

Enhertu-induced resistant tumor models



WuXi Biology, Oncology & Immunology Unit



2023.09

Outline

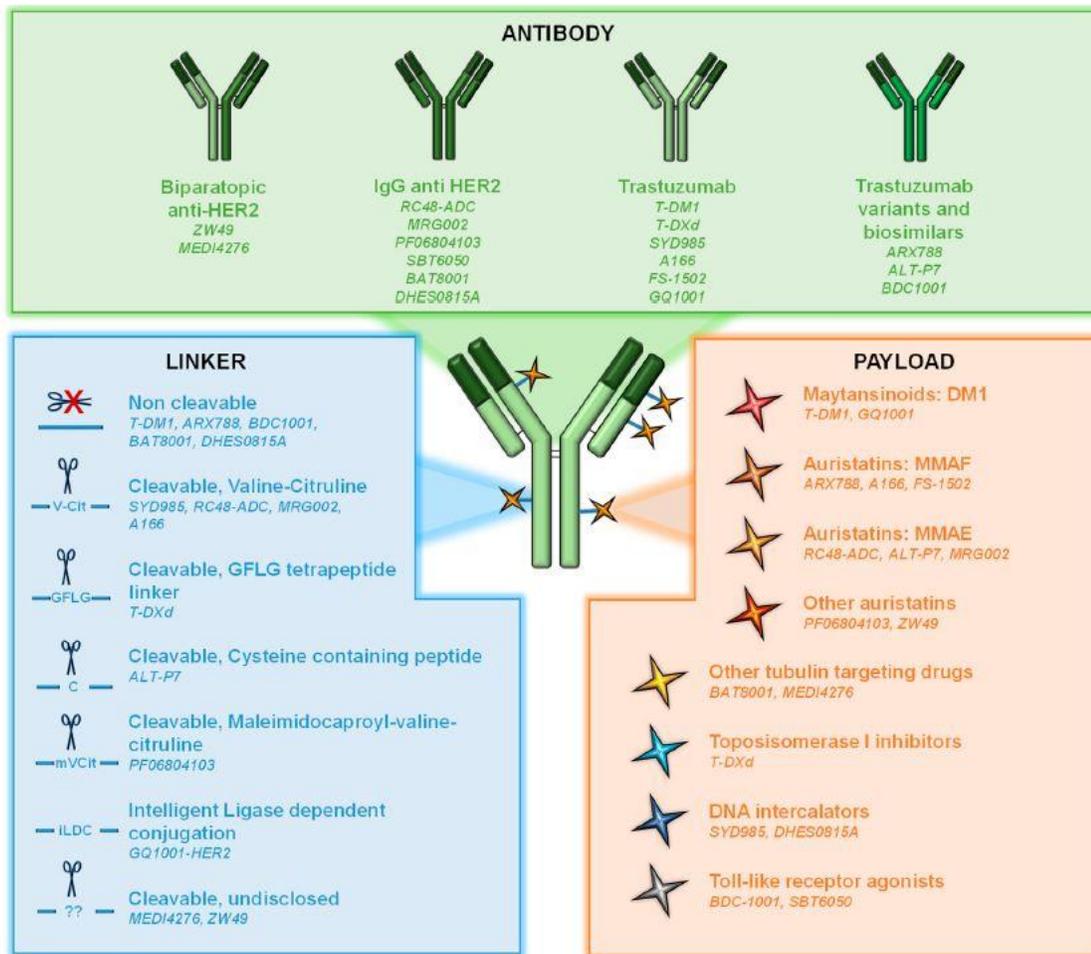
■ Background

- HER2 Targeted Antibody-drug Conjugates (ADCs)
- Mechanism of action of HER2 ADCs
- Mechanism of resistance to HER2 ADCs

■ Enhertu-induced resistant tumor cell lines and *in vivo* models

- Payload dysfunction: NCI-N87/Enhertu-R
- HER2 expression down-regulation: HCC1954/Enhertu-R and NCI-H2170/Enhertu-R

HER2-targeted antibody-drug conjugates (ADCs)



■ Trastuzumab emtansine (T-DM1), Trastuzumab deruxtecan (T-DXd, DS8201) and Disitamab vedotin (RC48) have been approved by the FDA.

TABLE 3 | Summary of anti-HER2 ADCs in clinical investigation.

