

# Precision Immunotherapy Utilizing Adapter CAR-T Cells (AdCAR-T) in Metastatic Breast Cancer Leads to Target Specific Lysis

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**Table S1.** List of BC patient data from whom the organoid lines were established. All patients were female aged between 52 and 70. Abbreviation: NST (no special type).

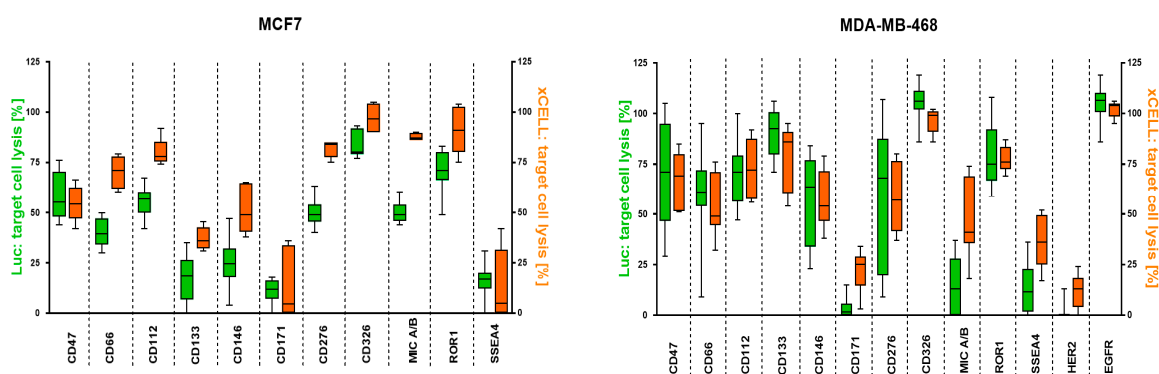
MBC-PDO	#03	#04	#06	#07
<b>Primary tumor</b>	NST (2008)	NST (2009)	NST (2019)	NST (2019)
<b>Therapy of primary tumor</b>	Trastuzumab; Aromasin; Carboplatin; Taxol; Avastin (until 2019)	FEC + Docetaxel; Tamoxifen (until 2013)	Epirubicin/Cyclophosphamide (aborted)	Doxorubicin
<b>Metastasis</b>	Hepatic and pleural metastasis (2019)	Lymph nodes (2017) Hepatic metastasis (2021)	Hepatic and osseous metastasis (2020) Pulmonary and medullary metastasis (2021)	Cutaneous, lymphogenic and osseous metastasis (2020-2021)
<b>Therapy of Metastasis</b>	Avastin	Ribociclib + Letrozole; Palbociclib + Letrozole	Letrozole + Goserelin + Abemaciclib	Paclitaxel; Eribulin; Abemaciclib + Letrozole
<b>Therapy prior to drainage</b>	Avastin; Nab-Paclitaxel; Eribulin (2020)	Abemaciclib + Fulvestrant (2020-2021)	Paclitaxel (2021)	Capecitabine (2021)
<b>Drainage</b>	Pleura (2021)	Pleura (2021)	Pleura (2021)	Pleura (2021)
<b>Follow-up therapy</b>	Palbociclib + Letrozole; Vinorelbine + Trastuzumab + Pertuzumab	Everolimus + Aromasin; Doxorubicin	None, patient did not survive	Carboplatin + Olaparib; Olaparib
<b>Additional information</b>	Hepatic and pleural metastasis (2019): NST infiltration, <i>PIK3CA</i> H1047R mutation	-	-	NST (2019): <i>BRCA1/2</i> deletion, <i>AKT1</i> E17K mutation

