

AACR VIRTUAL SPECIAL CONFERENCE

TUMOR IMMUNOLOGY AND IMMUNOTHERAPY

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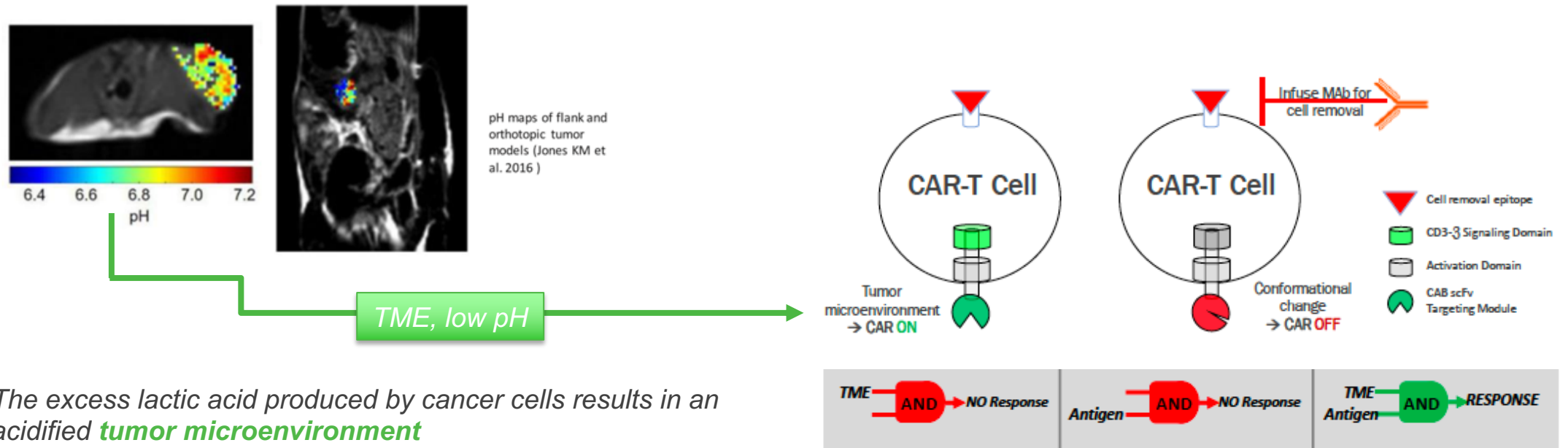
Logic-gating HER2 CAR-T to the tumor microenvironment mitigates on-target, off-tumor toxicity without compromising cytotoxicity against HER2-over-expressing tumors

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Background: Using the tumor microenvironment (TME), the Warburg effect can be used to activate “logic gated” CAR-T cells

- With its elevated receptor copy number and relatively homogeneous expression following gene amplification, HER2 represents an attractive antigen to target via CAR-T; unfortunately, severe toxicity related to off-tumor binding of the CAR-T to HER2 present in normal tissue may limit the use of HER2 CAR-T therapy
- To circumvent this issue, a “logic-gated” HER2-targeted CAR-T was designed that preferentially recognizes HER2 in the tumor microenvironment (TME), thereby limiting on-target toxicity of low HER2 levels expressed in normal tissue
- Most cancer cells rely on aerobic glycolysis to generate energy for cellular processes thereby producing an over-abundance of lactic acid and acidifying the surrounding TME, a process known as the “Warburg effect”



- The excess lactic acid produced by cancer cells results in an acidified **tumor microenvironment**
- This activates the CAR-T cells so that they are in the ON state

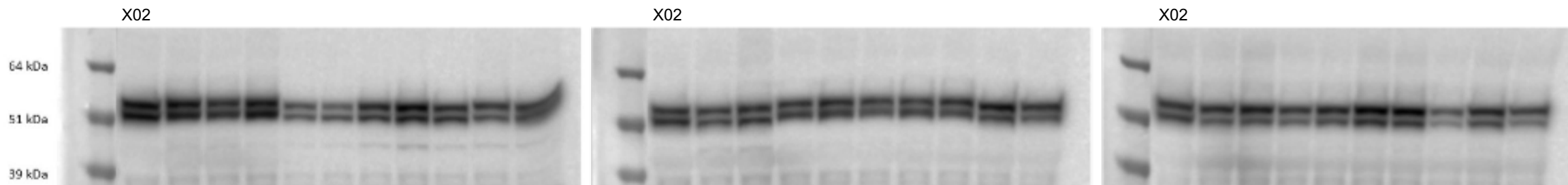
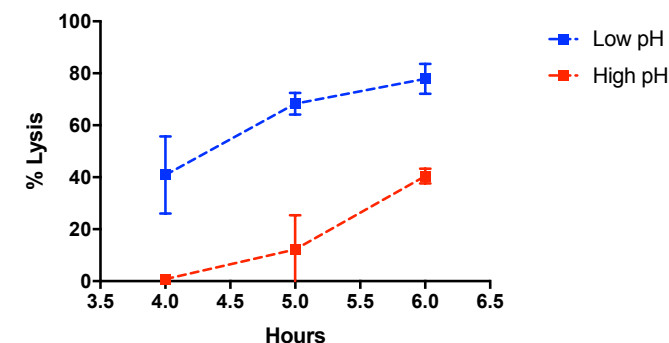
Hu et al. 2019 AACR Abs #3189