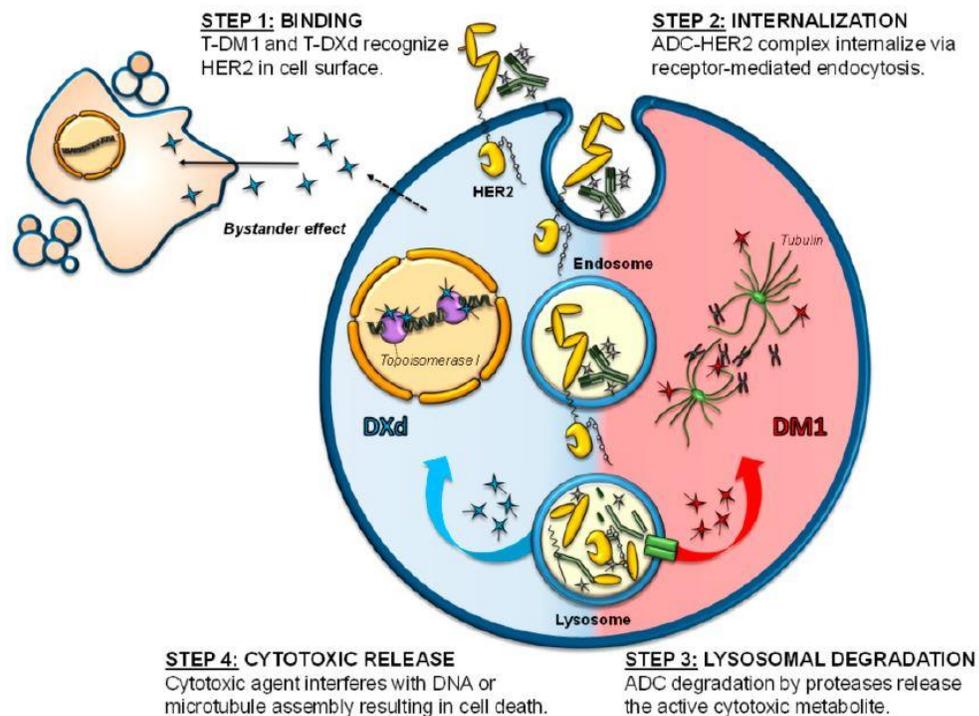
ADC	mAb	Payload	Linker	DAR	Conditions	Clinical phase	Company
Trastuzumab duocarmazine (SYD985)	Trastuzumab	seco-DUBA	Cleavable vc linker	2.7	Breast cancer, endometrial cancer	I (solid tumors, HER2-positive, HER2-low and metastatic breast cancer) VII (metastatic breast cancer) II (endometrial cancer)	Byondis
ARX-788	Anti-HER2 mAb (ARX269)	MMAF	Non-cleavable linker conjugated to pAcF	1.9	Breast cancer, gastric cancer	I (breast neoplasms, gastric neoplasm, solid tumors) II (metastatic breast cancer, breast and gastric neoplasm cancer, breast cancer with low expression of HER2)	Ambrx
A166	Anti-HER2 mAb	Duostatin-5	Cleavable vc linker	n/a	Solid tumors expressing HER2 or having amplified HER2 gene	I/II	Klus
MRG002	Anti-HER2 mAb	MMAE	Cleavable vc linker	3.8	Breast cancer, gastric cancer, gastroesophageal junction cancer, non-small cell lung cancer, urothelial cancer, biliary tract cancer	I (advanced solid tumor) II (locally advanced gastric cancer and metastatic gastroesophageal junction cancer, non-small cell lung cancer, advanced or metastatic breast cancer, locally advanced or metastatic urothelial cancer, advanced or metastatic biliary tract cancer)	Miracogen
ALT-P7	Trastuzumab biobetter (HM2)	MMAE	Cleavable cysteine-containing peptide linker	2	Breast cancer	I	Alteogen
GQ1001	Trastuzumab	DM1	n/a	n/a	Breast cancer, gastric cancer, advanced solid tumor	I	GeneQuantum
SBT6050	Anti-HER2 mAb	Toll-like receptor 8 agonist	n/a	n/a	Breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer	I (solid tumors), II (breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer)	Silverback

Díaz-Rodríguez E et al. *Cancers*. 2021 Dec

Frontiers in Molecular Biosciences. February 2022

■ HER2 has been found to be amplified and overexpressed in a number of human cancers, contributing to tumor development, cell cycle progression, and cellular motility and growth.

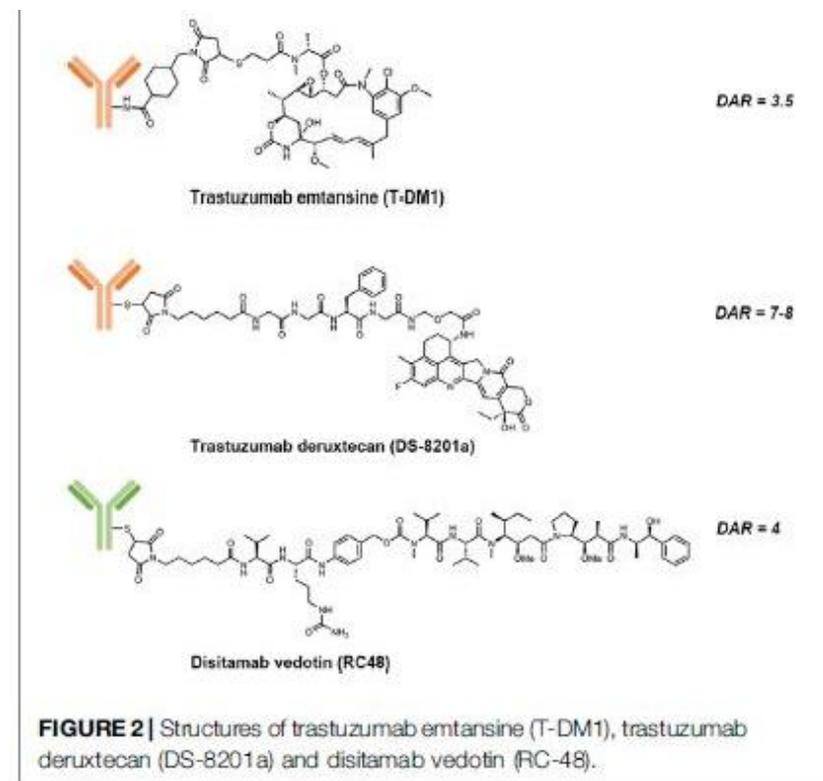
Mechanism of action of HER2 ADCs



- **Specific binding:** binding of the monoclonal anti-HER antibody component to HER2 expressed tumor cells on the cell surface;
- **Internalization:** ADC-HER2 complex is internalized by endocytosis;
- **Linker cleavage:** linker cleavage by lysosomal proteases, the payload is released
- **Cytotoxic effect:** high drug-to-antibody ratio(DAR) increases anti-tumor effect
- **ADCC effect:** mediating killer cells to kill cancer cells directly
- **Bystander killing effect:** the payload kills neighboring cancer cells with a low HER2 expression

