

**Supplementary Table S1. Patient characteristics.**

<b>Cancer type</b>	Primary CRC	Metastatic CRC peritoneum	Metastatic CRC liver	Primary EC	HNSCC	NSCLC
<b>N</b>	17	25	3	23	5	4
<b>Gender (female)</b>	4 (24%)	11 (44%)	0 (100%)	5 (22%)	1 (20%)	0 (0%)
<b>Age mean (range)</b>	67 (53-77)	62 (41- 79)	67 (62- 71)	67 (43-79)	62 (48-74)	69 (60-76)
<b>Stage</b>						
I	5 (29%)			1 (4%)		
II	1 (6%)			4 (17%)		1 (25%)
III	6 (35%)			12 (52%)		2 (50%)
IV	7 (41%)	25 (100%)	3 (100%)	2 (9%)	5 (100%)	1 (25%)
Unknown				4 (17%)		
<b>Treatment</b>						
Chemotherapy	1 (6%)	11 (44%)	2 (67%)			1 (25%)
Radiotherapy		2 (8%)	1 (33%)			1 (25%)
Chemoradiotherapy		1 (4%)				
Untreated	16 (94%)	11 (44%)		23 (100%)	5 (100%)	2 (50%)
<b>Mutational status</b>						
No RAS/ BRAF mutation		1 (4%)				
KRAS mutation	1 (6%)	1 (4%)				
BRAF mutation		4 (16%)				
Unknown	16 (94%)	19 (76%)	3 (100%)	23 (100%)	5 (100%)	4 (100%)
<b>MSI</b>						
MMR proficient/ MSS	1 (6%)	12 (48%)		6 (26%)		
MSI		3 (12%)				
Unknown	16 (94%)	10 (40%)	3 (100%)	17 (74%)	5 (100%)	4 (100%)

Abbreviations: CRC: colorectal cancer, EC: esophageal cancer (n=18 adenocarcinomas and n=5 squamous cell carcinomas), HNSCC: head and neck squamous cell carcinoma, NSCLC: non-small cell lung cancer, AC: adenocarcinoma, SCC: squamous cell carcinoma.

