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Supplementary Methods

Cell lines

The murine gastric carcinoma cell line mGC8 was provided by W. Zimmermann (Munich, Germany) [14], the murine breast cancer cell line 4T1 by M. Wartenberg (Jena, Germany) and the murine T cell line B3Z, the packaging cell line Plat-E and the HEK-derived cell line 293T by W. Uckert (Berlin, Germany). The human melanoma cell lines Mel624.38 was provided by M. Panelli (Bethesda, USA) and MelA375 was obtained from ATCC (CRL1619). The colon carcinoma cell line LS174T was obtained from ATCC (CLL 188). The human T cell line Jurkat was obtained from Life Technologies. The tyrosinase specific T cell clone IVSB was obtained from T. Wölfel (Mainz, Germany). mGC8, Plat-E and 293T cells were cultured in DMEM with 10 % fetal bovine serum (FBS, Life Technologies, USA), 1 % penicillin and streptomycin (PS) and 1% L-glutamine (all from PAA, Germany). 10 µg/ml puromycin and 1 µg/ml blasticidin (Sigma, Germany) was added to the Plat-E medium. 4T1, Mel624.38, MelA375, LS174T, B3Z, Jurkat cell lines and primary murine and human T cells were cultured in RPMI 1640 with 10% FBS, 1% PS and 1% L-glutamine. 1% sodium pyruvate, 1 mM HEPES and 50 µM β-mercaptoethanol were added to the T cell medium. IVSB T cell clone was cultured in AIM V medium (Life technologies, Germany) with 10% human AB serum (Bavarian Red Cross, Germany) 1% L-glutamine, 1% PS, 1% sodium pyruvate and 50 U/ml of interleukin-2 (Novartis, Germany). Phenotypic testing was performed for all cell lines to ensure authenticity.

Antibody generation

The BiAb against human EGFR and murine EpCAM (ER-Ep BiAb) was designed in a two-plus-two tetravalent BiAb format as a murine IgG2a construct based on the sequences of MAB225 [28] and of the rat anti-murine EpCAM G8.8

(obtained from the Developmental Studies Hybridoma Bank). Sequences of the variable regions of MAB225 were cloned into two mammalian expression vectors including the mouse IgG2a framework and the kappa light chain backbone constant regions. Murinized G8.8 and MAB225 were generated in the same backbone. To generate the bispecific construct, a disulfide bridge-stabilized single chain Fv G8.8 VH-$(G4S)_3$-VL was designed via gene synthesis and fused by a $(G4S)_2$ connector to the C-terminus of the MAB225 heavy chain. The bispecific antibody against EGFR and c-Met (ER-Met BiAb) was generated from cetuximab and the c-Met antibody 5D5, respectively [29]. The bispecific antibody specific for EGFR and for digoxigenin (ER-Dig BiAb) was generated from cetuximab and the digoxigenin antibody (WO2011/003557) [30].

Image analysis

Immunofluorescence slides were analyzed using ImageJ software (version 10.2, National Institutes of Health, USA).

Surface plasmon resonance analysis of binding to EGFR and EpCAM by BiAb

All experiments were performed on Biacore B3000, T100 and T200 instruments in running buffer PBS containing 0.05 % (v/v) Tween20. Dilution buffer consisted of running buffer supplemented with 1 mg/mL BSA. Standard amine coupling to ECD/NHS-activated chip surfaces was performed as recommended by the provider (GE Healthcare). Signals were double referenced against blank buffer and a flow cell containing no ligand. Kinetic constants were calculated from fitting to a 1:1 Langmuir binding model (RI = 0). Single antigen binding were assayed at 37°C, simultaneous binding at 25°C. Antigen was amine-coupled on a C1 sensor chip. For simultaneous binding, EGFR was coupled to the chip surface, bispecific antibody was injected and

upon reaching saturation, a second injection with recombinant EpCAM was performed. An additional injection of EpCAM did not further raise the response level.

