

TNF Superfamily

Key Cytokines in B & T Cell Immuno-Regulation

The tumor necrosis factor (TNF) and TNF receptor (TNFR) superfamilies (TNFSF/TNFRSF) include 20 ligands and 30 receptors that play important roles in the modulation of cellular functions. TNFSF/TNFRSF members regulate cellular differentiation, survival and programmed death, but their most critical functions pertain to the immune system. Both innate and adaptive immune cells are controlled by TNFSF/TNFRSF members in a manner that is crucial for the coordination of various mechanisms driving either co-stimulation or co-inhibition of the immune response. Dysregulation of these same signaling pathways has been implicated in inflammatory and autoimmune diseases, highlighting the importance of their tight regulation. The TNF Superfamily ligands are typically membrane bound, although some can signal as soluble species as well. The ligands self-assemble into non-covalent trimeric complexes, have a key role in cell-cell interactions and bind to 3 distinct groups of receptors, that can either induce apoptosis, activate NF- κ B, JNK, Erk or Akt signaling or act as decoy receptors.

TNFSF/TNFRSF together with the CD28–B7 family are two major co-signal pathways in T cell activation. CD28, ICOS, GITR, 4-1BB and OX40 pathways co-stimulate T cell activation. CTLA-4, PD-1, BTLA and CD160 pathway co-inhibit T cell activation. Most co-signal ligands are expressed or induced on antigen-presenting cells (APCs), such as dendritic cells (DCs), polymorphonuclear leukocytes (PMNs, including neutrophils, eosinophils, basophils, and mast cells), B cells and macrophages, while most co-signal receptors on T cells are constitutively expressed or induced on T cells after T Cell Receptor (TCR) activation (see Figure 1).

Investigation of the control of TNFSF/TNFRSF activities has led to the development of therapeutics with the potential to reduce chronic inflammation or promote anti-tumor immunity as immune-oncology targets.