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Design and selection of logic-gated HER2 CARs: Top 28 variant screen

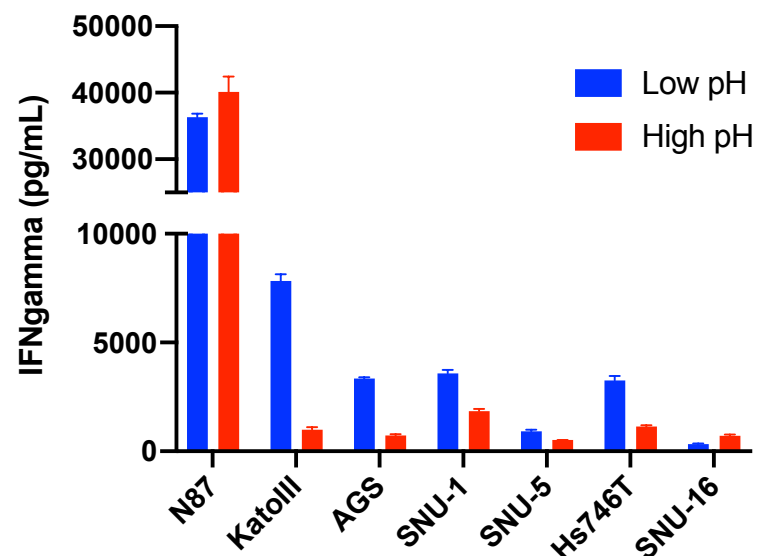
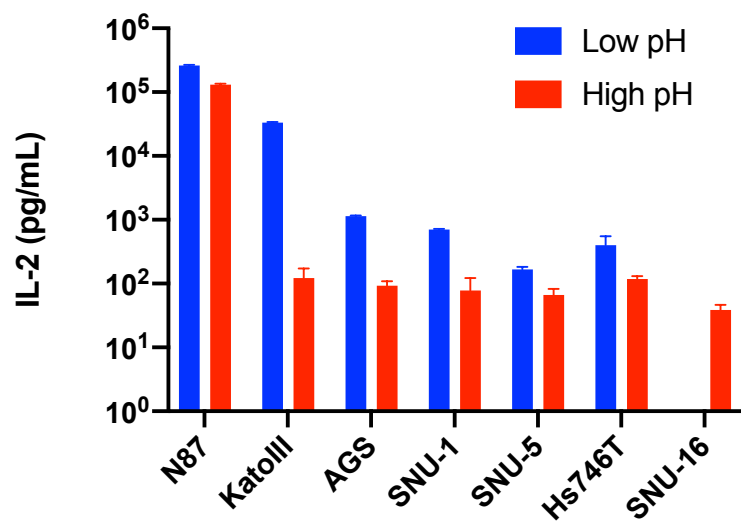
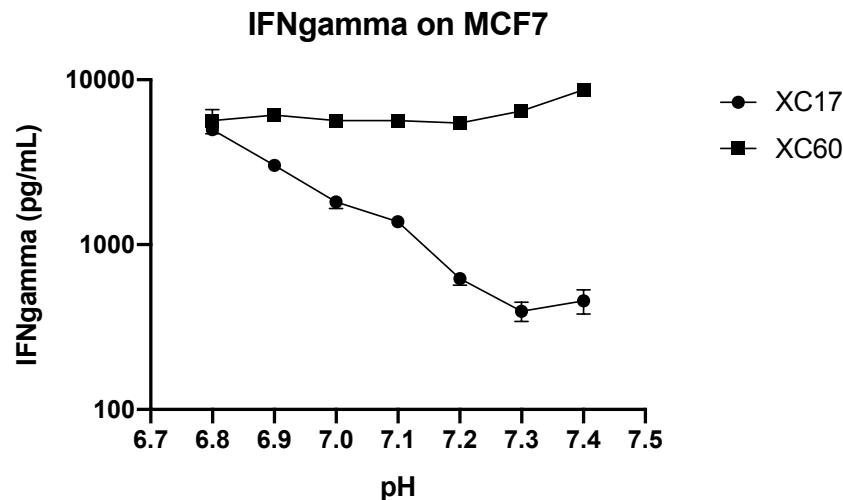
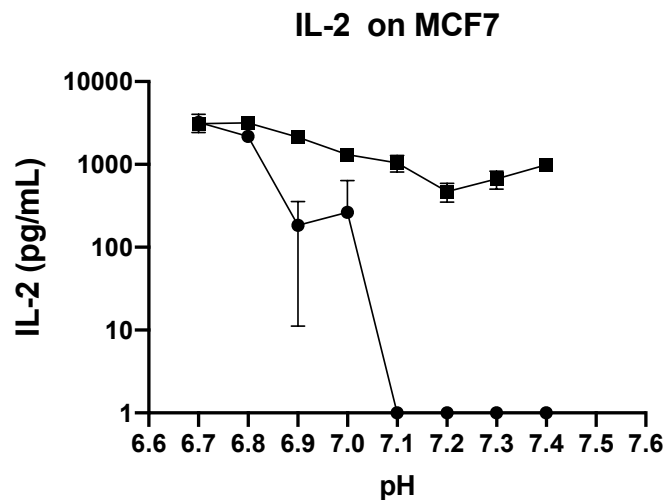
- HER2 CARs were evolved for activity at low pH; the design was further optimized to remove proteolytic sites
- The top 28 variants were then selected from a total of 96 based on their cytolytic activity at low pH vs. high pH
 - Variants were further subjected to additional screens such as cytokine release assays, cell killing by RTCA at various E:T ratios, proliferation assays, etc.

