

Supplemental table I. TNFRSF receptor-specific antibodies evaluated in this study

Antigen	Isotype	Clone	Function	Applications	Company	Order-no.
4-1BB	mIgG1	4B4-1		FACS	BD Pharmingen TM	555956
CD27	mIgG1	57703	neutralization	FACS, WB	R&D Systems	MAB382
CD40	IgG1	G28.5 ^a				
CD40	mIgG1	HB14		FACS	Milteny Biotec	130-099-385
CD40	mIgG2A	LOB-11		FACS , IF, IHC, IP, WB	Santa Cruz	sc-13528
CD95	IgG1	E09 ^a		FACS		
CD95	mIgG1	DX2		IHC, WB	R&D Systems	MAB142
Fn14	hIgG1	18D1 ^c	blocking			
Fn14	mIgG2B	ITEM-4	neutralization	FACS, IF, WB	Invitrogen	12-9018-42
Fn14	hIgG1	P4A8 ^d	blocking			
Fn14	hIgG1	PDL192 ^e				
GITR	mIgG1	DT5D3		FACS	Milteny Biotec	130-099-088
LTβR	mIgG2B	31G4D8	agonist	ELISA , FACS, IP, WB,	Novusbio	NB100-78050
LTβR	mIgG1	71315	agonist	n.m.	R&D Systems	MAB6291
LTβR	mIgG1	71319		FACS , WB	R&D Systems	MAB629
LTβR	IgG1	BHA10 ^f				
Ox40	mIgG1	BER-Act35		FACS,IF, IHC, IF, WB	Santa Cruz	sc-20073
TNFR1	mIgG1	16803	neutralization	FACS, WB	R&D Systems	MAB225
TNFR1	mIgG1	MABTNFR1-A1		ELISA (capture)	BD Pharmingen TM	552535
TNFR1	mIgG2A	H398 ^g	blocking			
TNFR1	mIgG1	htr9	agonist, inhibits TNF-binding	FACS, IHC, WB	Acris	BM4053
TNFR2	mIgG2A	22235		FACS	R&D Systems	FAB226P
TNFR2	mIgG1	MR2-1		FACS , IHC, IP, WB	LSBio	LS-C139940
TNFR2	mIgG1	utr1	blocking	FACS , IHC, IP, WB	Acris	BM4054
TNFR2				ELISA (capture) ^h	R&D Systems	
TRAILR1	mIgG1	DJR1		FACS, IF	Biologend ®	307202
TRAILR1	mIgG1	HS101	inhibition	FACS, ICC, IP	AdipoGen ®	AG-20B-0022
TRAILR2	mIgG1	D-6		ELISA , IF, IP, WB	Santa Cruz	sc-166624
TRAILR2	mIgG1	DJR2-2		FACS , IF	Biologend ®	307302

^a AJ853736 (PDB entry)

^b 3TJE_H+L (PDB entry)

^c sequence on request by Argenx

^d Garber E, Burkly L, Michealson J, Lugovskoy A, Hsu YM, Hanf K. Anti-Fn14 antibodies and uses thereof. WO2009140177A2. 2009

^e Culp P. Therapeutic use of anti-TWEAK antibodies. WO2009020933A2. 2009

^f Michaelson et al., 2009 [1]

