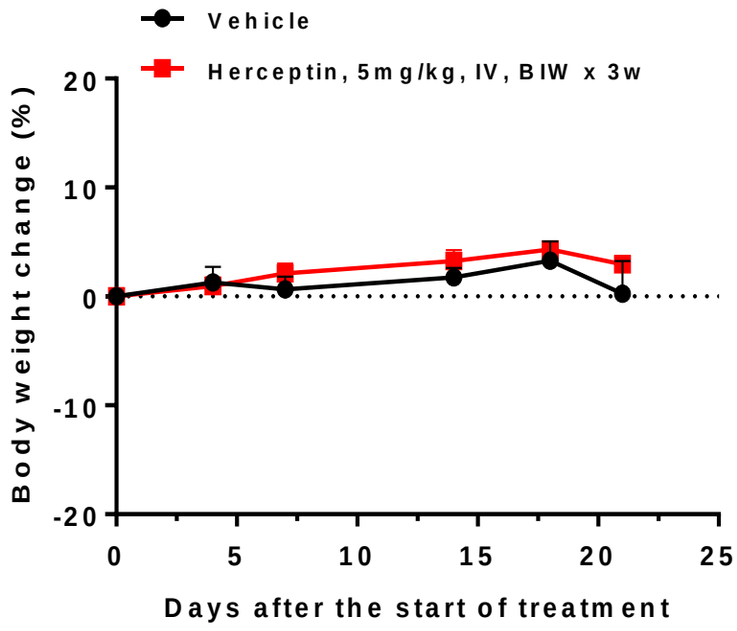




Herceptin in BR-05-0434 Breast Cancer PDX Model

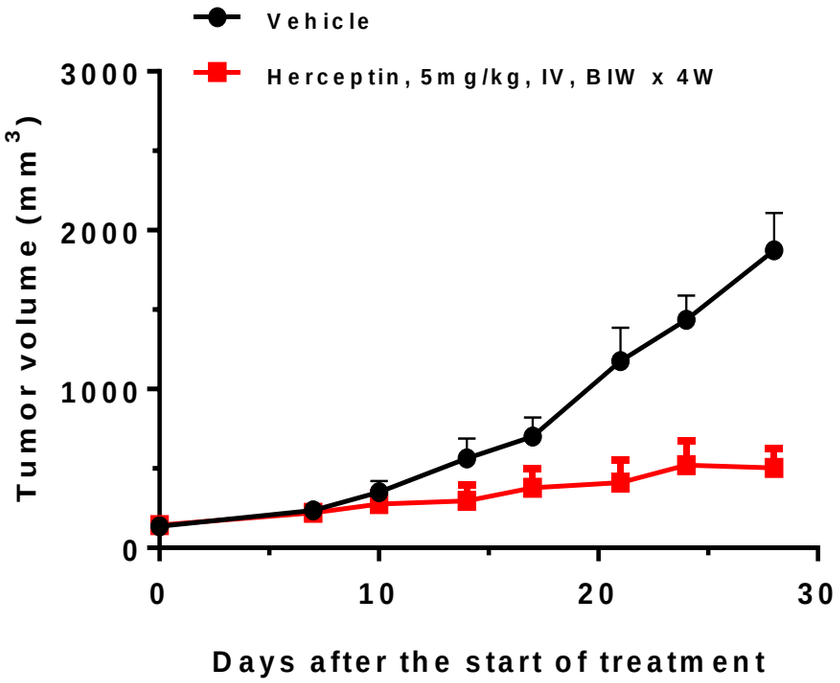
Model ID	Cancer Type	Pathology diagnosis	Pathology diagnosis (PDX)	Gender	Age	Tumor Grade	Tumor Stage
BR-05-0434	Breast Cancer	ER(-), PR(-), HER2(in situ+, infiltrating 3+)	HER2 positive	Female	41	High grade	NA

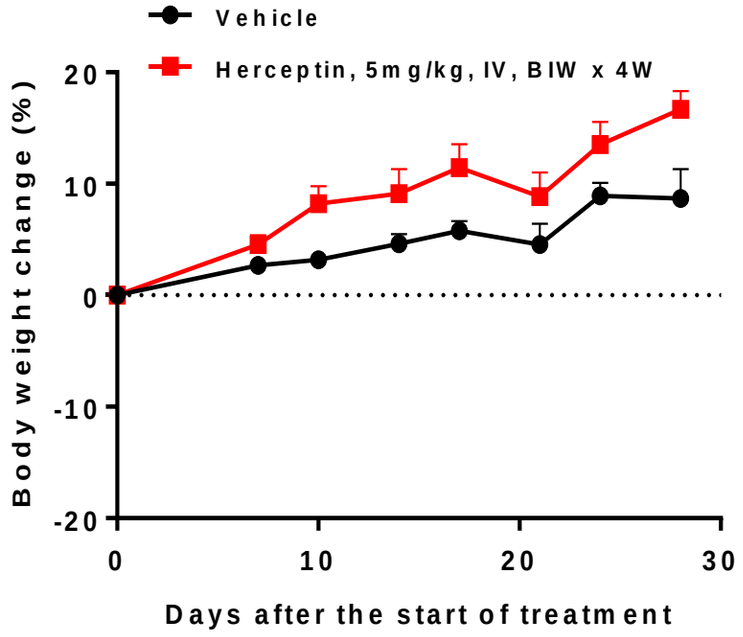




Herceptin in BR-05-0452E Breast Cancer PDX Model

Model ID	Cancer Type	Pathology diagnosis	Pathology diagnosis (PDX)	Gender	Age	Tumor Grade	Tumor Stage
BR-05-0452E	Breast Cancer	ER(-), PR(-), HER2(3+)	HER2 positive	Female	NA	III	NA





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