Luciferase cell killing example



	XC01	XC02	XC03	XC04	XC05	XC06	XC07	XC08	XC09	XC10	XC11	XC12	XC13	XC14	XC15	XC16	XC17	XC18	XC19	XC20	XC21	XC22	XC23	XC24	XC25	XC26	XC27	XC28
Transd. Eff.	50%	40%	50%	20%	20%	35%	40%	30%	30%	35%	40%	37%	35%	40%	37%	35%	40%	40%	37%	35%	35%	20%	25%	40%	35%	12%	30%	30%
Protein L MFI	63,581	65,257	58,271	71,019	77,134	82,040	75,183	58,122	62,516	54,436	45,970	43,899	63,855	76,353	100,833	71,596	71,622	118,638	94,497	107,663	86,743	83,384	108,890	122,862	122,725	85,109	95,654	90,549
Lysis ratio	5.74	2.66	1.03	1.12	1.50	1.87	1.84	1.13	4.15	4.32	1.94	2.62	2.65	1.06	3.50	2.00	1.33	1.14	1.89	9.64	1.27	1.24	1.28	NA	1.17	3.50	1.73	7.50

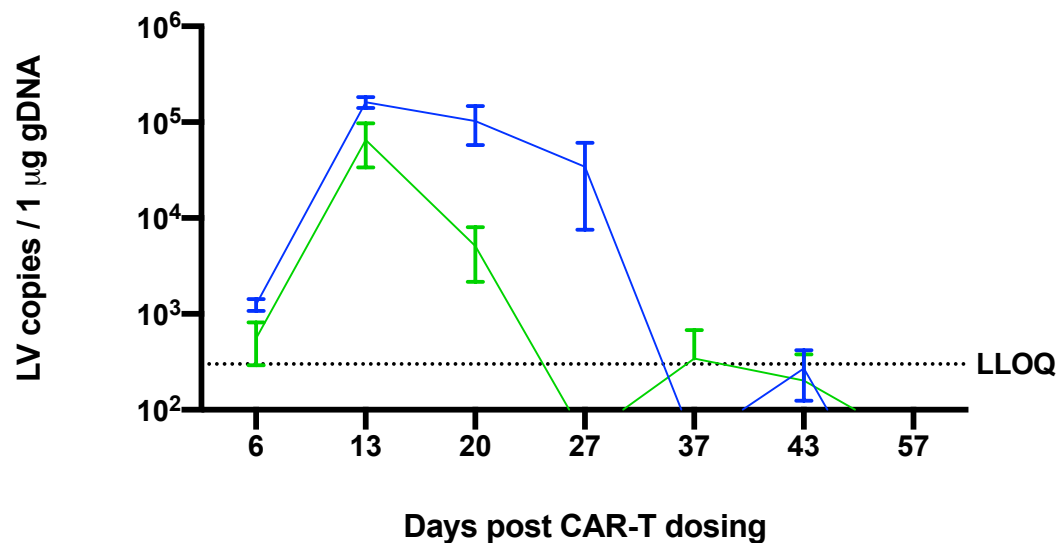
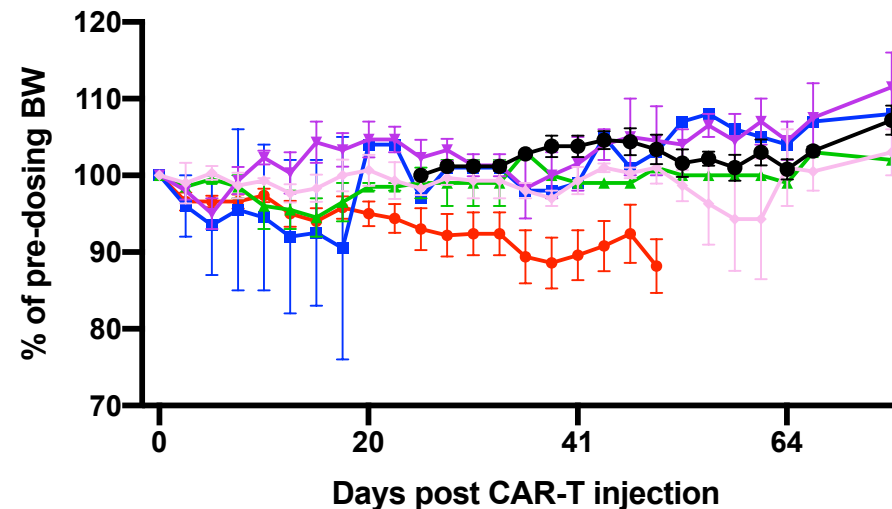
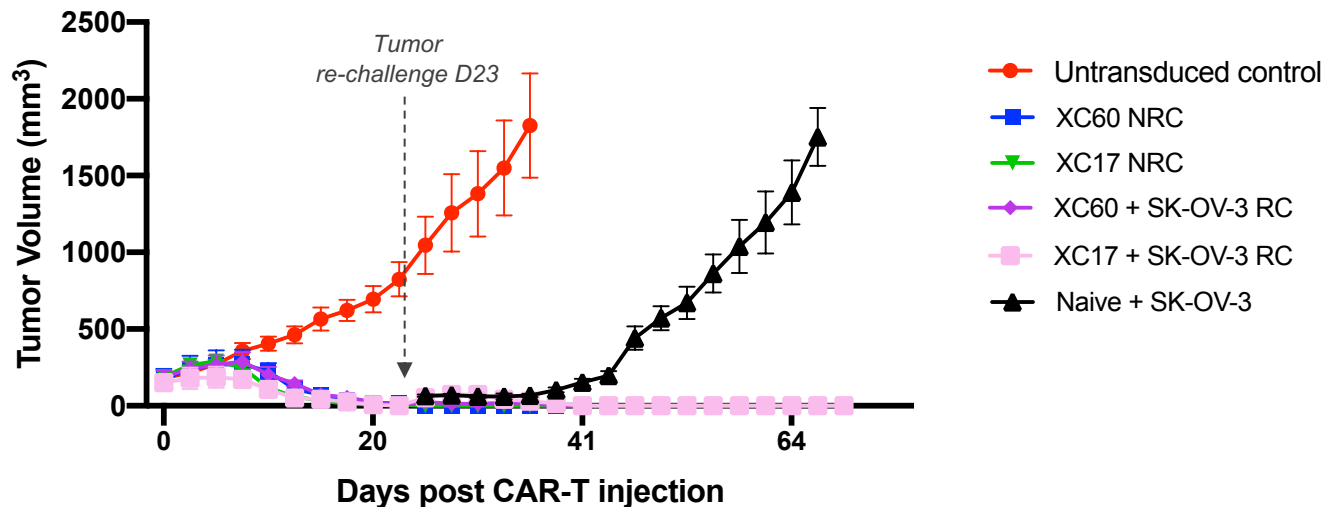
Cytokine release of top variant XC17 by pH and receptor density on high and low HER2-expressing tumor cell lines



Cell line	HER2 PE molecules/cell	Level
NCI-N87	254,432	High
KATO-III	80,460	Medium
AGS	9,625	Med/Low
SNU-1	5,832	Med/Low
SNU-5	4,652	Med/Low
Hs746T	3,504	Med/Low
SNU-16	1,049	Low