Immunohistochemistry and immunofluorescence

Immunohistochemistry and immunofluorescence were performed as described [14]. In brief, tumors were removed, embedded in tissue tek OCT medium (Leica, Germany) and snap frozen. Tumors were cut in 7 µm sections and transferred to microscopy slides which were then fixed with acetone and blocked with 10 % donkey serum (Miltenyi). Nuclei were stained using hemalaun (Carl Roth, Karlsruhe, Germany) or DAPI hydrochloride (Life Technologies, USA). For detection of antigens, anti-EpCAM rat antibody (clone G8.8, Santa Cruz Biotechnology, USA), anti-CD3 Syrian hamster antibody (clone 500A2, BD Pharmingen, USA), anti-MHC class I (clone ER-HR52, AbD Sero Tec, Düsseldorf, Germany) and anti-EGFR antibody cetuximab (Merck-Serono, Germany) were used. Anti-rat IgG-Cy2, anti-Syrian hamster IgG-biotin, streptavidin-AP and anti-human IgG-FITC were from Jackson Immunoresearch, USA.

Flow cytometric analysis of T cell distribution

Non-tumor bearing mice were treated with the combination of ER-Ep BiAb and ΔEGFR-transduced T cells or T cells with murine anti-EpCAM antibody G8.8. One week after treatment, animals were euthanized and organs (spleen, liver, lung, liver and small bowel) collected to obtain single cell suspensions. Cell suspensions were stained with anti-CD3-FITC and anti-CD8-APC antibodies (both ebioscience, clones 145-2C11 and clone 53.67). Staining was analyzed using BD FACS Canto II (BD, Germany).

Supplementary Tables

Supplementary Table 1: summary of the p values for Figure 2B

Condition	ER-EpBiAb + TCR + 4T1	ER-Ep BiAb + TCR + B16	ER-Dig BiAb + TCR + mGC8	ER-Ep BiAb + OT1 + mGC8	ER-Ep BiAb + WT + mGC8	TCR + mGC8
ER-Ep BiAb + T cell + mGC8	0.003	0.001	<0.001	0.040	<0.001	<0.001

ER-Ep BiAb, anti-EGFR x anti-EpCAM bispecific antibody; TCR, T cell receptor; WT, wild type

Supplementary Table 2: summary of the p values for Figure 3A

	Days										
Comparison	42	44	48	49	50	53	54	55	56	57	60
ER-Ep BiAb + ΔEGFR T cells vs PBS	0.039	0.016	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001 until day 62
ER-Ep BiAb + ΔEGFR T cells vs anti-EpCAM antibody	ns	ns	0.004	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
ER-Ep BiAb + ΔEGFR T cells vs anti-EpCAM antibody + ΔEGFR T cells	ns	ns	ns	ns	ns	ns	0.0137	0.010	0.009	<0.001	<0.001 until the end

ER-Ep BiAb, anti-EGFR x anti-EpCAM bispecific antibody

Supplementary Table 3: summary of the p values for Figure 3B

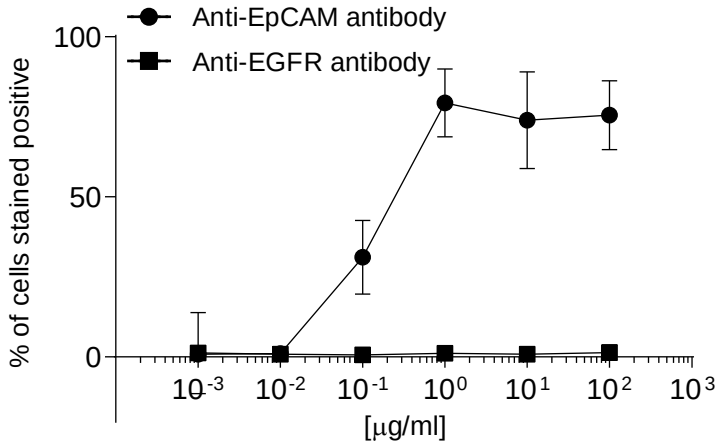
	Days								
Comparison	41	43	47	50	54	61	63	65	68
ER-Ep BiAb + ΔEGFR T cells vs PBS	0.026	0.016	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
ER-Ep BiAb + ΔEGFR T cells vs anti-EpCAM antibody	ns	ns	0.020	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ER-Ep BiAb + ΔEGFR T cells vs ER-Ep BiAb	ns	ns	ns	ns	0.015	0.001	<0.001	<0.001	<0.001 until day 72
ER-Ep BiAb + ΔEGFR T cells vs murinized anti-EpCAM antibody + ΔEGFR T cells	ns	ns	ns	ns	0.038	0.017	0.002	<0.001	<0.001 until the end

ER-Ep BiAb, anti-EGFR x anti-EpCAM bispecific antibody.

