Supplementary Materials and Methods

Human OMTX005 Antibody Generation and Production

The OMTX005 antibody was generated from a humanized scFv antibody fragment directed against human and mouse FAP protein (26). The V_H and V_L sequences of the humanized scFv anti-FAP fragment were cloned into vectors containing the human heavy and light constant regions for full length IgG1 kappa antibody expression into the GS Xceed® Gene Expression System (Lonza Ltd). The GS Xceed double gene construct containing full length antibody genes were transfected into CHOK1SV GS-KO cells for OMTX005 antibody expression (Lonza Biologics plc). Supernatant of stable pool transfectant cells was HPLC Protein A purified (HiTrap MabSelect SuRE column, GE Healthcare) and the OMTX005 antibody obtained resuspended in PBS buffer pH 7.2 for *in vitro* or *in vivo* use and for OMTX705 conjugation.

OMTX005 Conjugation and Analysis

OMTX005 antibody was reduced with 2.5x molar excess of 2mM TCEP (tris(2-carboxyethyl) phosphine) prior to the addition of 5.5x molar excess of 1mM TAM 558 and the mixture incubated 2h at 25ºC. After 40 min incubation a sample was taken for HPLC hydrophobic interaction chromatography (HIC) to confirm conjugation. The OMTX705 obtained was diafiltered by tangential flow filtration (TFF), concentrated to 10 mg/mL into 10 mM Sodium succinate, 6% w/v Sucrose pH 5.5 and analyzed by size exclusion chromatography (SEC) and HIC analysis to determine final OMTX705 purity and DAR (drug antibody ratio) (Piramal Pharma Solutions). HPLC HIC analysis was performed in 100 µg conjugation mixture on an Agilent 1100 series and Protein Pak Hi Res HIC 2.5µm column, over 18 minute gradient between mobile phase A (1.5 M Ammonium sulphate, 25 mM sodium phosphate, pH 6.95) and mobile phase B (75% 25 mM sodium phosphate, 25% IPA, pH 6.95) (0 to 100% B) at 0.8 mL/min, 25ºC, and 280 nm detection channel. HPLC SEC analysis was performed injecting 20 µg conjugation mixture on an Agilent 1100 series using XBridge Protein BEH SEC 200A column, and mobile phase (85% 0.25 M potassium perchlorate, 0.2 M potassium phosphate, pH 6.85, 15% v/v IPA) at 0.8 mL/min, 25ºC and 280 nm detection channel.

Antigen Recognition (Supplement Figure S1-A&B)

Antigen recognition of the antibody OMTX005 in comparison to the ADC OMTX705 was determined by ELISA using immobilized human FAP recombinant protein at 3 µg/ml and the OMTX005 or the OMTX705 titrated from 100 nM (15 µg/ml) to 1 pM (150 ng/ml). Bound primary antibodies were detected with an HRP-conjugated anti-human Fc secondary antibody (Sigma Aldrich A0170) diluted 1:5000. FAP antigen recognition was evaluated in HT1080 wild type (HT1080-WT), human or murine FAP-transfected HT1080 (HT1080-FAP or HT1080-moFAP, respectively), and CAFs from patients by flow cytometry analysis. Cells were incubated with either OMTX005, OMTX705 or control IgG1-kappa-TAM558 (30nM) and bound antibodies were detected with a PE-labeled anti-human Fc antibody (Sigma P9170; 1:500). Cells were analyzed using a Milteny MACS-Quant.

Antibody Internalization Analysis (Supplement Figure S1-C)

The internalization of pHrodo-labeled antibodies, OMTX705 and isotype IgG1 kappa-TAM558, into CAF07 and HT1080-FAP cells were analyzed via flow cytometry, as pHrodo only shows fluorescence in acidic milieu, e.g. in the lysosome for internalized antibodies. The day before the assay, cells were seeded (20.000 cells/well) in 100 µl medium per well (96-well plate) and cultivated over night at 37 °C. Medium was removed and substituted with 100 µl fresh medium (4 °C or 37 °C) containing 30 nM of the respective pHrodo-labeled antibody and incubated either at 4 °C or 37 °C. After 5 minutes, 45 minutes, 120 minutes, 240 minutes, 360 minutes, and 24 hours of incubation, medium was removed, cells were washed with 100 µl PBS and detached from the plate by adding 50 µl trypsin (37°C). Finally, 100 µl of ice-cold PBA (PBS containing 2 % FCS and 0.02 % Na-azide) was added to suspended cells, which were then analyzed via flow cytometry using a Milteny MACS-Quant.

Pharmacokinetic Study

Animal care and all experiments performed were in accordance with Federal and European guidelines and have been approved by university and German state authorities.