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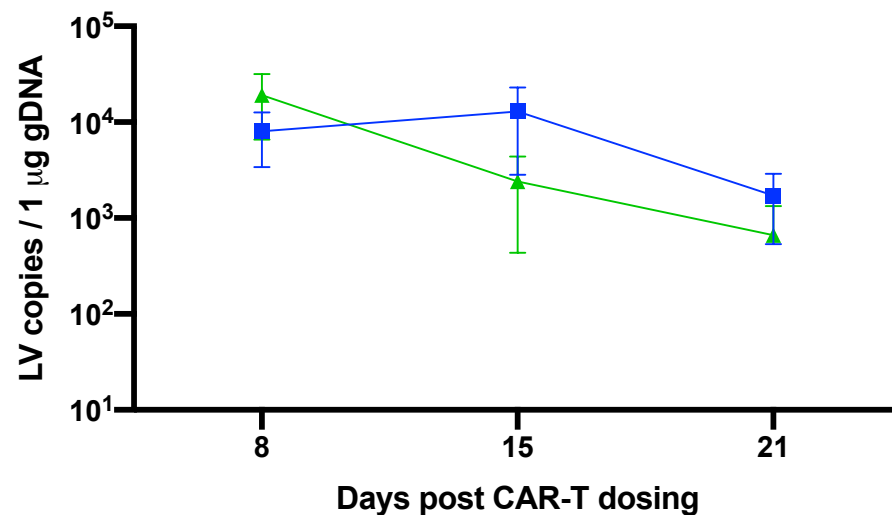
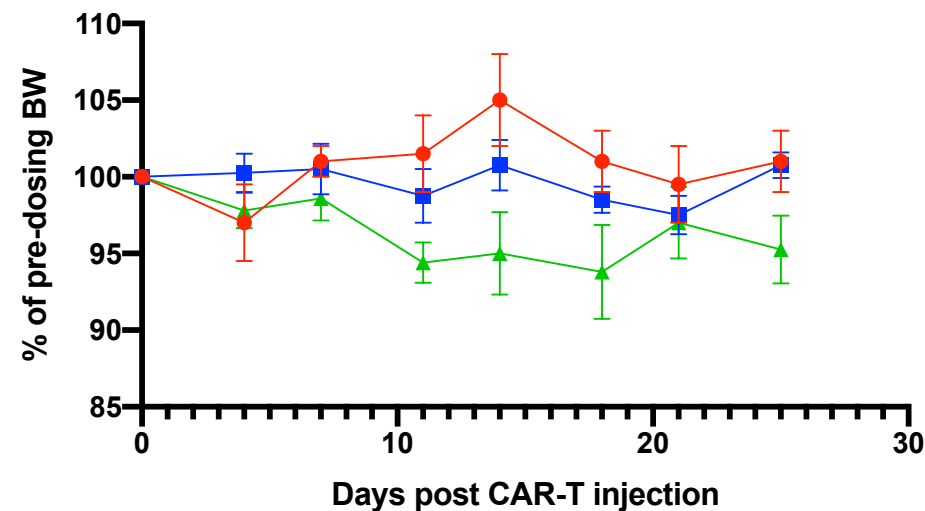
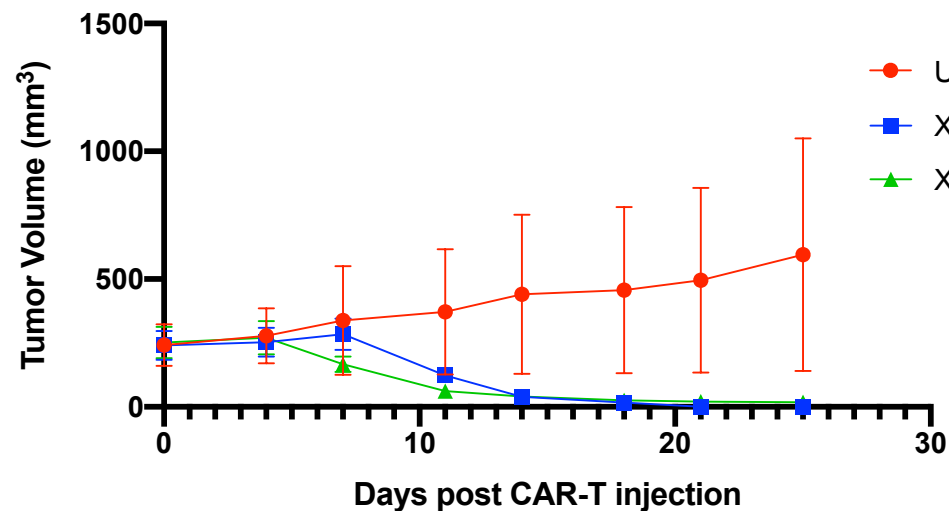
Logic-gated HER2 CAR-T eradicates ovarian (SK-OV-3) tumors *in vivo* and exhibits persistence & engraftment via tumor re-challenge



NRC = not re-challenged
RC = re-challenged
Error bars represent SEM
N=6 mice per group, n=3 per group for re-challenge