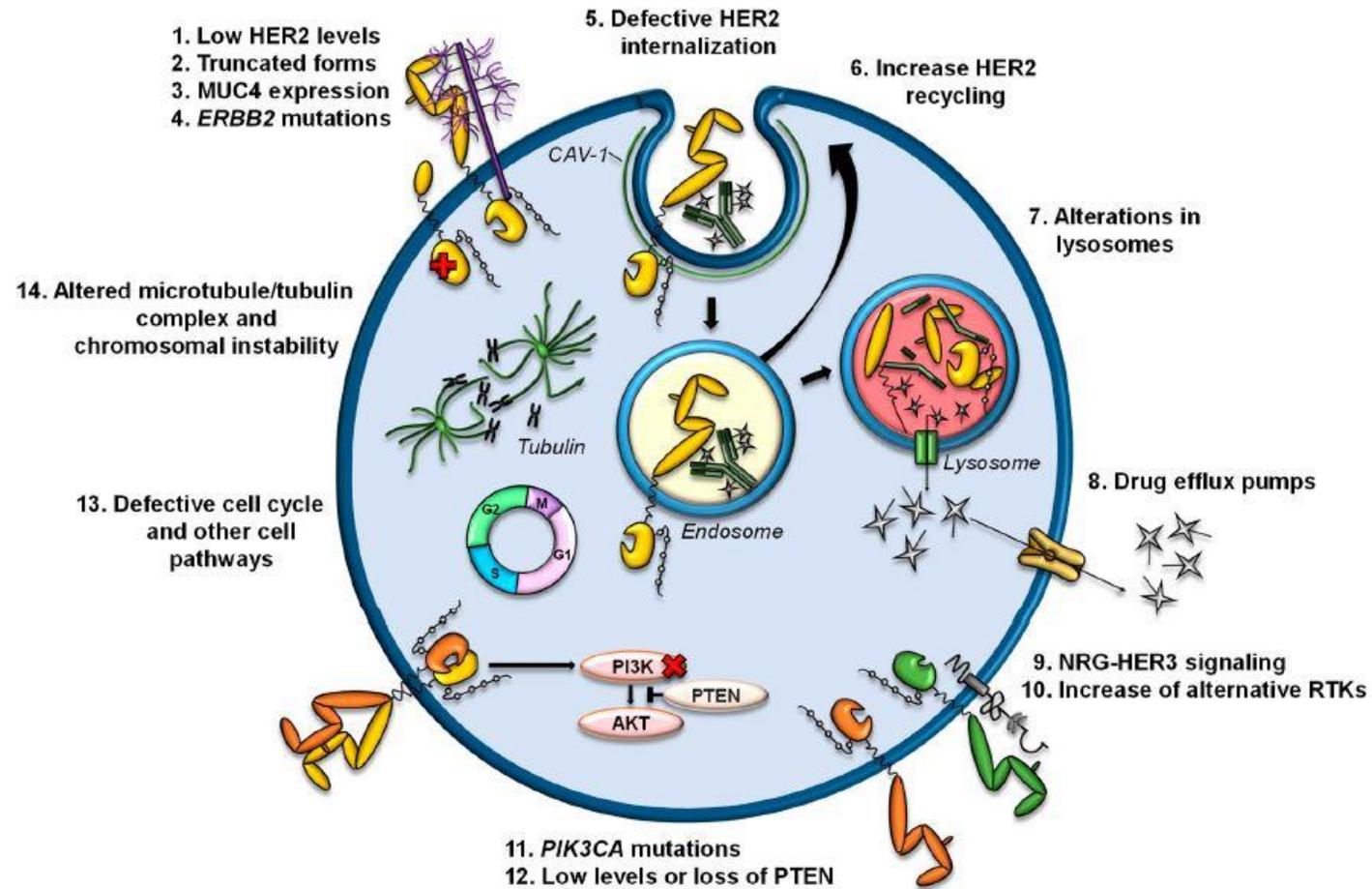
MoA of Enhertu

1. HER2 signaling blockade
2. Bystander cytotoxicity
3. Payload-mediated DNA relegation prevention, generating single and double-strand breaks