**Table S2.** Composition of Breast Cancer Medium (BCM). Composition was previously described [24, 27].

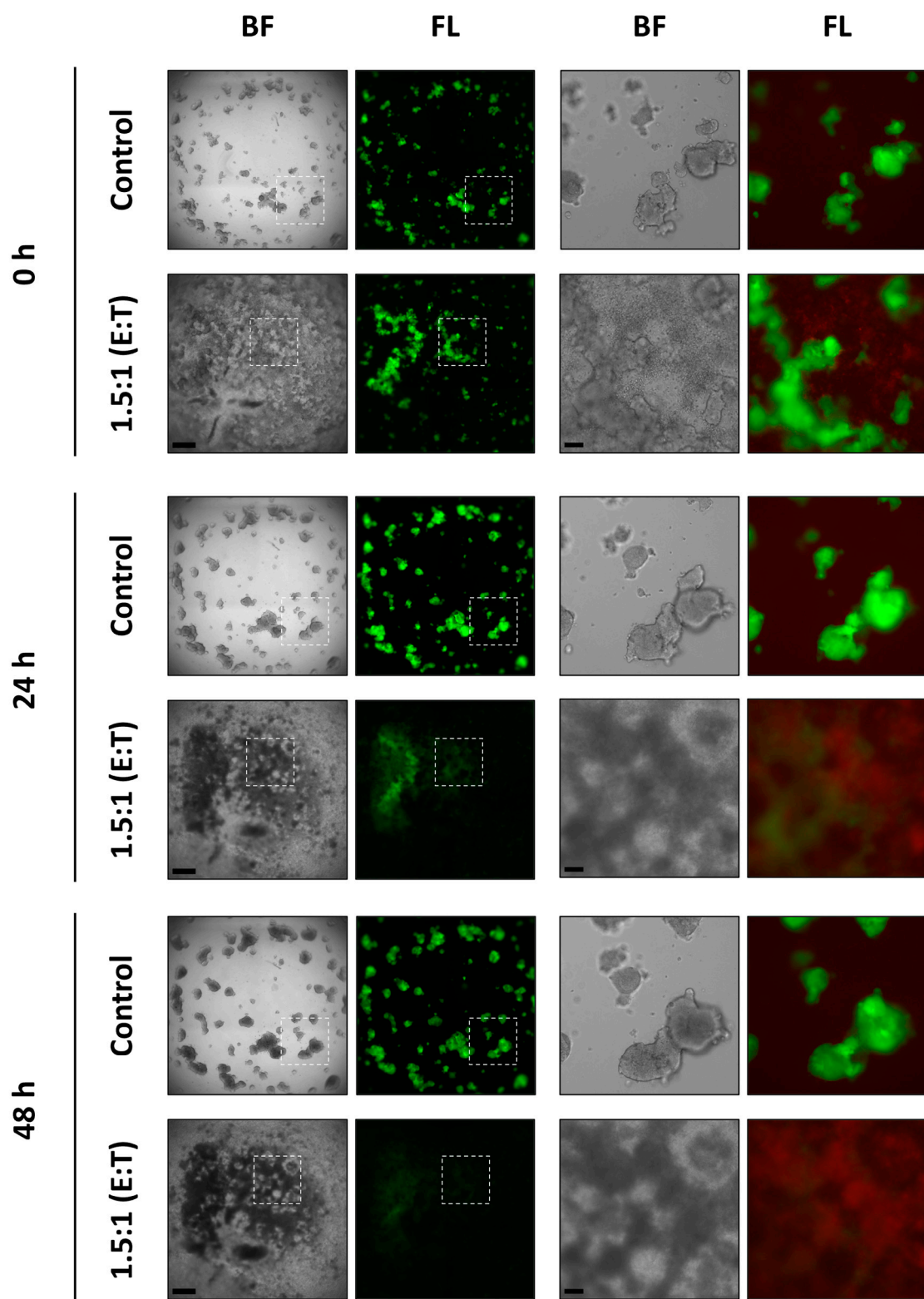
Component	Final concentration	Company	Catalog number
L-WRN conditioned medium	50%	Home-made	-
Neuregulin 1	5 nM	Peptotech (Cranbury, NJ, USA)	100-03
FGF7	5 ng/mL	Peptotech (Cranbury, NJ, USA)	100-19
FGF10	20 ng/mL	Peptotech (Cranbury, NJ, USA)	100-26
EGF	5 ng/mL	Peptotech (Cranbury, NJ, USA)	AF-100-15
A83-01	500 nM	Tocris Bioscience (Bristol, UK)	2939
Y-27632	5 $\mu$ M	Hölzel Diagnostika Handels GmbH (Köln, Germany)	TMO-T1725-50 mg
SB202190	500 nM	Sigma-Aldrich (St. Louis, MO, USA)	S7067
B-27™ supplement	1x	Thermo Fisher Scientific (Waltham, MA, USA)	17504-44
Nicotinamide (NIC)	5 mM	Sigma-Aldrich (St. Louis, MO, USA)	NO636
N-Acetylcysteine (NAC)	1.25 mM	Sigma-Aldrich (St. Louis, MO, USA)	A9165-5G
Primocin	50 $\mu$ g/mL	InvivoGen (San Diego, CA, USA)	Ant-pm-1
AdvDMEM +++ (1% Pen/Strep, 1x GlutaMAX™-I, 10 mM HEPES)	1x	Thermo Fisher Scientific (Waltham, MA, USA)	12634-028; 15140-122; 35050-038; 15630-056