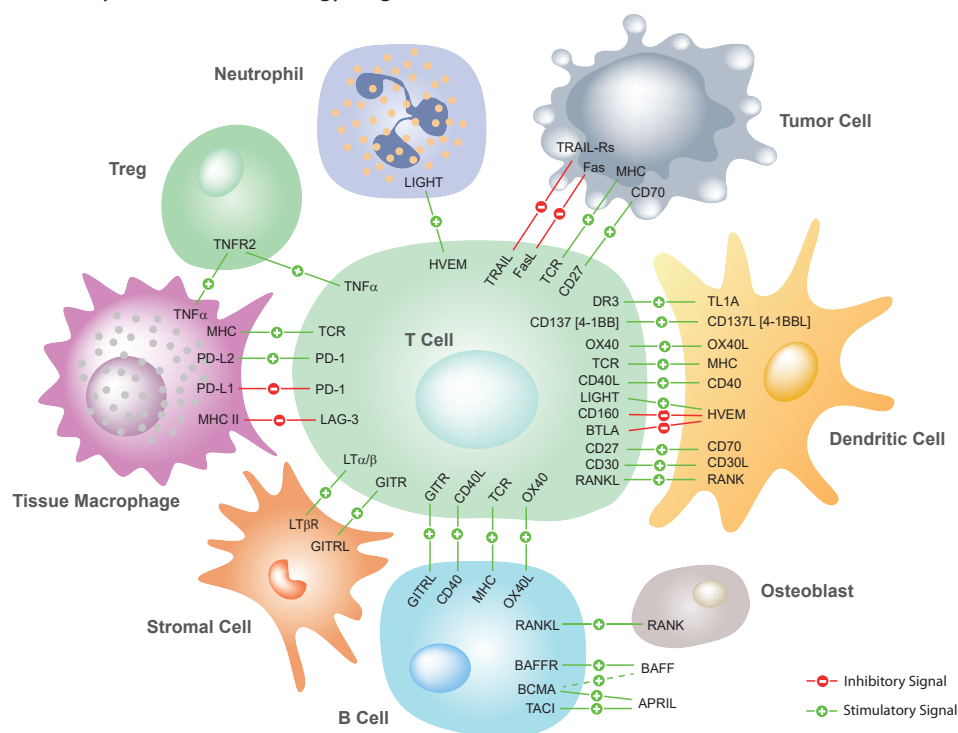
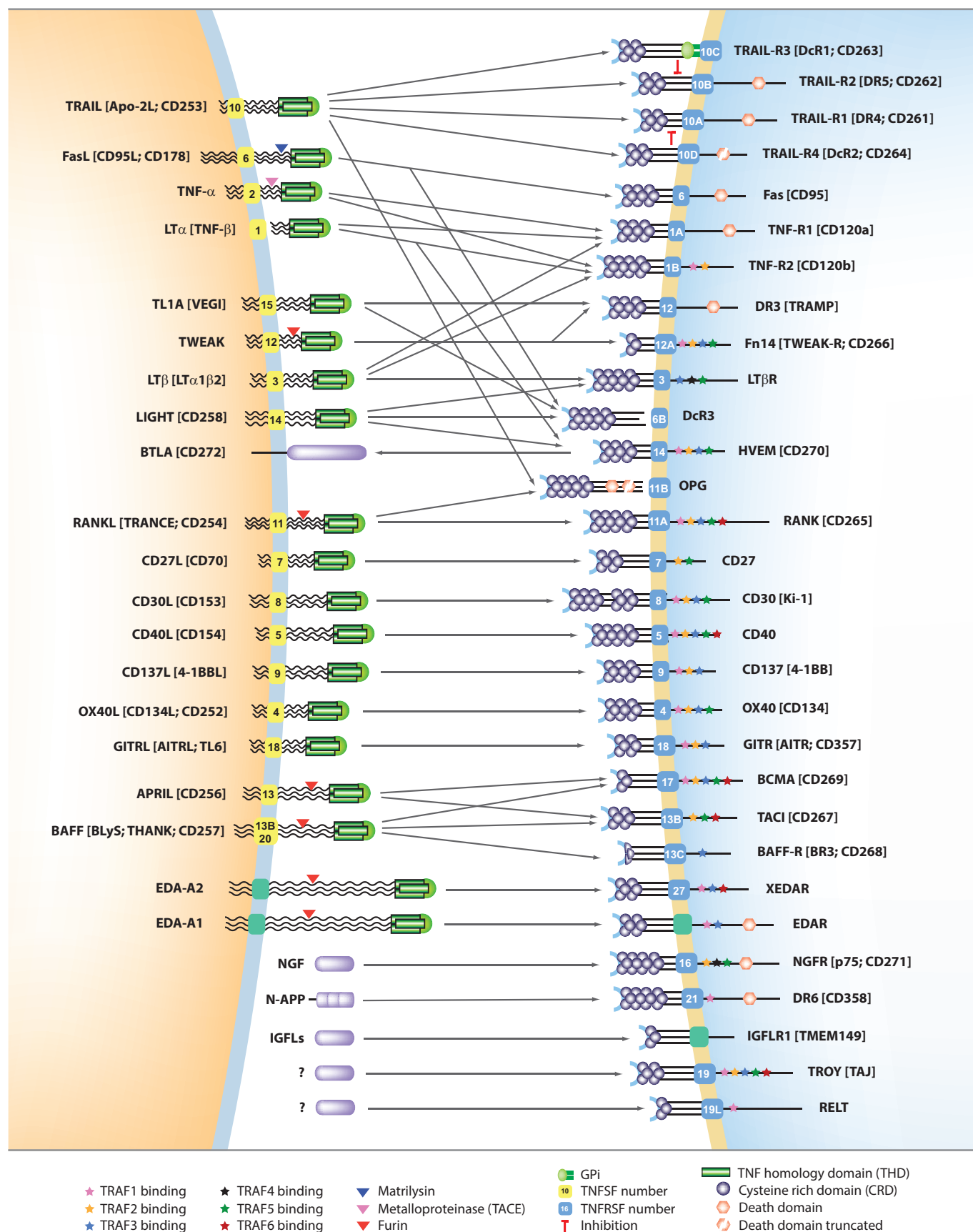


FIGURE: TNF Superfamily Members Involved in Immune Responses.