Frontiers in Molecular Biosciences. February 2022

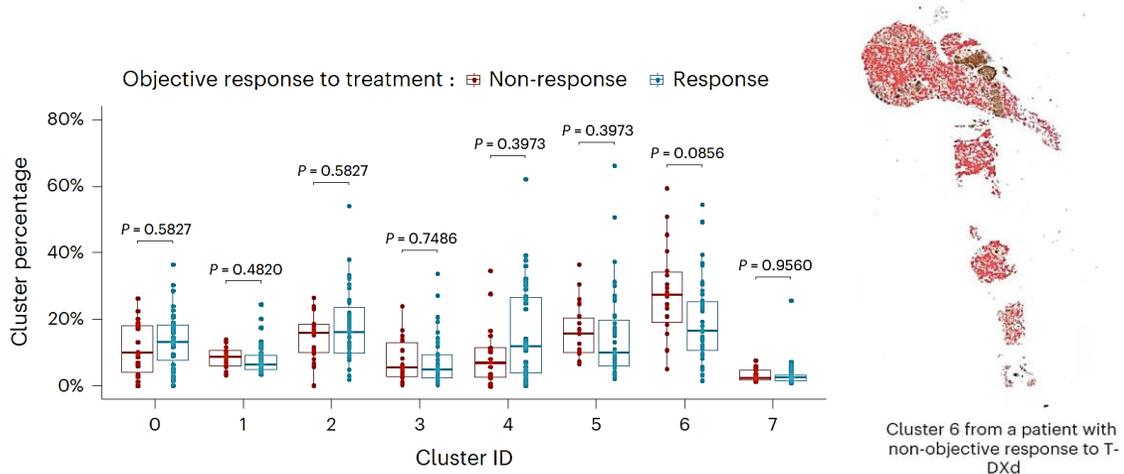
Mechanism of resistance to HER2 ADCs



Díaz-Rodríguez E et al. *Cancers*. 2021 Dec

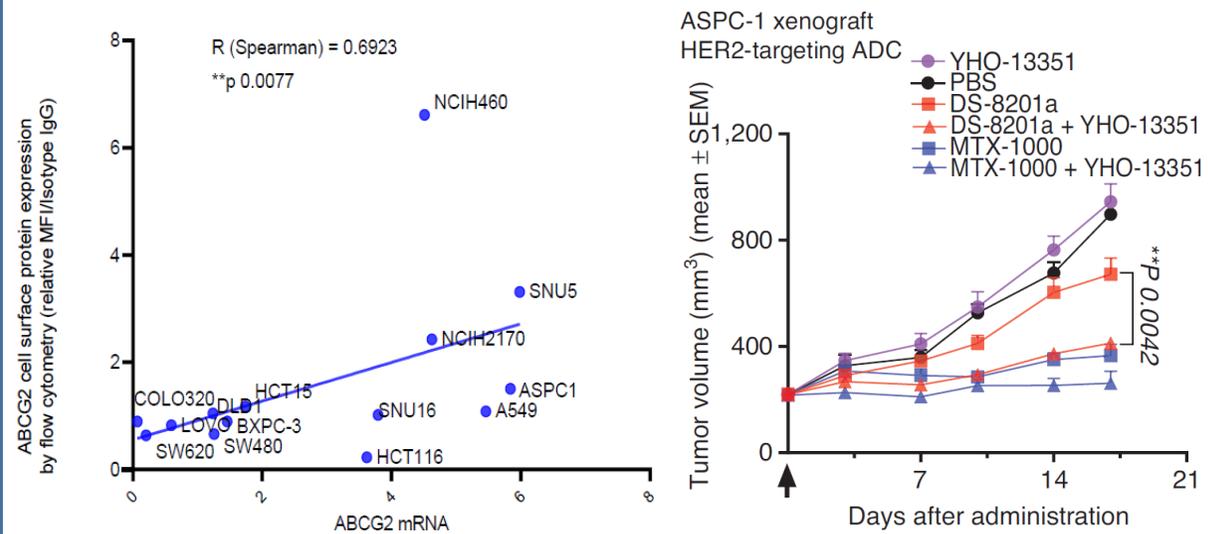
Mechanism of resistance to HER2 ADCs

➤ Some patients who have decreased HER2 expression showed resistance to T-DXd.



Mosele F et al. Nat Med. 2023 Aug

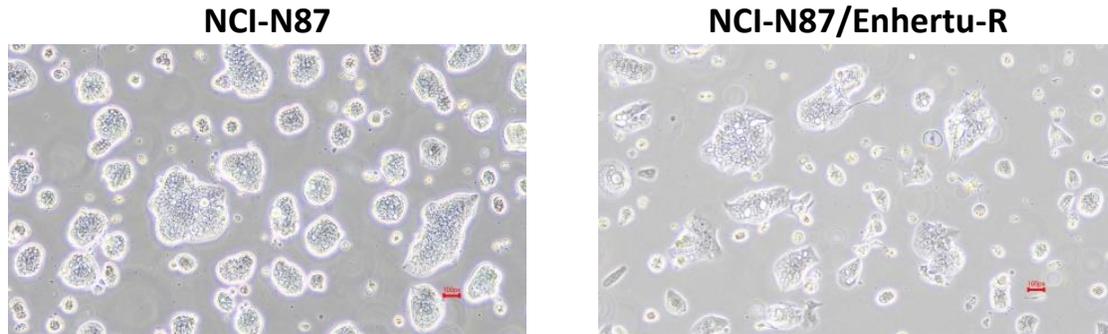
➤ Resistance to T-DXd may also be caused by the upregulation of multidrug-resistant (MDR) signals or the metabolism of payload.



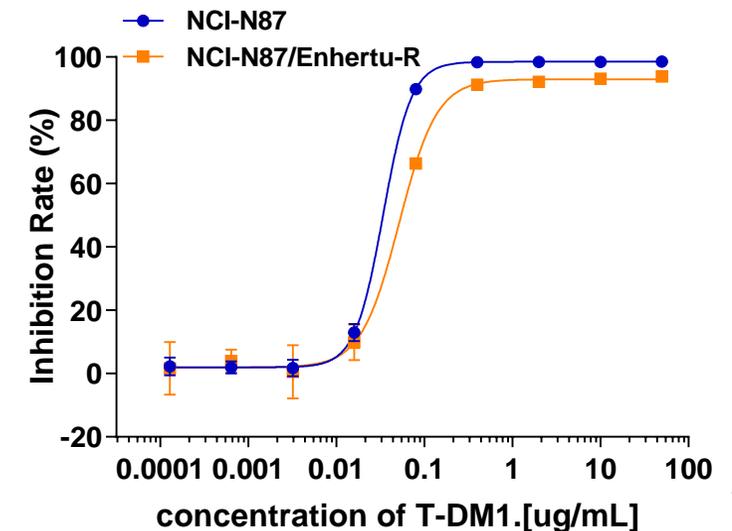
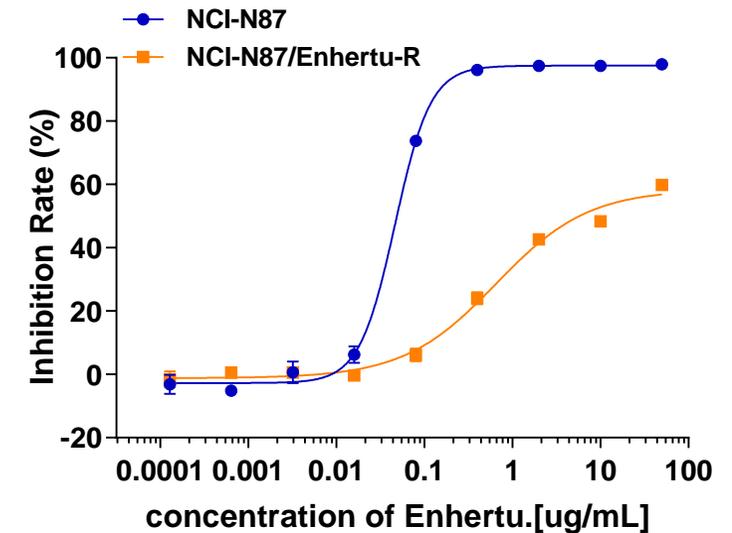
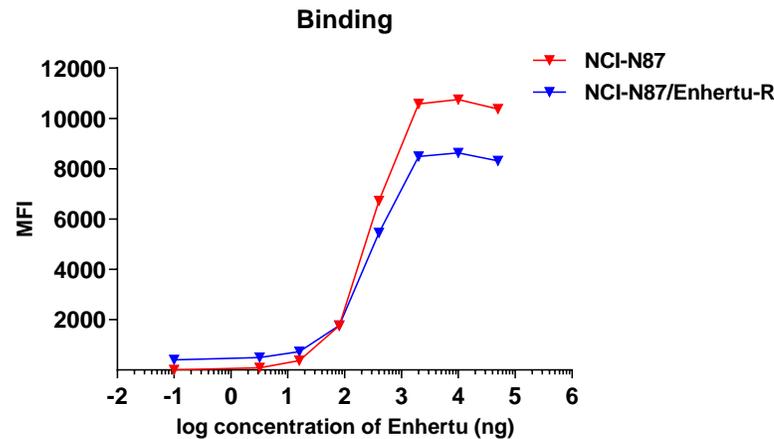
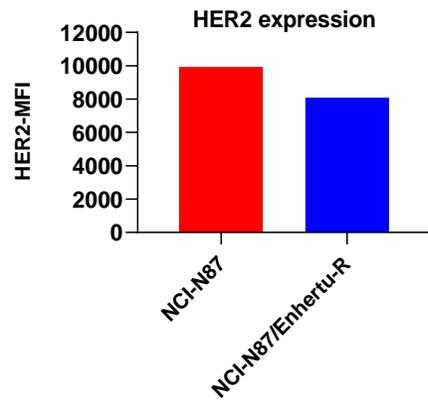
Weng W et al. Cancer Discov. 2023 Apr