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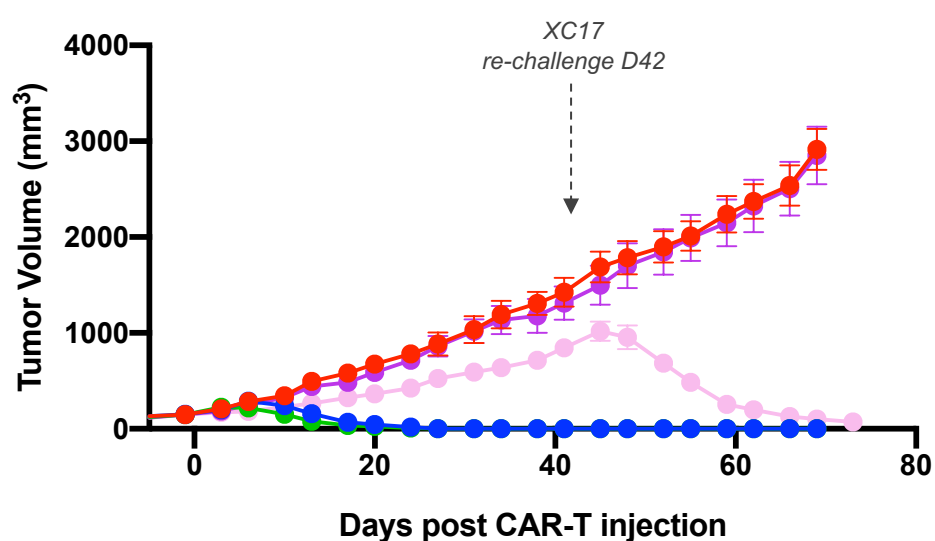
Logic-gated HER2 CAR-T eliminates high HER2-expressing breast cancer (BT-474) tumors *in vivo*



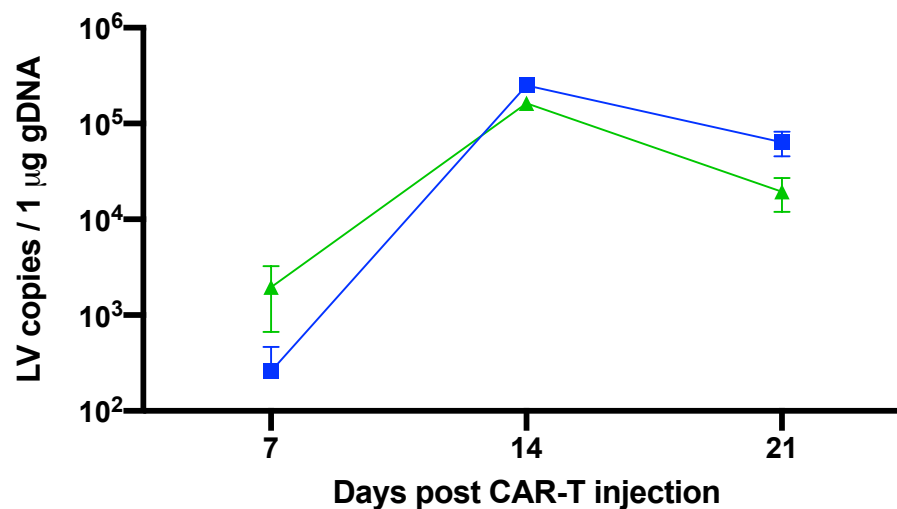
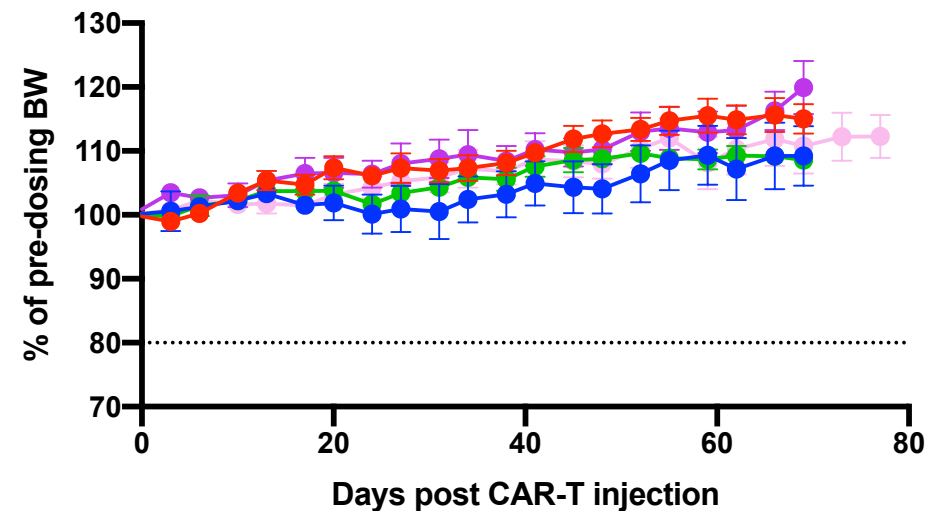
Error bars represent SEM
N=6 mice per group

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Logic-gated HER2 CAR-T eradicates gastric (NCI-N87) tumors *in vivo* and is also effective against trastuzumab progressors