^g kind gift of Klaus Pfizenmaier

^h no additional information available

Supplemental table II. Primary structure of antibody chains used in this study

	CONSTRUCT	ARCHITECTURE
[1]	α4-1BB(HBBK4)-LC	$L^a - F^b - V_L$ (US 2009/0041763 A1_H4B4-L) – C_L^c
[2]	α4-1BB(HBBK4)-IgG1(N297A)-HC-scFv:CD20	$L^a - F^b - V_H$ (US 2009/0041763 A1_H4B4-H) – C_H^d – scFv:CD20 ^f
[3]	αCD40(G28.5)-LC	$L^a - F^b - V_L$ (aa: 148-259 [AAB81500.1]) – C_L^c
[4]	αCD40(G28.5)-IgG1-HC	$L^a - F^b - V_H$ (aa: 21-132 [CAH69220.1]) – C_H^d
[5]	αCD40(G28.5)-IgG1(N297A)-HC-scFv:CD20	$L^a - F^b - V_H$ (aa: 21-132 [CAH69220.1]) – C_H^e – scFv:CD20 ^f
[6]	αCD95(E09)-LC	$L^a - F^b - V_L$ (aa: 1-111 [3TJE_L]) – C_L^c
[7]	αCD95(E09)-IgG1-HC	$L^a - F^b - V_H$ (aa: 1-132 [3TJE_H]) – C_H^d
[8]	αCD95(E09)-IgG1(N297A)-HC-scFv:CD20	$L^a - F^b - V_H$ (aa: 1-132 [3TJE_H]) – C_H^e – scFv:CD20 ^f
[9]	αFn14(18D1)-LC	$L^a - F^b - V_L^h - C_L^c$
[10]	αFn14(18D1)-LC (murin)	$L^a - F^b - V_L^h - C_L$ (aa: 3-111 [WP_077942483.1])
[11]	αFn14(18D1)-LC-GpL	$L^a - F^b - V_L^h - C_L^c - GpL$ (aa: 63-230 [AEX33289.1])
[12]	αFn14(18D1)-IgG1-HC	$L^a - F^b - V_H^h - C_H^d$
[13]	αFn14(18D1)-IgG2-HC	$L^a - V_H^h - C_H$ (aa: 137-464 [BAC85395.1])
[14]	αFn14(18D1)-IgG3-HC	$L^a - V_H^h - C_H$ (aa: 141-519 [AAH89421.1])
[15]	αFn14(18D1)-IgG4-HC	$L^a - V_H^h - C_H$ (aa: 118-446 [ASY91977.1])
[16]	αFn14(18D1)-mIgG1-HC	$L^a - V_H^h - C_H$ (aa: 135-460 [BAQ25543.1])
[18]	αFn14(18D1)-mIgG2A-HC	$L^a - V_H^h - C_H$ (aa: 136-467 [4WEB_H])
[19]	αFn14(18D1)-IgG1(N297A)-HC	$L^a - F^b - V_H^h - C_H^e$
[20]	αFn14(18D1)-Fab1-HC-scFv:CD20	$L^a - V_H^h - C_H$ (aa: 117-232 [4OQT_H]) – scFv:CD20 ^f
[21]	αFn14(18D1)-IgG1(N297A)-HC-scFv:CD20	$L^a - F^b - V_H^h - C_H^e - scFv:CD20^f$
[22]	αFn14(18D1)-IgG2-HC-scFv:CD20	$L^a - V_H^h - C_H$ (aa: 137-464 [BAC85395.1]) – scFv:CD20 ^f
[23]	αFn14(18D1)-IgG3-HC-scFv:CD20	$L^a - V_H^h - C_H$ (aa: 141-519 [AAH89421.1]) – scFv:CD20 ^f
[24]	αFn14(18D1)-IgG4-HC-scFv:CD20	$L^a - V_H^h - C_H$ (aa: 118-446 [ASY91977.1]) – scFv:CD20 ^f
[26]	αFn14(P4A8)-LC	$L^a - F^b - V_L$ (aa: 311-421 [AIL25364.1]) – C_L^c
[27]	αFn14(P4A8)-IgG1-HC	$L^a - F^b - V_H$ (aa: 437-557 [AIL25364.1]) – C_H^d
[28]	αFn14(PDL192)-LC	$L^a - F^b - V_L$ (WO 2013/177386 AI_PDL192 V_L) – C_L^c
[29]	αFn14(PDL192)-IgG1-HC	$L^a - F^b - V_H$ (WO 2013/177386 AI_PDL192 V_H) – C_H^d
[30]	αLTβR(BHA-10)-LC	$L^a - F^b - V_L$ (aa: 1-107 [1]) – C_L^c
[31]	αLTβR(BHA-10)-IgG1-HC	$L^a - F^b - V_H$ (aa: 1-113 [1]) – C_H^d
[32]	αTNFR2(C4)-LC	$L^a - F^b - V_L^i - C_L^c$