Establishment of Enhertu-induced resistant NCI-N87 cell line

In vitro validation



- NCI-N87/Enhertu-R cell line was established through chronic exposure to 100 ng/mL of Enhertu.

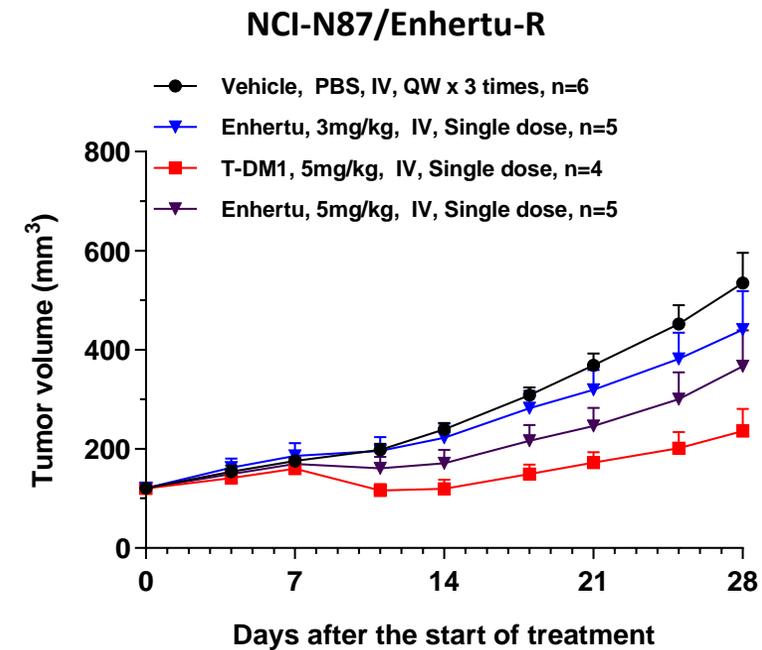
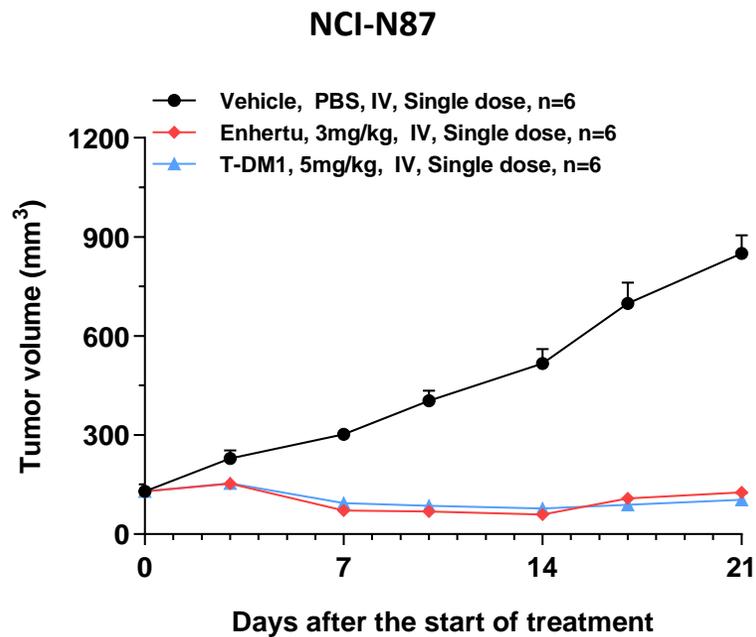


ADC	Cell line	ReIC50 (µg/ml)	AbsIC50 (µg/ml)	Bottom (%)	Top (%)
Enhertu	NCI-N87	0.0464	0.0487	-2.73	97.52
	NCI-N87/Enhertu-R	0.651	5.761	-1.21	58.42
T-DM1	NCI-N87	0.0340	0.0339	1.95	98.51
	NCI-N87/Enhertu-R	0.0516	0.0545	1.96	92.98

Establishment of Enhertu-induced resistant NCI-N87 cell line

In vivo validation

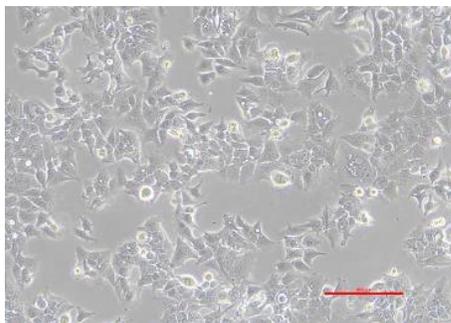
Model ID	Cancer type	Drugs tested	Dosage	TGI
NCI-N87	Lung cancer	T-DM1	5 mg/kg, single dose	103.5%
		Enhertu	3 mg/kg, single dose	100.36%
NCI-N87/Enhertu-R	Lung cancer	T-DM1	5 mg/kg, single dose	71.89%
		Enhertu	3 mg/kg, single dose	22.73%
		Enhertu	5 mg/kg, single dose	40.63%