**Supplementary Table S2. Details flow-cytometry antibodies.**

<b>Marker</b>	<b>Fluorochrome</b>	<b>Clone</b>	<b>Catalogue number</b>	<b>Company</b>	<b>Host</b>
Anti-Illama	FITC	Polyclonal	BET A160-100F	Bioke	Goat
Anti-VHH	iFluor555	96A3F5	A01863	Genscript	Rabbit
Anti-VHH	iFluor 647	45H8	18L001648	Genscript	Rabbit
Anti-his-tag	PE	A32	A568	Biolegend	Mouse
Streptavidin	APC	Not applicable	17-4317-82	eBioscience	Not applicable
V $\delta$ 2-TCR	BV711	B6	331412	Biolegend	Mouse
V $\delta$ 2-TCR	FITC	IMMU389	PN IM1464	Beckman Coulter	Mouse
V $\gamma$ 9-TCR	APC	B3	331310	Biolegend	Mouse
V $\gamma$ 9-TCR	FITC	IMMU360	IM1463	Beckman Coulter	Mouse
TCR pan $\gamma\delta$	PE	B1	331210	Biolegend	Mouse
TCR pan $\gamma\delta$	BV421	B1	B324431	Ozyme	Mouse
CD3	BV421	SK7	563798	BD Horizon	Mouse
CD3	PerCPy5.5	SK7	332771	BD Biosciences	Mouse
CD3	PerCPy5.5	SP34-2	552852	Becton Dickinson	Mouse
CD3	PE	SK7	345765	BD Biosciences	Mouse
CD3	AF700	SP34-2	557917	BD Biosciences	Mouse
CD3	BUV395	SP34-2	564117	BD Biosciences	Mouse
CD4	FITC	345768	SK3	BD Biosciences	Mouse
CD4	AF700	RPA-T4	557922	BD Pharmingen	Mouse
CD8	V500	SK1	561618	BD Horizon	Mouse
CD8	FITC	345772	SK1	BD Biosciences	Mouse
CD16	BV650	3G8	563692	BD Biosciences	Mouse
CD16	FITC	555406	3G8	BD pharmingen	Mouse
CD25	PE	M- A251	555432	BD pharmingen	Mouse
CD25	APC	2A3	340907	BD Biosciences	Mouse
CD25	PE-Cy7	M-A251	356108	Biolegend	Mouse
CD25	PE-Dazzle 594	M-A251	B327560	Becton Dickinson	Mouse
CD45	AF700	HI30	304024	Biolegend	Mouse
CD45	BV786	D058-1283	563861	BD Biosciences	Mouse
CD45RA	APC-Cy7	HI100	560674	BD Pharmingen	Mouse
CD56	FITC	NCAM16.2	345811	BD Biosciences	Mouse
CD69	APC-Fire750	FN50	310946	Biolegend	Mouse
CD69	BV711	FN50	B322918	Ozyme	Mouse
CD107a	PE	eBioH4A3	12-1079-42	eBioscience	Mouse
EpCAM	FITC	324204	9C4	Biolegend	Mouse
PD-1	BV786	563789	EH12.1	BD Horizon	Mouse
LAG-3	PE-Cy7	25-2239-42	3DS223H	eBioscience	Mouse
TIM-3	BV421	F38-2E2	345008	Biolegend	Mouse
CTLA-4	PE-CF595	BNI3	562742	BD Horizon	Mouse
FoxP3	PE	PCH101	12-4776-42	eBioscience	Rat
7AAD	PerCPy5.5	Not applicable	A9400-1MG	Sigma	Not applicable

**Supplementary Table S3. Toxicokinetic parameters for EGFR-Vy9 bsTCE following a single intravenous infusion to female cynomolgus monkeys.**

Animal ID	Dose (mg/kg)	T <sub>max</sub> (h)	C <sub>max</sub> (µg/ml)	nC <sub>max</sub> (µg/ml)/(mg/kg)	T <sub>1/2</sub> (h)	AUC <sub>0-∞</sub> (µg·h/ml)	nAUC <sub>0-∞</sub> (µg·h/ml)/(mg/kg)	C1 (L/h/kg)	V <sub>z</sub> (L/kg)
L08	0.03	0.5	0.25	8.37	13.7	2.48	82.7	0.012	0.24
J08	0.1	1	0.50	5.04	NC	NC	NC	NC	NC
Y06	0.3	1	1.38	4.59	7.93	2.53	0.12	0.12	1.36

Abbreviations: T<sub>max</sub>: time after dosing at which the maximum concentration was observed. C<sub>max</sub>: the maximum concentration observed after dosing. nC<sub>max</sub>: the C<sub>max</sub> normalized for the dose received. AUC<sub>0-∞</sub>: the area under the concentration versus time curve from time-point t=0h to infinity. nAUC<sub>0-∞</sub>: the AUC<sub>0-∞</sub> normalized for the dose received. T<sub>1/2</sub>: the elimination half-life, determined by linear regression of at least three data points on the terminal phase of the log (Concentration) vs time plot. C1: clearance. V<sub>z</sub>: apparent volume of distribution. NC: not calculated, not enough time point on the elimination phase.