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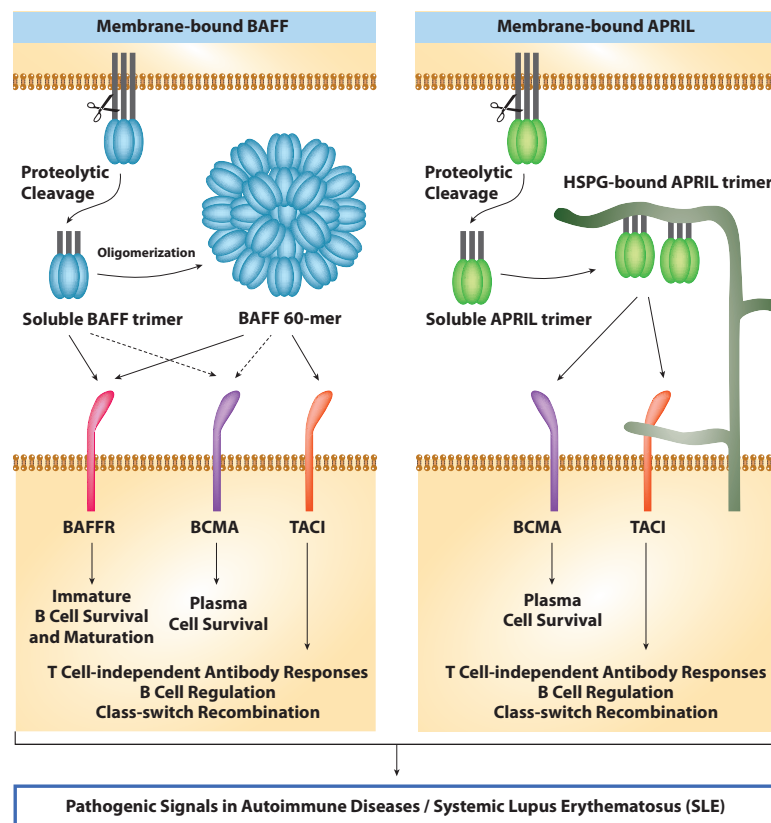
TNF Superfamily Ligands and Receptors Overview



APRIL & BAFF Pathways

The B cell-stimulating molecules, BAFF (B cell activating factor also known as BlyS; TALL-1; CD257 or TNFSF13B) and APRIL (a proliferation-inducing ligand, also known as CD256 or TNFSF13), are critical factors in the maintenance of the B cell pool and humoral immunity. APRIL binds to TACI (CD267; TNFRSF13B), BCMA (CD269; TNFRSF17) and heparan sulfate proteoglycans (HSPG). APRIL can interact with carbohydrate side chains of proteoglycans that may trigger cross-linking. BAFF is proteolytically processed by furin to be released as soluble BAFF that exists either as trimers (BAFF 3-mer) or as BAFF 60-mer. BAFF 3-mer and BAFF 60-mer both signal through BAFF-R (CD268; TNFRSF13C), only TACI (and BCMA) respond to BAFF 60-mer and not to BAFF 3-mer.

BAFF and APRIL are implicated in several human autoimmune diseases with autoreactive B cell involvement, including systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), IgA nephropathy (IgAN), and rheumatoid arthritis (RA). APRIL might also function in enhancing proliferation of some tumor cells, especially B cell malignancies and has a protective role in atherosclerosis. BAFF levels are also increased in some lymphoid cancers.



Best-In-Class APRIL & BAFF ELISA Kits

UNIQUE

NEW APRIL (human) ELISA Kit

AG-45B-0012

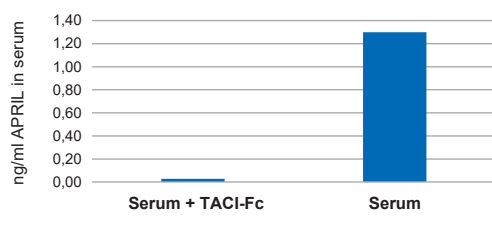
96 wells

Sensitivity: 1 pg/ml

Range: 3.9 to 250 pg/ml

Sample: Cell Culture Supernatant, Plasma, Serum

Specificity: Serum from a healthy patient is left untreated or treated with 1 µg/ml of the APRIL receptor, TACI (human):Fc (human) (Prod. No. AG-40B-0079). APRIL levels were measured using the APRIL (human) ELISA Kit (Prod. No. AG-45B-0012).