- Untransduced control
- XC60
- XC17
- trastuzumab (low dose)
- trastuzumab (high dose) + XC17

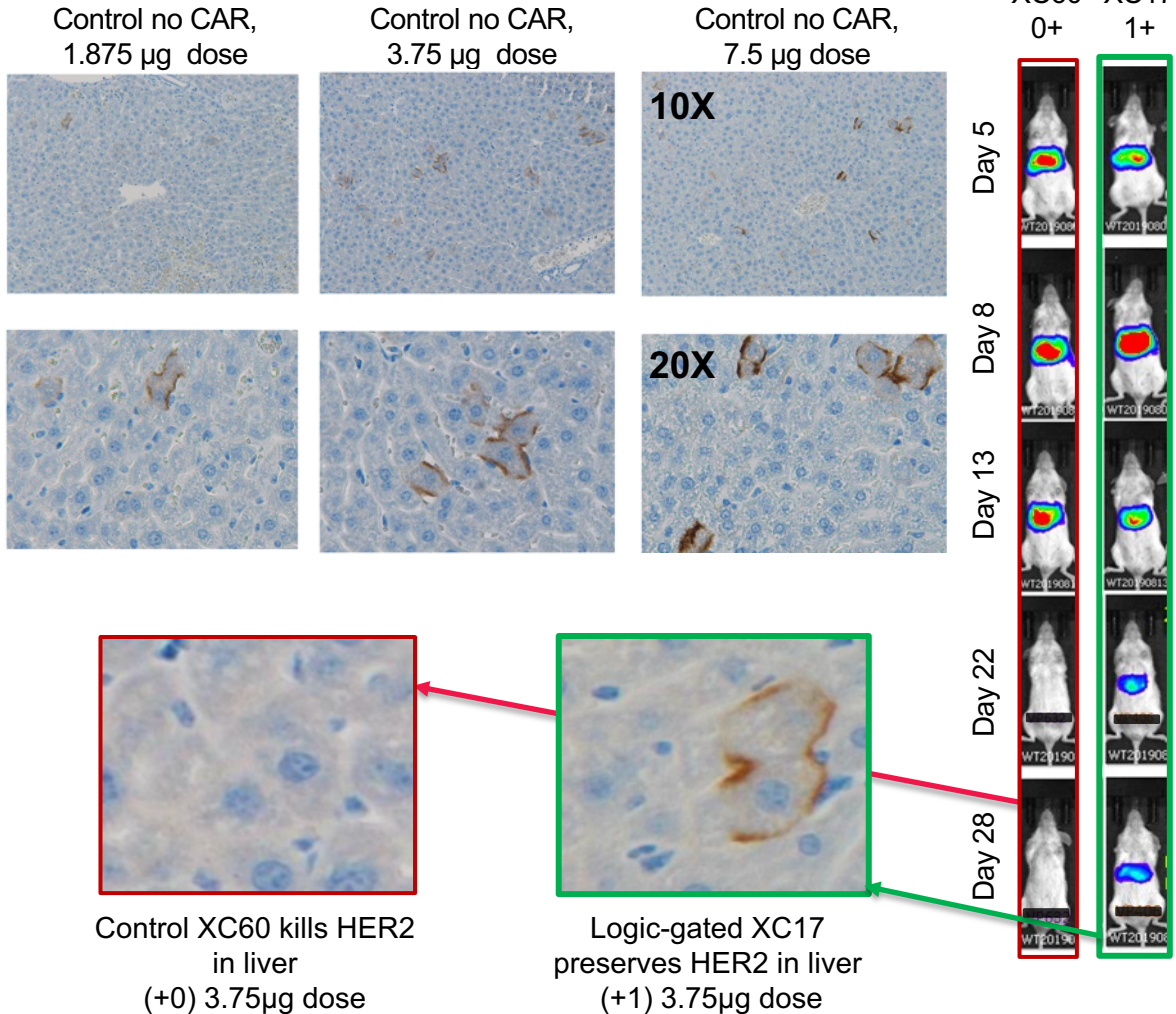


Error bars represent SEM
N=6 mice per group
Low dose trastuzumab = 4 mg/kg
High dose trastuzumab = 30 mg/kg

