

Supplementary Tables

Table S1: Biochemical properties of scTRAIL molecules.

Molecule	Cal. MW [kDa]	Yield [mg/l]	S _r [nm]	T _M [°C]
scTRAIL	58	0.7	3.4	52
Db-scTRAIL	168	3.3	5.5	55
EHD2-scTRAIL	141	2.3	5.6	58
scFv-EHD2-scTRAIL	194	3.8	6.0	58
Fc-scTRAIL	169	6.9	6.0	58
scFv-Fc-scTRAIL	222	10.4	6.4	58

Table S2: Binding properties of scTRAIL molecules. EC₅₀ values [nM with respect to scTRAIL].

Molecule	EGFR	huTRAIL-R2	moTRAIL-R2	Colo205	HCT116
scTRAIL	-	1.8 ± 0.4	3.3 ± 0.2	6.7 ± 1.4	2.2 ± 0.2
Db-scTRAIL	0.8 ± 0.1	0.8 ± 0.1	3.9 ± 1.2	0.9 ± 0.3	0.6 ± 0.03
EHD2-scTRAIL	-	2.0 ± 0.2	2.0 ± 0.3	>100 ^a	>100 ^a
scFv-EHD2-scTRAIL	1.2 ± 0.1	2.1 ± 0.7	3.8 ± 0.8	0.6 ± 0.1	0.4 ± 0.02
Fc-scTRAIL	-	2.5 ± 0.3	4.1 ± 1.0	>100 ^a	>100 ^a
scFv-Fc-scTRAIL	0.9 ± 0.01	1.6 ± 0.4	3.5 ± 0.7	1.7 ± 1.1	0.5 ± 0.1

^a, no saturation reached.

Table S3: EC₅₀ values [pM] of cell death induction in the presence of blocking antibody. Effects on Colo205 cells were analyzed in the absence and presence of bortezomib (250 ng/ml). EGFR binding was blocked by preincubation with a 200-fold molar excess of cetuximab. EC₅₀ values correspond to concentration of scTRAIL.

Molecule	Colo205	
	w/o BZB	+ BZB
scTRAIL	–	36.0 ± 9.6
Db-scTRAIL	100.6 ± 12.0	14.1 ± 2.1
EHD2-scTRAIL	44.6 ± 8.4	6.0 ± 2.2
scFv-EHD2-scTRAIL	69.3 ± 11.6	6.8 ± 0.9
Fc-scTRAIL	118.1 ± 8.7	14.1 ± 4.0
scFv-Fc-scTRAIL	66.0 ± 12.2	6.0 ± 2.1

–, less than 50 % of cells dead.

Table S4: Pharmacokinetic properties. Initial and terminal half-lives ($t_{1/2\alpha}$, $t_{1/2\beta}$) and areas under the curve (AUC) were determined in non-tumor-bearing CD-1[®] mice after i.v. injection of 25 µg protein.

Molecule	$t_{1/2\alpha}$ [h]	$t_{1/2\beta}$ [h]	AUC [µg/ml*h]
scTRAIL	0.7 ± 0.03	2.2 ± 0.1	14 ± 2
Db-scTRAIL	1.1 ± 0.2	2.4 ± 0.1	22 ± 4
EHD2-scTRAIL	1.3 ± 0.04	8.6 ± 0.4	61 ± 1
scFv-EHD2-scTRAIL	1.5 ± 0.1	6.5 ± 0.2	45 ± 7
Fc-scTRAIL _{8M}	1.5 ± 0.02	21.6 ± 3.8	46 ± 9
Fc-scTRAIL	5.0 ± 1.5	14.5 ± 0.8	68 ± 4
scFv-Fc-scTRAIL	2.0 ± 0.2	17.4 ± 3.1	71 ± 7
scFv-Fc	2.7 ± 0.8	111.9 ± 23.3	637 ± 121
cetuximab	4.1 ± 1.0	219.2 ± 25.3	761 ± 24

Table S5: Drug exposure of scTRAIL fusion proteins in tumor-bearing mice. Areas under the curve (AUC) were calculated based on the concentrations [nM scTRAIL units] determined after the first and last treatment of the first PD experiment, respectively.

Molecule	AUC _{0-48h} [nM*h]	AUC _{0-48h} [nM*h]
	first treatment	last treatment
Db-scTRAIL	3181 ± 287	2250 ± 697
scFv-EHD2-scTRAIL	2805 ± 100	2017 ± 454
Fc-scTRAIL	5207 ± 737	5803 ± 730
scFv-Fc-scTRAIL	6636 ± 904	5694 ± 1273