[33]	α TNFR2(C4)-LC (murin)	$L^a - F^b - V_L^i - C_L$ (aa: 2-111 [WP_077942483.1])
[34]	α TNFR2(C4)-IgG1-HC	$L^a - V_H^j - C_H^d$
[35]	α TNFR2(C4)-IgG2-HC	$L^a - V_H^j - C_H$ (aa: 137-464 [BAC85395.1])
[36]	α TNFR2(C4)-IgG3-HC	$L^a - V_H^j - C_H$ (aa: 141-519 [AAH89421.1])
[37]	α TNFR2(C4)-IgG4-HC	$L^a - V_H^j - C_H$ (aa: 118-446 [ASY91977.1])
[38]	α TNFR2(C4)-mIgG1-HC	$L^a - V_H^j - C_H$ (aa: 137-460 [BAQ25543.1])
[39]	α TNFR2(C4)-mIgG2A-HC	$L^a - V_H^j - SSA - C_H$ (aa: 136-467 [4WEB_H])
[40]	α TNFR2(C4)-IgG1(N297A)-HC-muGITRL	$L^a - V_H^j - C_H^e - \text{muGITRL}$ (aa: 50-177 [EDL39313.1])
[41]	α TNFR2(C4)-IgG1(N297A)-HC-muIL2	$L^a - V_H^j - C_H^e - \text{PGT} - \text{muIL2}$ (aa: 21-169 [AAA39281.1])
[42]	α TNFR2(C4)-IgG1(N297A)-HC-scFv:CD19	$L^a - V_H^j - C_H^e - \text{scFv:CD19}^{k[2]}$
[43]	α TNFR2(C4)-IgG1(N297A)-HC-scFv:CD20	$L^a - V_H^j - C_H^e - \text{scFv:CD20}^f$
[44]	α TNFR2(C4)-IgG1(N297A)-HC-scFv:CD70	$L^a - V_H^j - C_H^e - V_H$ (US20090028872A1_2H5-VH) - $LK^g - V_L$ (US20090028872A1_2H5-VK)
[45]	α TNFR2(C4)-IgG1(N297A)-HC-scGITRL	$L^a - V_H^j - C_H^e - \text{GGGSG} - \text{GITRL}^l - LK^g - \text{GITRL}^l - LK^g - \text{QF} - \text{GITRL}^l$
[46]	α TNFR2(C4)-IgG1(N297A)-HC-sc(mu)4-1BBL	$L^a - V_H^j - C_H^e - \text{GGGSG} - \text{mu4-1BBL}^m - LK^g - \text{mu4-1BBL}^m - LK^g - \text{mu4-1BBL}^m$

^a L = Leader: MNFGSLIFLVLVKGQCEVKLVPR

^b F = Flag: DYKDDDDK

^c C_L = constant light (aa: 105-214 [BAA97671.1])

^d C_H = constant heavy IgG1 (aa: 145-476 [AAA02914.1])

^e IgG1(N297A): with A to N mutation at position 327 of constant heavy IgG1 ^d

^f scFv:CD20 (aa: 38-280 [AUI40871.1]): V_H (Rituximab) - $LK^g - V_L$ (Rituximab)

^g LK = Linker GGGGSGGGGSGGGGS

^h sequence on request by Argenx

ⁱ V_L -C4: DIVMTQSHKFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDYTLTISSVQAEDLARYYCQQYYSPPTFGGGTKL

^j V_H -C4: QVQLLQSGPELVKPGASVKLSCKASGYSFTSYDINWVVKRPGQGLEWVWGIYPRDGTKYNEKFKGKAILTVDTSNTAYMNLHSLTSEDSAVYFCARLTGPIWYFDVWGTGTTVTVSS

^k kind gift of Prof. Helfrich (University of Groningen)

^l GITRL (aa: 50-177 [AAD22634.1])

^m mu4-1BBL (aa: 105-309 [NP_033430.1])

1. Michaelson JS, Demarest SJ, Miller B, Amatucci A, Snyder WB, Wu X, et al. Anti-tumor activity of stability-engineered IgG-like bispecific antibodies targeting TRAIL-R2 and LTbetaR. *MAbs*. 2009;1:128-41.
2. Stieglmaier J, Bremer E, Kellner C, Liebig TM, ten Cate B, Peipp M, et al. Selective induction of apoptosis in leukemic B-lymphoid cells by a CD19-specific TRAIL fusion protein. *Cancer immunology, immunotherapy : CII*. 2008;57:233-46.