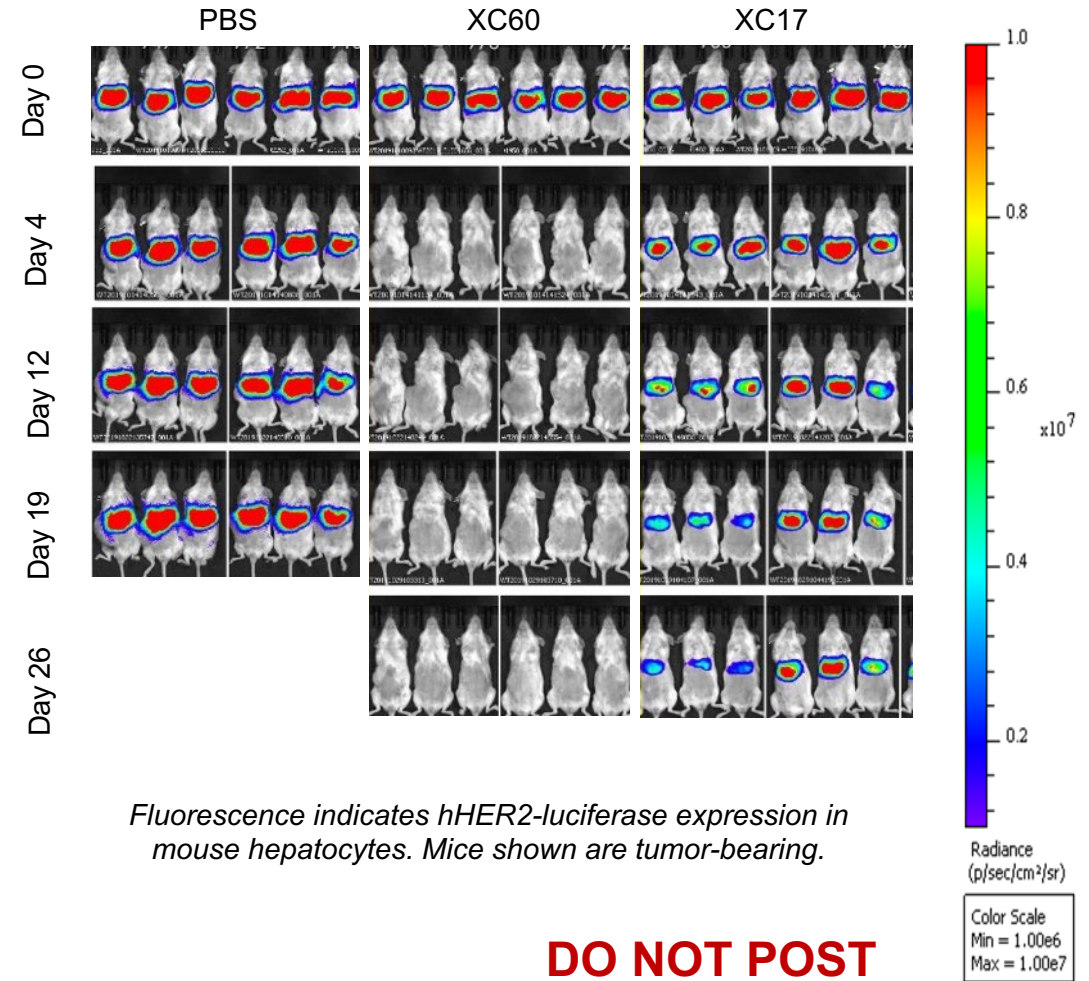
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Hydrodynamic gene delivery (HGD) of human HER2-luc to mouse liver allows for testing CAR safety against normal tissue

Pilot HGD Dose-finding study



Logic-gated XC17 preserves hHER2 in the mouse livers whereas control non-logic-gated XC60 kills hHER2-expressing livers



- A “logic-gated” HER2-targeted CAR-T was designed that preferentially recognizes HER2 in the tumor microenvironment (TME), thereby limiting on-target toxicity of low HER2 levels expressed in normal tissue
- HER2 scFvs with pH-restricted binding towards physiologic levels of HER2 were screened as CARs in primary T cells and demonstrated pH dependent cytotoxicity and cytokine release *in vitro*; the pH-dependence was also preserved in the context of HER2 CAR-Ts vs. ungated HER2 CARs.
- Antitumor activity and cellular kinetics were assessed in NSG mice bearing human HER2-amplified xenografts. Logic-gated HER2 CARs were capable of regressing established gastric (NCI-N87), breast (BT-474), and ovarian (SK-OV-3) tumors with HER2 amplification.
 - Importantly, logic-gated HER2 CAR-T cells were also capable of completely regressing large established gastric carcinoma xenografts that had progressed on prior trastuzumab therapy.
- On-target, off-tumor safety of the CAR-Ts was assessed in NSG mice with enforced expression of human HER2 and luciferase in hepatocytes using a hydrodynamic gene delivery (HGD) model. Compared to ungated HER2 CAR-T constructs, logic-gated HER2 CAR-Ts did not eliminate hepatocyte luciferase expression with human HER2 +1 staining in mouse livers as determined by Herceptest scoring of livers at necropsy.
- **In conclusion, these results demonstrate that a logic-gated HER2-targeted CAR-T can eliminate established HER2-amplified malignancies in a xenograft model, while mitigating potential on-target, off-tumor toxicity**