BAFF, Soluble (human) ELISA Kit (hypersensitive)

AG-45B-0001

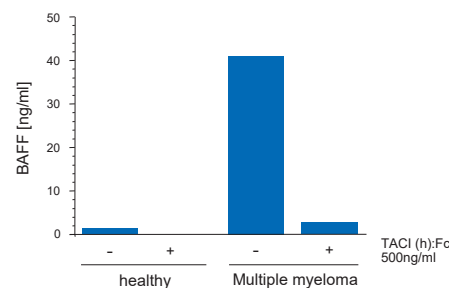
96 wells

Sensitivity: 8 pg/ml

Range: 15.6 to 500 pg/ml

Sample: Cell Culture Supernatant, Plasma, Serum

Specificity: Serum from a healthy patient or patient with multiple sclerosis is left untreated or treated with 0.5 µg/ml of a BAFF receptor, TACI (human):Fc (human) (AG-40B-0079). BAFF levels are measured using the BAFF, Soluble (human) ELISA Kit (hypersensitive) (AG-45B-0001).



Also available

BAFF, Soluble (human) Matched Pair Detection Set (Prod. No. AG-46B-0001)

BAFF and APRIL Blocking Antibodies

UNIQUE POTENT

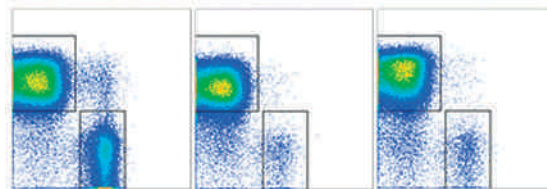
iV anti-BAFF (mouse), mAb (blocking) (Sandy-2)

AG-20B-0063 100 µg
AG-20B-0063PF Preservative Free 100 µg | 500 µg

This monoclonal antibody recognizes mouse BAFF and works specifically in IP and Functional Application. This antibody inhibits mouse BAFF binding to BAFF-R and TACI. It is highly potent in blocking mouse BAFF *in vivo* and induces B cell depletion and generates a phenotype similar to that observed in BAFF^{-/-} mice.

FIGURE: anti-BAFF (mouse), mAb (Sandy-2) (Prod. No. AG-20B-0063) blocks the action of endogenous BAFF *in vivo*.

METHOD: Wild type C57BL/6 mice were treated at day 0 (single administration) with monoclonal antibody anti-BAFF (mouse), mAb (Sandy-2) (at 2mg/kg). Lymph nodes were prepared at week 2 and analyzed by FACS for the presence of T (CD3) and B (CD19) cells. Untreated BAFF WT and KO mice were analyzed in parallel.



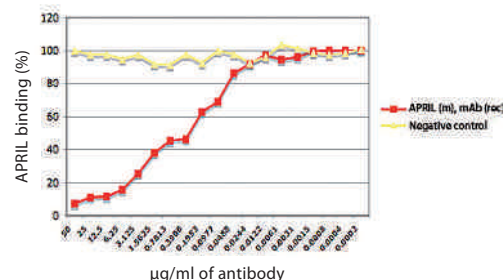
iV anti-APRIL (mouse), mAb (rec.) (blocking) (Apy-1-1)

AG-27B-0001 100 µg
AG-27B-0001PF Preservative Free 100 µg | 500 µg
AG-27B-0001B Biotin 100 µg

This recombinant monoclonal antibody recognizes mouse APRIL and works specifically in IP and Functional Application. The antibody inhibits mouse APRIL binding to BCMA and TACI. It is highly potent in blocking mouse APRIL *in vitro* and *in vivo*. In addition it promotes the binding of APRIL to HSPGs and confers atheroprotection.

FIGURE: Binding of APRIL (mouse) to BCMA is inhibited by anti-APRIL (mouse), mAb (rec.) (blocking) (Apy-1-1) (Prod. No. AG-27B-0001).

METHOD: BCMA:Fc was coated on an ELISA plate at 1 µg/ml. anti-APRIL (mouse) mAb (rec.) (blocking) (Apy-1-1) or an unrelated mAb (recombinant) (Negative control) were added (starting at 50 µg/ml with a twofold serial dilution) together with 0.1 µg/ml of MultimericAPRIL (mouse) (Prod. No. AG-40B-0089). After incubation for 1 h at RT, the MultimericAPRIL (mouse) binding was detected using an anti-FLAG[®] antibody (HRP). The percentage of binding is shown.



iV **NEW** anti-APRIL (mouse), mAb (blocking) (Centotto-1)

AG-20B-0083PF Preservative Free 100 µg | 500 µg

This monoclonal antibody recognizes mouse APRIL and works specifically in IP and Functional Application. This antibody potentially depletes mouse APRIL.