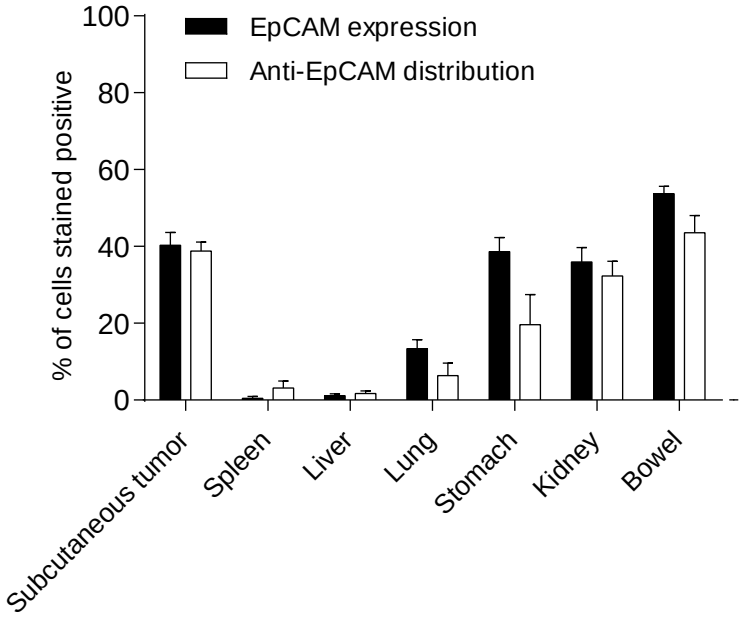
Supplementary Figure 1: EpCAM is a target for antibody-based therapy in a subcutaneous tumor model of a murine gastric cancer. (A) Expression analysis of EpCAM on the murine gastric cancer cell line mGC8 by flow cytometry. mGC8 cells were stained with increasing concentrations of anti-EpCAM antibody G8.8 or anti-EGFR antibody MAB225. Results shown are the mean of biological triplicates of one experiment, which is representative for three independent experiments. Error bars represent standard deviation (SD) **(B)** Expression of EpCAM and distribution of rat anti-EpCAM antibody G8.8 injected i.v. in tumor bearing C57BL/6 mice. For expression analysis of EpCAM (black bars), sections of embedded frozen organs were stained with rat anti-EpCAM antibody G8.8 followed by anti-rat IgG labelled with Cy2. For analysis of rat anti-EpCAM antibody distribution, mice were injected i.p. with G8.8 antibody (300 µg) and organs were isolated 48 h later. Rat G8.8 distribution was detected with an anti-rat IgG labelled with Cy2. Quantification in both settings was performed using ImageJ software. For each organ mean values of three mice are shown with SD. **(C)** Representative staining of tissues taken from mice injected with rat anti-EpCAM antibody G8.8 or PBS. Tissues were stained with anti-rat IgG antibody labelled with Cy2. Data for the mGC8 subcutaneous tumor and for the kidney are shown. Scale bars represent 100 µm.