25 or 150 µg of OMTX705 or OMTX005 were injected into the tail vein of female CD1 mice (3 to 6 animals per construct) in a total volume of 100 µl. Blood samples were taken at different time-points up to 7 days (3 min, 30 min, 1 h, 2 h, 6 h, 24 h, 72 h, 168 h) after injection and incubated immediately on ice to obtain serum samples after centrifugation (16,000 x g, 4 °C, 20 min). Serum samples were stored at -20 °C until analysis. Antibody concentrations of different serum samples were measured by sandwich ELISA using FAP-Flag (3 µg/ml) as immobilized antigen. Bound antibodies were detected either with HRP-conjugated anti-human Fc secondary antibody or with rabbit anti-TAM558 cytolysin-p153 polyclonal antibody in combination with HRP-conjugated anti-rabbit antibody. Data were analyzed with the program PKsolver

FAP mRNA Expression

FAP mRNA expression was evaluated by RT-qPCR. RNA from the different tumor models was isolated from frozen tumor samples using the gentleMACS Dissociator (Milteny Biotec) and micro RNeasy kit (Qiagen) according to instructions from the manufacturer. The corresponding synthesized cDNA was amplified using M-MLV reverse transcriptase (Invitrogen, 28025-103) and specific forward (5'-TACACCCACATGACCCACTT-3´) and reverse (5´-GCAAACTGTCTGAGGGGTTTAT-3´) primers for human FAP sequence or specific forward (5´-GCTTCACATGGACGACTTGG-3´) and reverse (5´-GATGTGTGGCTCCCCTTTTC-3´) primers for mouse FAP sequence.

Flow Cytometry Analysis on Tumor Samples

Flow cytometry analysis in tumors of treated mice was performed after mice were euthanized. Tumors were collected into MACS media and processed by enzymatic digestion until generation of single cell suspension. For flow cytometry analysis the following panel of antibodies was used: anti-mCD45 APC conjugated(30-F11), anti-hCD45 BV510 conjugated (H130), anti-hCD3 FITC conjugated (UCHT1), anti-hCD8 BV421 conjugated (RPA-T8), anti-hCD4 PEVio770 conjugated (M-T321), anti-hCD19 PE conjugated (HIB19), anti-hCD25 APC-Cy7 conjugated (BC96) and cell viability was assessed with 7-AAD staining. Flow cytometry acquisition was performed with a

MacsQuant Flow Cytometer. Values were expressed as mean +/- SE and p values were calculated using Mann-Whitney U test.

Immunocytochemistry and Confocal Microscopy Analysis of Payload Intracellular Release

HT1080-FAP (1 x 10^4 cells) were cultivated for 24 hours at 37 °C on a collagen R-coated cover slip. Cells were incubated at 37 °C with medium (RPMI supplemented with 10 % FCS) containing OMTX005 or OMTX705 (40 nM) for 30 minutes and subsequently (after washing cells twice with PBS) with medium for 60 minutes. Then, cells were fixed with 3.7 % paraformaldehyde (PFA) for 15 minutes at room temperature (RT), quenched with 1 M glycine for 10 minutes at RT, permeabilized with 0.2 % Triton X-100 for 2 minutes at RT, and finally blocked with PBS containing 5 % FCS for 30 minutes at RT. Cells were incubated with primary antibody (rabbit anti-Rab7 #2094, Cell Signaling; 1 to 50 diluted) for 2 hours at RT, and secondary antibodies (FITC-labeled anti-human Fab #F5512, Sigma-Aldrich, 1 to 60 diluted; PE-labeled anti-rabbit #ab50677, Abcam, 1 to 500 diluted) was added for 1 hour at RT. All antibodies were diluted in PBS supplemented with 5 % FCS. After each incubation step, cells were washed twice with PBS for 5 minutes at RT. Finally, cover slips were mounted with Mowiol 4.88 (Polysciences, Eppelheim, Germany) supplemented with bis-benzimide and analyzed at RT using a confocal laser scanning microscope (LSM 710, Carl Zeiss) equipped with a Plan Apochromat 63x/1.40 DIC M27 (Carl Zeiss) oil-immersion objective. Green channel was excited with the 488 nm line of an Argon laser (emission detected from 496 – 582 nm). Red channel was excited with a 561 nm DPSS laser (emission detected from 568 – 690 nm). Blue channel was excited with a 405 nm diode laser (emission detected from 410 – 495 nm). Pinhole was set to 60 µm which represents 1 AU in the red channel.