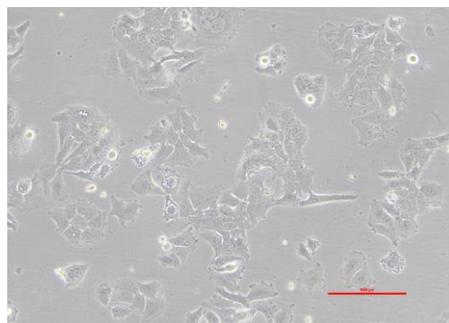
Establishment of Enhertu-induced resistant HCC1954 cell line

In vitro validation

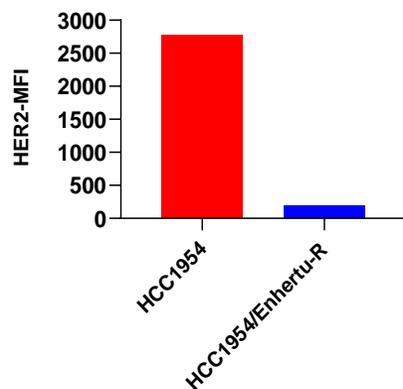
HCC1954



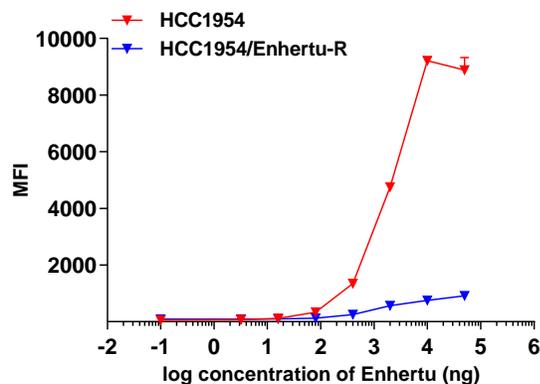
HCC1954/Enhertu-R



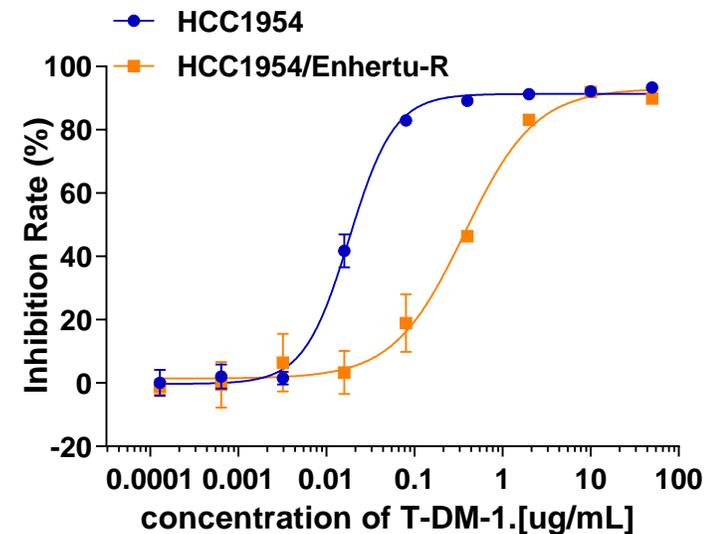
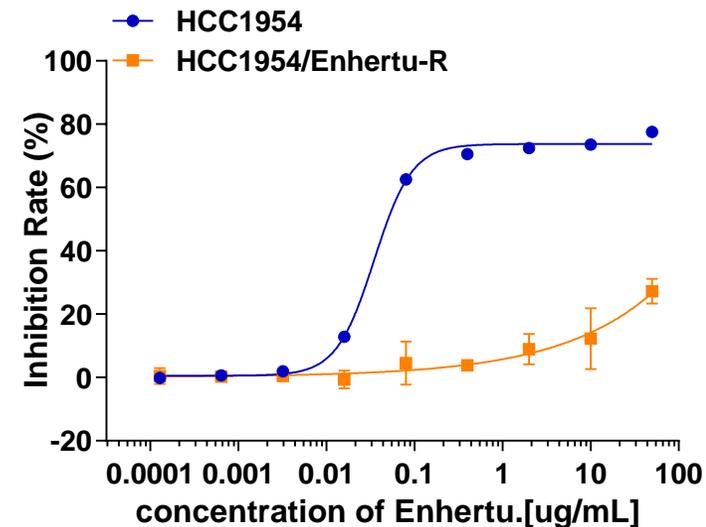
HER2 expression



Binding assay



- HCC1954/Enhertu-R cell line was established through chronic exposure to 45 ng/mL of Enhertu.



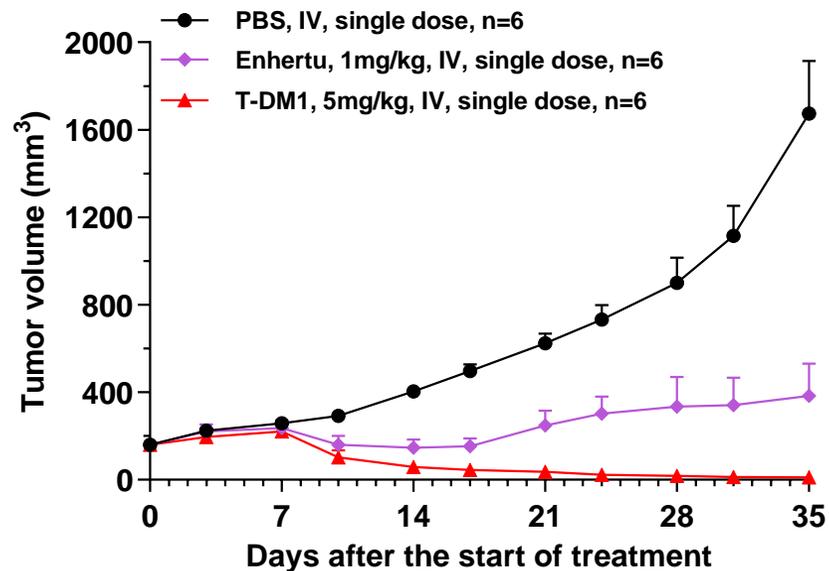
ADC	Cell line	ReIC50 (μg/ml)	AbsIC50 (μg/ml)	Bottom (%)	Top (%)
Enhertu	HCC1954	0.0501	0.0349	0.56	73.74
	HCC1954/Enhertu-R	244.55	>50	0.15	27.23
T-DM1	HCC1954	0.0181	0.0203	-0.29	91.34
	HCC1954/Enhertu-R	0.3689	0.4126	1.42	93.06