**Supplementary Table S4. Toxicokinetic parameters for EGFR-Vy9-Fc bsTCE following multiple intravenous infusions to female cynomolgus monkeys.**

Animal ID	Dose (mg/kg)	Day	T <sub>max</sub> (h)	C <sub>max</sub> (µg/ml)	T <sub>1/2</sub> (h)	AUC <sub>0-tlast</sub> µg·h/mL	AUC <sub>0-∞</sub> (µg·h/ml)	C1 (ml/h/kg)	V <sub>z</sub> (ml/kg)
4916	1	0	1	17.3	110.5	910.0	1,383.7	0.72	115.2
4916	1	7	0.5	22.7	106.1	1,189.7	1,785.3	0.56	85.7
4916	1	14	2.5	16.4	NC	730.2	NC	NC	NC
4916	1	21	1	6.6	NC	25.5	NC	NC	NC
4917	5	0	1	102.0	103.0	6,213.0	9,190.3	0.54	80.8
4917	5	7	1	132.2	114.6	7,894.1	12,228.1	0.41	67.6
4917	5	14	2.5	98.6	NC	7,956.7	NC	NC	NC
4917	5	21	1	126.8	NC	2,161.5	NC	NC	NC
4918	23	0	1	482.0	127.4	30,268.9	49,412.4	0.47	85.5
4918	23	7	1	663.3	113.6	41,377.1	64,409.5	0.36	58.5
4918	23	14	2.5	548.5	NC	47,061.6	NC	NC	NC
4918	23	21	1	792.5	NC	13,911.3	NC	NC	NC

Abbreviations: AUC<sub>0-tlast</sub>: the area under the concentration versus time curve from time-point t=0h to the last measurable concentration.

Supplementary Table S5. Clinical, hematological and biochemical parameters in NHP pre- and post-administration of EGFR-V $\gamma$ 9 bsTCE.

	Day	EGFR-V $\gamma$ 9 bsTCE		
		0.03 mg kg <sup>-1</sup>	0.1 mg kg <sup>-1</sup>	0.3 mg kg <sup>-1</sup>
BW (kg)	-14	3.9	4.58	4.8
	-7	3.9	4.5	4.62
	0	3.94	4.48	4.7
	7	3.9	4.4	4.68
RBC (x10 <sup>12</sup> L <sup>-1</sup> )	-14	5.81	7.03	6.66
	-7	5.77	6.61	6.15
	1	5.47	5.96	6.16
	2	5.38	5.78	5.92
	3	4.9	5.57	5.72
	4	4.63	5.28	5.72
	5	4.42	4.96	5.04
	6	4.4	4.92	4.74
	7 + 24h	4.21	4.61	4.65
PLT (x10 <sup>9</sup> L <sup>-1</sup> )	-14	551	372	372
	-7	561	338	367
	1	702	382	425
	2	736	389	420
	3	740	397	427
	4	722	424	426
	5	733	403	420
	6	727	398	447
	7 + 24h	744	414	442
PT (s)	-14	9.2	10	9.3
	-7	9.2	9.6	9.8
	3	9.4	9.7	9.5
	7	9.1	9.3	9.4
Total bilirubin ( $\mu$ mol L <sup>-1</sup> )	-14	3.4	6.8	3.1
	-7	2.6	7.2	3
	2	2.1	8.8	3.6
	5	2.1	7.9	2.1
	7	2.3	7.4	2.1
ALP (U L <sup>-1</sup> )	-14	133	120	135
	-7	142	128	118
	2	175	138	148
	5	162	142	143
	7	150	134	175
GGT (U L <sup>-1</sup> )	-14	47.1	43.6	38.8
	-7	46.6	41.8	34.5
	2	43.7	37.9	31.3
	5	39.3	37.2	27.3
	7	40.1	34.4	26.9
ALT (U L <sup>-1</sup> )	-14	28.6	23.3	96.2
	-7	90.5	20.3	74.5
	2	54	27.9	111.4
	5	40.2	24.1	77.6
	7	34	21.4	66.5

<b>Creatinine (<math>\mu\text{mol L}^{-1}</math>)</b>	-14	55	50	65
	-7	59	49	66
	2	52	44	65
	5	53	48	61
	7	50	49	68

Abbreviations: BW: body weight. RBC: red blood cells. PLT: platelets. PT: prothrombin time test ALP: alkaline phosphatase. GGT: gamma-glutamyltransferase. ALT: alanine aminotransferase. The gradual decrease in RBC over time is considered to be caused by the repeated blood draws.