Immunohistochemistry of PDX Tumors from Treated Mice

Tumor tissue slides were stained on a Ventana autostainer based on indirect approaches using DAB as the reporter marker and the Discovery XT3 Chromo MAP HRP / OmniMap DAB Rb kit from Ventana. Staining was analyzed with ScanScope® (Aperio/Leica) system and ImageScope® (v12.3.2.5030) using the analysis algorithm "positive pixel

count V9" for surface quantification and "Nuclear V9" for nuclear quantification (CitoxLab). OMTX705 detection with an anti-human IgG antibody was analyzed in frozen tumor sections using Rb monoH169-1-5 anti-Human IgG (AB125909, Abcam). Payload TAM558 detection was performed in formalin-fixed paraffin embedded samples using a rabbit polyclonal anti TAM 558-153 generated at Oncomatryx. Ki67 staining was performed in formalin-fixed paraffin embedded samples using an anti-Ki-67 (30-9) rabbit monoclonal primary antibody (Ventana). Active caspase 3 staining was performed in formalin-fixed paraffin embedded samples using a Rabbit polyclonal to active caspase 3 (AB2302, Abcam). aSMA and COL11A1 staining were performed in formalin-fixed paraffin embedded samples using an anti-alpha smooth muscle actin antibody (AB5694, Abcam) and a rabbit polyclonal anti murine COL11A1-158 generated at Oncomatryx, respectively. Values were expressed as mean +/- SE. Statistical analysis and p values were calculated using Student’s t test.

	PATIENT				ORIGIN								TREATMENT		
	Age	Gender	Ethnic backgd	Smoking history	Tumor type	Tumor AP	HLA type	Origin	Harvest site	Tumor grade	Stage	Diagnosis	At tumorgraft collection	Neo- adjuvant	Adjuvant
CTG-0860	70	Female	Caucasian	Former smoker	NSCLC	Adenocarcinoma	A11, A31	Local metastatic	Lung	Poorly differentiat	IV	Recurrent	Not Available		

MOLECULAR CHARACTERIZATION					
MUTATIONS				IHC	FISH
BRCA2	KRAS	NTRK3	RPTOR		
Mutated	Mutated	Mutated	Mutated	N/A	N/A

	PATIENT				ORIGIN								TREATMENT		
	Age	Gender	Ethnic backgd	Smoking history	Tumor type	Tumor AP	HLA type	Origin	Harvest site	Tumor grade	Stage	Diagnosis	At tumorgraft collection	Neo- adjuvant	Adjuvant
Panc007	71	Male	Caucasian	N/A	Pancreas	Adenocarcinoma	*	Primary Tumor	Pancreas		pT2N1M0	*	None	None	Gemcitabine Apr2007

MOLECULAR CHARACTERIZATION					
MUTATIONS	IHC				FISH
	ERCC1	E-Cadherine	Vimentin	Thymidylate synthase	
N/A	(+) 10% (negative)	(2+) 91%	(3+) 99%	(2+) 62%	N/A

	PATIENT				ORIGIN								TREATMENT		
	Age	Gender	Ethnic backgd	Smoking history	Tumor type	Tumor AP	HLA type	Origin	Harvest site	Tumor grade	Stage	Diagnosis	At tumorgraft collection	Neo- adjuvant	Adjuvant
Lung024	48	Male	Cau casian	Former smoker	Lung	Epidermoid	*	Retroperineal Mtx	*		IV	*	Not Available		

MOLECULAR CHARACTERIZATION									
MUTATIONS		IHC						FISH	
EGFR	KRAS	ERCC1	Pan-keratin AE1/AE3	CK-7	TTF-1	p63	p56	EGFR	ALK
WT	mutated (G12C)	(+) (2+; 73%)	(+)	(+)	(-)	(-)	(+) (2+; 74%)	Not amplified	Not translocated

	PATIENT				ORIGIN								TREATMENT		
	Age	Gender	Ethnic backgd	Smoking history	Tumor type	Tumor AP	HLA type	Origin	Harvest site	Tumor grade	Stage	Diagnosis	At tumorgraft collection	Neo- adjuvant	Adjuvant
Breast 014	60	Female	Caucasian	N/A	Triple Negative Breast	Infiltrating ductal carcinoma	*	Primary tumor	Breast		cT2cN0M0		Neoadjuvant: Taxol (x10) + 5FU-epirubicin-cyclophosphamide (x4) Surgery and then RT		

MOLECULAR CHARACTERIZATION								
MUTATIONS	IHC							FISH
	ki-67	E-cadher	Pan-keratin AE1/AE3	Estrogen receptors	Progesterone recpts	p63	p53	HER2
N/A	62%	(+)	(-)	(-)	(-)	(+) in isolated cells	Not detected	Not amplified

Table S1. Description of PDX models

Data from Patient, tumor at collection, previous treatment and molecular characterization.