Supplementary figure 1

A



B



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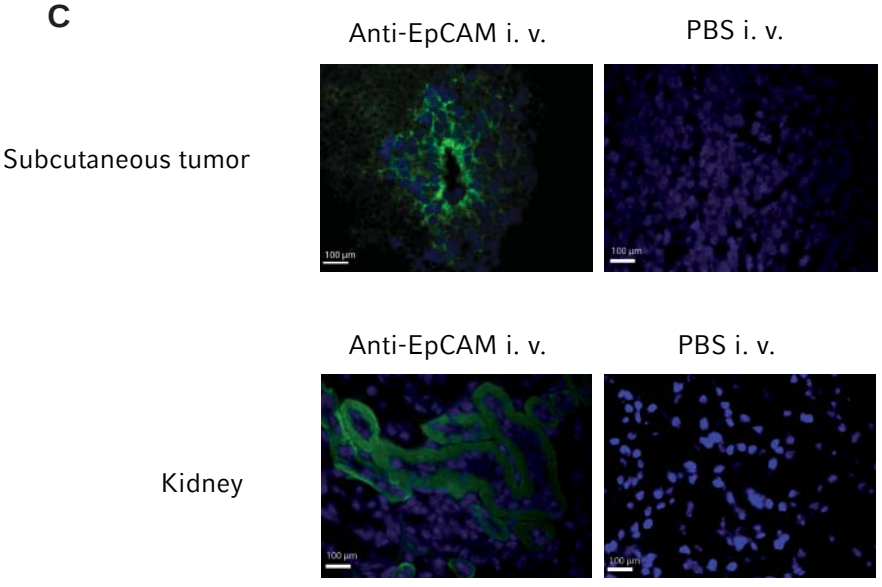
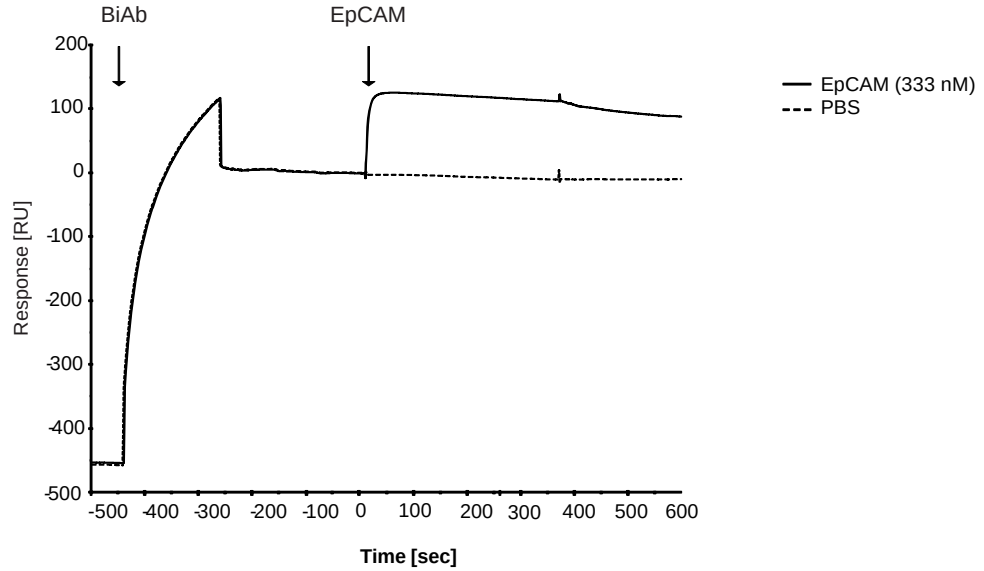


Supplementary Figure 2: (A) Simultaneous binding of human EGFR and murine EpCAM ectodomains by the BiAb analyzed by surface plasmon resonance. Human EGFR ectodomain was amine-coupled to the sensor chip. ER-Ep BiAb was injected at time point -450 s. At 0 s, recombinant EpCAM (333 nM) or saline was injected. **(B) ∆EGFR expression after T cell transduction.** Primary murine T cells or the T cell line (B3Z) were retrovirally transduced with ∆EGFR and stained with monoclonal anti-EGFR antibody. Histogram is representative for all cell transductions performed in the presented experiments.