Establishment of Enhertu-induced resistant HCC1954 cell line

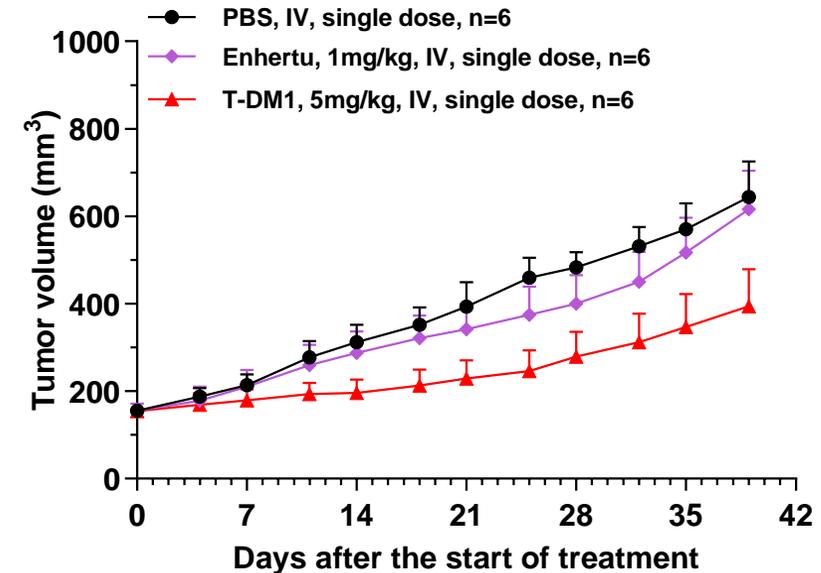
In vivo validation

Model ID	Cancer type	Drugs tested	Dosage	TGI
HCC1954	Breast cancer	T-DM1	5 mg/kg, single dose	109.88%
		Enhertu	1 mg/kg, single dose	85.25%
HCC1954/Enhertu-R	Breast cancer	T-DM1	5 mg/kg, single dose	51.11%
		Enhertu	1 mg/kg, single dose	5.71%

HCC1954



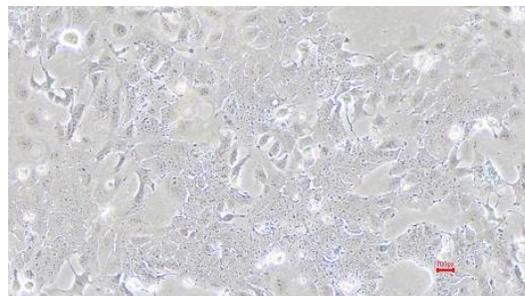
HCC1954/Enhertu-R



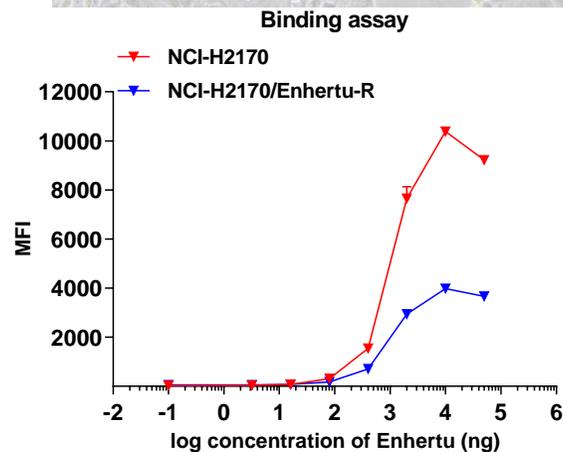
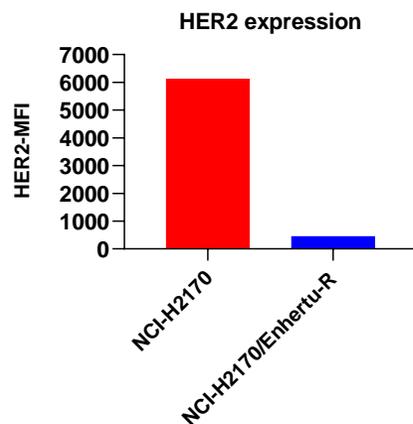
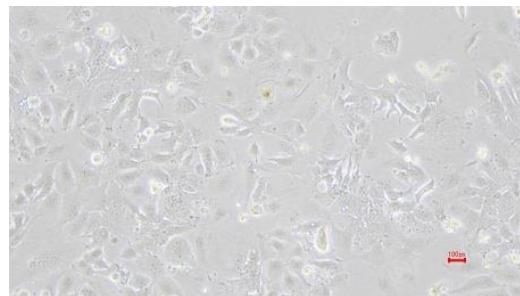
Establishment of Enhertu induced resistant NCI-H2170 cell line