**Table S3.** Antibody information chart.

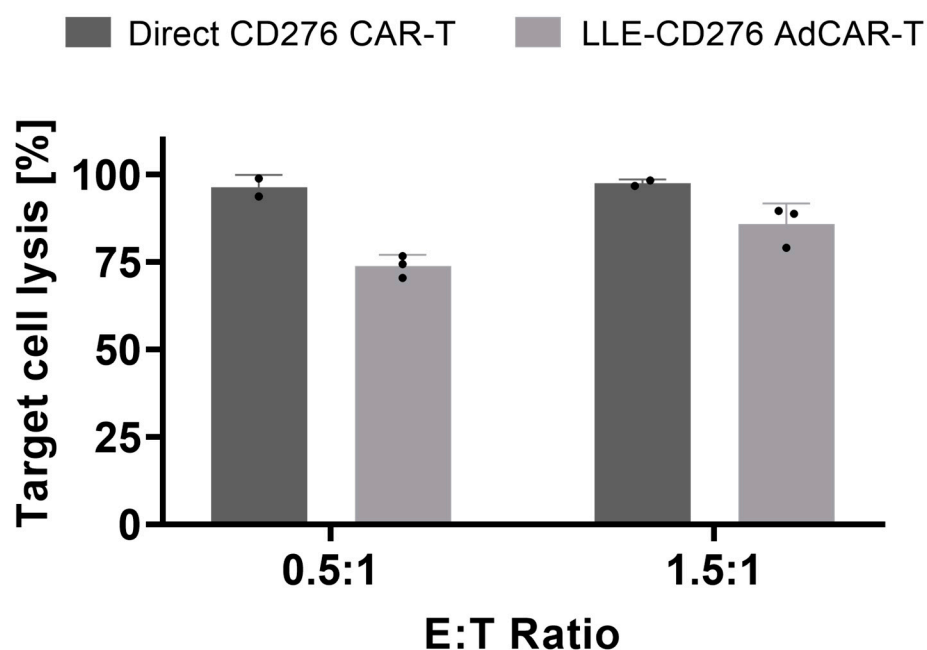
Antibody	Clone	Host	Isotype	Conjugation	Company	Cat No.
Anti-CD47	REA220	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-101-342
anti-CD66	TET2	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-093-156
anti-CD112	R2.525	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-109-000
anti-CD133	REA820	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-112-193
anti-CD146	541-10B2	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-092-850
anti-CD171	REA163	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-100-702
anti-CD276	REA1094	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-118-579
anti-ROR1	REA1051	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-118-018
anti-CD326	REA764	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-111-114
anti-SSEA	REA101	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-098-339
anti-TROP2	REA916	human Hybridoma cell line	recombinant human IgG1	Biotin	Miltenyi Biotec	130-115-096
anti-Biotin	REA746	human Hybridoma cell line	recombinant human IgG1	APC	Miltenyi Biotec	130-111-069
anti-HER2	Trastuzumab, Kanjinti	Chinese Hamster Ovarian	IgG1 Isotype	Biotin	Pharmacy University clinics Tübingen UKT	1144554A
anti-EGFR	Cetixumab, Erbixux	murine myeloma cell line	IgG1 Isotype	Biotin	Pharmacy University clinics Tübingen UKT	G0157D



**Figure S1.** Correlation between LCA and real-time ICA. Comparison of target cell lysis of MCF-7 and MB-MDA-468 via LCA (green bars) or ICA (red bars). Target cell lysis with an E:T ratio of 2:1 via LCA and ICA was determined after 48h and 24h respectively. Data is represented by mean values ( $n=6$ )  $\pm$  SD.



**Figure S2.** Direct CD276 CAR-T cell treatment of MCF-7 organoids expressing GFP. Brightfield (BF) and fluorescence (FL) images of GFP-expressing MCF-7 organoids (green) treated with mCherry-expressing CD276 CAR-T cells (red) in an E:T ratio of 1.5:1 for 48 h. Control conditions represent MCF-7 organoids alone. Scale bar of left two columns: 500  $\mu$ m. Scale bar of right two columns: 100  $\mu$ m.



**Figure S3.** Comparison of target cell lysis between direct CD276 CAR-T and LLE-CD276 AdCAR-T on MCF-7 organoids at an E:T ratio of 0.5:1 and 1.5:1 after 48h. Target cell lysis was normalized to MCF-7 organoids alone. Data shown represents the mean  $\pm$  SD of biological duplicates ( $n = 2$ ) and triplicates ( $n = 3$ ) for direct CD276 CAR-T and LLE-CD276 AdCAR-T respectively.

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