LIT: APRIL limits atherosclerosis by binding to heparan sulfate proteoglycans: D. Tsiantoulas, et al.; Nature 597, 92 (2021)

NEW

VALIDATED Antibodies for BAFF and APRIL Research

ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
NEW APRIL (human), mAb (blocking) (Mahya-1) (PF)	AG-20B-0078PF	100 µg 500 µg	Mouse IgG1κ	ELISA, IP, FUNC (Blocking)	Hu
iV APRIL (mouse), mAb (rec.) (blocking) (Apy-1-1)	AG-27B-0001 *	100 µg	Mouse IgG2bλ	ELISA, IP, FUNC (Blocking)	Ms
iV NEW APRIL (mouse), mAb (rec.) (blocking) (Apy-1-3)	AG-27B-0017	100 µg	Human IgG1λ	ELISA, IP, FUNC (Blocking)	Ms
BAFF (human), mAb (1-35-1)	AG-20B-0037	100 µg	Rat IgG2aκ	FACS	Hu
BAFF (human), mAb (2.81)	AG-20B-0018	100 µg	Rat IgG2b	ELISA, IP	Hu
BAFF (human), mAb (blocking) (4.62)	AG-20B-0017 *	100 µg	Rat IgG2a	ELISA, FUNC (Blocking), IP	Hu
BAFF (human), mAb (ANC2H3)	ANC-266-020 *	100 µg	Mouse IgG1κ	ELISA, FACS, FUNC (Blocking)	Hu
BAFF-R (human), mAb (HuBR9.1)	AG-20B-0016 *	100 µg	Mouse IgG1	FACS	Hu
BAFF-R (human), mAb (ANC268.2/6E6)	ANC-275-020 *	100 µg	Mouse IgG1κ	ELISA, FACS	Hu
iV BAFF (mouse), mAb (blocking) (Sandy-2)	AG-20B-0063 *	100 µg	Mouse IgG1	FUNC (Blocking, Depletion), IP	Ms
iV BAFF-R (mouse), mAb (9B9)	AG-20B-0034 *	100 µg	Rat IgG2b	FUNC (Depletion), FACS	Ms
BCMA (human), mAb (ANC3B1)	ANC-269-020 *	100 µg	Mouse IgG1κ	ELISA, FACS	Hu
TACI (mouse), mAb (1A-10)	AG-20B-0035 *	100 µg	Rat IgG2a	FACS	Ms

iV For *in vivo* Studies

APPLICATIONS: FACS: Flow Cytometry; FUNC: Functional Application; ICC: Immunocytochemistry; IHC: Immunohistochemistry IP: Immunoprecipitation; WB: Western blot

FORMULATION: PF = Preservative free
SPECIES: Hu = Human; Ms = Mouse; Rt = Rat; Rb = Rabbit; Prm = Primate

* Different Formats available!

BAFF-related Proteins

Processed human BAFF can either remain as a trimer, which is usual for TNF family ligands, or assemble into 60-mer composed of 20 trimers. Mouse BAFF 60-mer has been identified in the serum of BAFF transgenic mice. Despite the predominant functional role of processed BAFF *in vivo*, membrane-bound BAFF might also play a role. Indeed, soluble BAFF (3-mer) can trigger BAFF-R but not TACI or BCMA, whereas oligomeric forms of BAFF (BAFF 60-mer), which mimic membrane-bound BAFF, activate all BAFF receptors.

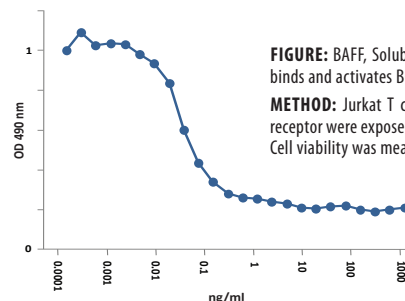


FIGURE: BAFF, Soluble (human) (60-mer) (AG-40B-0112) binds and activates BCMA receptor.