In vitro validation

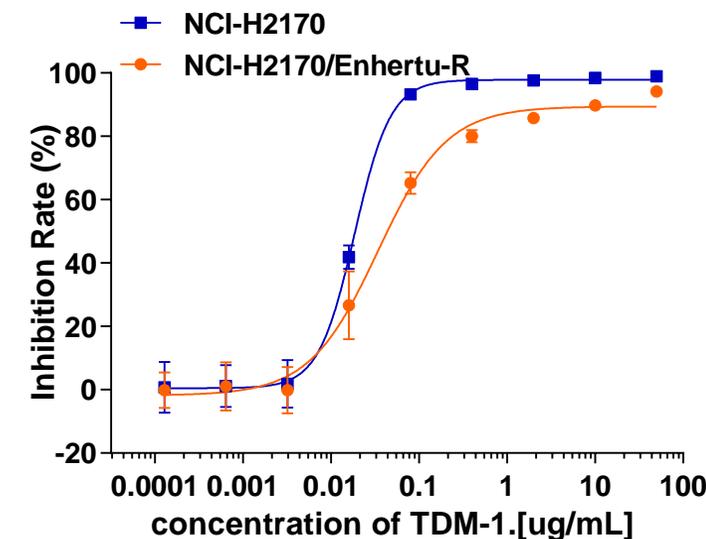
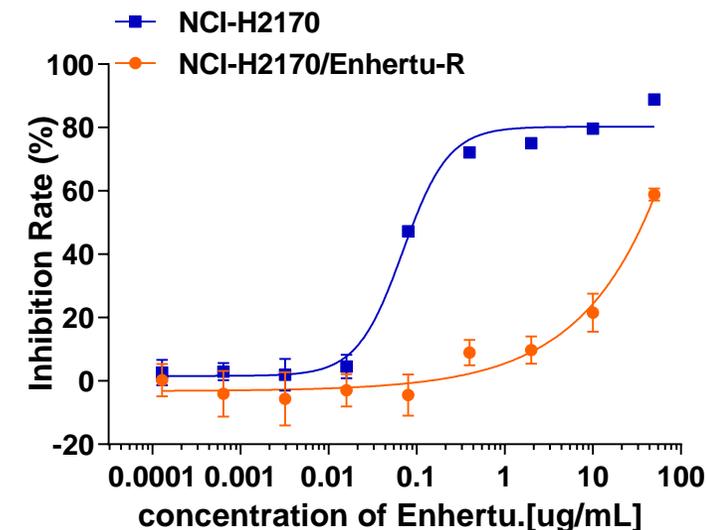
NCI-H2170



NCI-H2170/Enhertu-R



- NCI-H2170/Enhertu-R cell line was established through chronic exposure to 100 ng/mL of Enhertu.

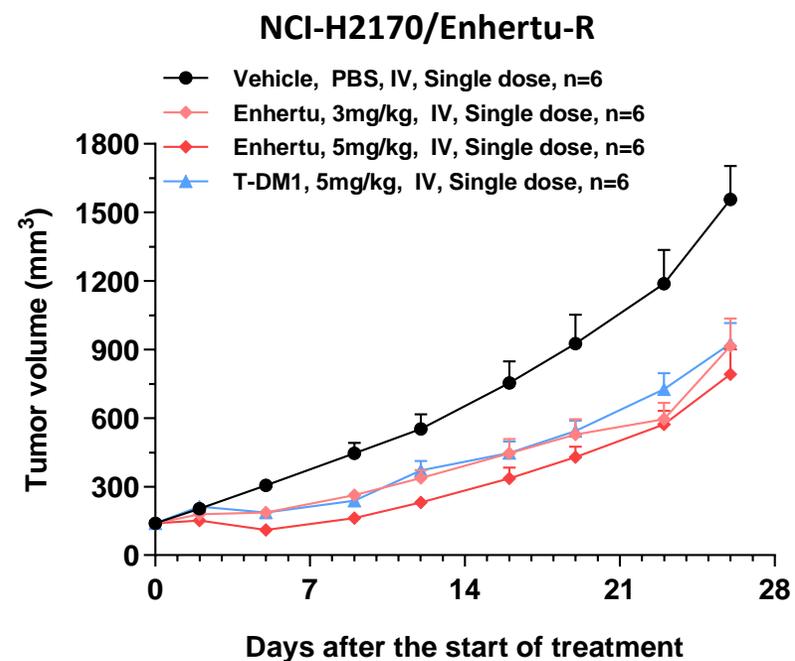
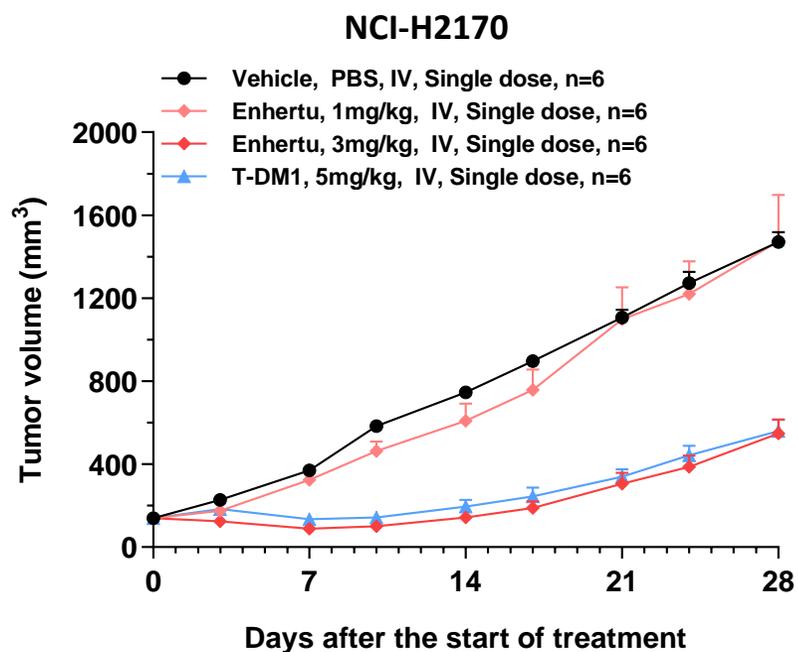


ADC	Cell line	ReIC50 (μg/ml)	AbsIC50 (μg/ml)	Bottom (%)	Top (%)
Enhertu	NCI-H2170	0.07	0.092	1.58	80.26
	NCI-H2170/Enhertu-R	>50	37.580	-3.22	100
T-DM1	NCI-H2170	0.0185	0.0188	0.47	97.84
	NCI-H2170/Enhertu-R	0.0342	0.0437	-1.82	89.43

Establishment of Enhertu induced resistant NCI-H2170 cell line

In vivo validation

Model ID	Cancer type	Drugs tested	Dosage	TGI
NCI-H2170	Lung cancer	T-DM1	5 mg/kg, single dose	68.47%
		Enhertu	1 mg/kg, single dose	-0.35%
		Enhertu	3 mg/kg, single dose	69.27%
NCI-H2170/Enhertu-R	Lung cancer	T-DM1	5 mg/kg, single dose	44.53%
		Enhertu	3 mg/kg, single dose	45.22%
		Enhertu	5 mg/kg, single dose	54.03%





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