Supplementary Table S6. Clinical, hematological and biochemical parameters in NHP pre- and post-administration of EGFR-Vy9-Fc bsTCE.

	Day	EGFR-Vy9-Fc bsTCE		
		1 mg kg <sup>-1</sup>	5 mg kg <sup>-1</sup>	23 mg kg <sup>-1</sup>
Temperature (°C)	6	38.3	38.5	38.2
	7	38.1	38.3	38.2
	7 + 6h	38.1	38.4	38.2
	7 + 24h	38.3	38.7	37.8
	7 + 48h	37.9	38.3	38.6
	7 + 72h	38.8	38.2	38.3
	14 - 48h	38.5	37.9	37.3
	14 - 24h	37.8	38.4	38
	14	37.3	37.4	37
	14 + 6h	38.5	38.7	37.2
	14 + 24h	38.2	38.5	37.5
	14 + 48h	37.8	38.7	37.7
	14 + 72h	38.6	38.1	37.5
	21 - 48h	38.1	38.3	37.5
	21 - 24h	38.6	39.2	38.2
	21	38.5	37.2	37.1
	21 + 6h	37.9	38.7	37
21 + 24h	38.1	38.2	37.8	
BW (kg)	-28	3.6	3.8	3.3
	-22	3.6	3.85	3.25
	-14	3.65	3.9	3.3
	-7	3.6	3.9	3.4
	1	3.65	3.8	3.35
	8	3.65	3.8	3.25
	15	3.5	3.9	3.35
	22	3.5	3.7	3.35
RBC (x10 <sup>12</sup> L <sup>-1</sup> )	-14	6.83	6.36	6.48
	-7	6.77	6.24	6.55
	0 + 72h	5.78	5.74	5.37
	7	5.89	5.71	6.07
	7 + 72h	5.75	5.37	5.59
	14	6.09	5.68	6.86
	14 + 72h	6.05	5.36	5.63
	21	6.21	5.95	6.06
	21 + 24h	5.13	5.65	5.37
PLT (x10 <sup>9</sup> L <sup>-1</sup> )	-14	236	247	246
	-7	164	246	151
	0 + 72h	187	325	312
	7	210	384	342
	7 + 72h	419	376	323
	14	249	316	344
	14 + 72h	343	285	309
	21	292	279	272
	21 + 24h	180	251	306
Total bilirubin (µmol L <sup>-1</sup> )	-14	4	4	5
	-7	5	5	2
	7	6	3	4

	7 + 72h	6	4	n.a.
	14	19	4	3
	21	7	4	3
	21 + 24h	5	5	2
<b>ALP (U L<sup>-1</sup>)</b>	-14	362	315	202
	-7	305	312	231
	7	262	271	211
	7 + 72h	308	261	n.a.
	14	210	258	200
	21	272	295	220
	21 + 24h	248	286	210
<b>GGT (U L<sup>-1</sup>)</b>	-14	78	68	70
	-7	73	69	74
	7	57	71	75
	7 + 72h	64	67	n.a.
	14	45	71	78
	21	59	73	79
	21 + 24h	59	72	79
<b>ALT (U L<sup>-1</sup>)</b>	-14	26	33	84
	-7	17	56	87
	7	41	64	116
	7 + 72h	28	86	n.a.
	14	26	82	167
	21	37	95	182
	21 + 24h	45	112	144
<b>Creatinine (μmol L<sup>-1</sup>)</b>	-14	64	95	78
	-7	61	94	66
	7	62	114	67
	7 + 72h	66	99	n.a.
	14	53	100	63
	21	60	80	74
	21 + 24h	66	84	65

Abbreviations: BW: body weight. RBC: red blood cells. PLT: platelets. ALP: alkaline phosphatase. GGT: gamma-glutamyltransferase. ALT: alanine aminotransferase. n.a.: not assessed. The gradual decrease in RBC over time is considered to be caused by the repeated blood draws.