Supplementary figure 2

A



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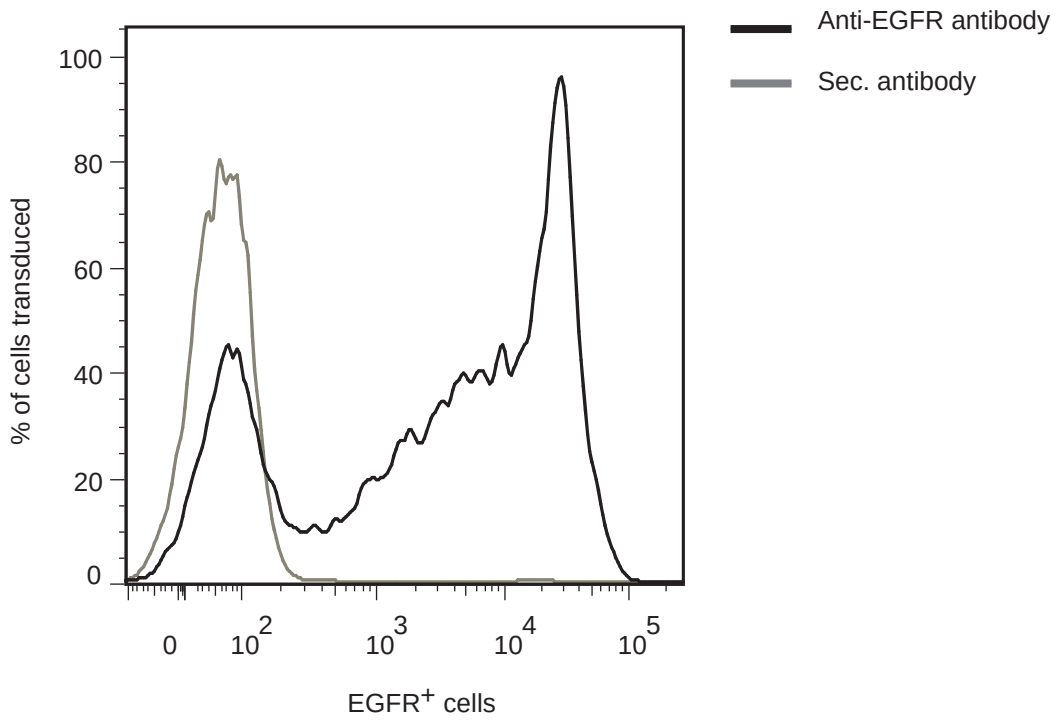

Supplementary Figure 3: Enhanced T cell infiltration in the tumors of mice treated with the combination of anti-EGFR x anti-EpCAM bispecific antibody and adoptive transfer of ΔEGFR-transduced T cells. (A) T cell infiltration in $EpCAM^+$ murine organs (spleen, liver, lung, kidney and small bowel) was analyzed by flow cytometry of tissue-derived cell suspensions. Mice (n = 3 for each condition) were treated with 10 mg/kg anti-EGFR x anti-EpCAM bispecific antibody (ER-Ep BiAb) and 10^7 ΔEGFR-transduced TCR-I T cells or with 10 mg/kg murinized anti-EpCAM antibody and 10^7 ΔEGFR-transduced TCR-I T cells. One week later, the animals were euthanized and single cell suspensions were stained for CD3 and CD8. No difference in the number of infiltrating T cells was found. Results are representative of two experiments performed with three mice per group. Each dot represents one mouse; bars represent SD. **(B)** Quantification of infiltrating CD3 positive T cells per 30 high power fields (HPF) in mice treated with PBS (n=11), anti-EGFR x anti-EpCAM bispecific antibody (n=6), anti-EpCAM antibody (n=13), anti-EpCAM antibody plus anti-EGFR antibody plus ΔEGFR-transduced T cells (n=12) or anti-EGFR x anti-EpCAM bispecific antibody plus ΔEGFR-transduced T cells (n=17). Tumors were harvested from mice which had to be euthanized due to tumor size. Data are pooled from experiments shown in Figure 3. Groups were compared using unpaired Student's t-test. Bars represent SD. **(C)** Representative immunohistochemistry of an untreated tumor in 40x magnification. Hemalaun and anti-CD3-AP co-staining. **(D)** Representative immunohistochemistry of a tumor treated with the combination of anti-EGFR x anti-EpCAM bispecific antibody and ΔEGFR-transduced T cells in 40x magnification. Hemalaun and anti-CD3-AP co-staining. Arrows indicate positively stained cells. Scale bars in C and D represent 100 µm.

Supplementary figure 3