METHOD: Jurkat T cells expressing a BCMA:Fas chimeric receptor were exposed to BAFF, Soluble (human) (60-mer). Cell viability was measured with the PMS/MTS assay.

	PRODUCT NAME	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
	BAFF (aa134-285), Soluble (human) (rec.)	AG-40B-0016	10 µg 3 x 10 µg	E. coli	<0.01EU/µg	Hu, Ms
	BAFF, Soluble (human) (60-mer) (rec.) (highly active)	AG-40B-0112	10 µg 3 x 10 µg	E. coli	<0.01EU/µg	Hu, Ms
	Fc (human):BAFF (human) (rec.)	AG-40B-0120	10 µg 3 x 10 µg 500 µg	CHO cells	<0.01EU/µg	Hu, Ms
iV	BAFF, Soluble (mouse) (rec.)	AG-40B-0022	10 µg 3 x 10 µg	HEK 293 cells	<0.01EU/µg	Ms, Hu
	BAFF (trn) (human)-muCD8 Fusion Protein	ANC-525-020 *	25 µg	CHO cells	n.d.	Hu
	BAFF-R (human):Fc (human) (rec.)	AG-40B-0027	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
	BAFF-R (human)-mulg Fusion Protein	ANC-524-020 *	25 µg	CHO cells	n.d.	Hu
	BCMA (human):Fc (human) (rec.)	AG-40B-0080	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
iV	BCMA (mouse):Fc (human) (rec.)	AG-40B-0076	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Ms, Hu
	BCMA (human)-mulg Fusion Protein	ANC-519-020 *	25 µg	CHO cells	n.d.	Hu
	TACI (human):Fc (human) (rec.)	AG-40B-0079	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms

Multimeric APRIL Proteins

	PRODUCT NAME	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
	APRIL (human) (H98) (multimeric) (rec.)	AG-40B-0088	10 µg 3 x 10 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
	APRIL (human) (multimeric) (rec.)	AG-40B-0017	10 µg 3 x 10 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
	APRIL (mouse) (H98) (multimeric) (rec.)	AG-40B-0035	10 µg 3 x 10 µg	HEK 293 cells	<0.01EU/µg	Ms, Hu
	APRIL (mouse) (multimeric) (rec.)	AG-40B-0089	10 µg 3 x 10 µg	HEK 293 cells	<0.02EU/µg	Ms, Hu

LATEST INSIGHT

TNF Ligands Multimeric Proteins *Higher Activity – Lower Endotoxin*

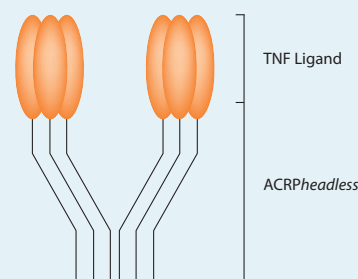
AdipoGen® Multimeric Proteins are high activity constructs in which two trimeric TNFSF ligands are linked via the oligomeric collagen domain of ACRP30 [ACRP30*headless*] and therefore mimic the membrane-bound forms of the proteins.

Endogenous TNF superfamily ligands are either active as membrane-form (e.g. FasL, TRAIL, CD40L, OX40L) or are secreted and activated through oligomerization by the binding of proteoglycans at the surface of cells (e.g. APRIL).

To mimic endogenous TNF ligands activity, the oligomerization of recombinant TNF ligands can be triggered:

- by fusing the TNF superfamily ligands, soluble form, to the collagen domain of the protein ACRP30 (which itself has no functional activity) to form a hexameric structure and therefore creating "Multimeric Proteins", or
- by adding a cross-linking antibody called "TNF Ligands Enhancer" (Prod. No. AG-35B-0001).

AdipoGen® Multimeric Proteins provide higher activity than monomeric proteins and are perfectly suitable for *in vitro* studies.



CD40 – CD40L Pathway

CD40 is a member of the TNF receptor family expressed by antigen-presenting cells (APCs) and B cells whereas its ligand, CD40L (CD154), is expressed by activated T cells. Interaction between CD40–CD40L stimulates cytokines secretion of B cells with subsequent T cell activation and antitumor immunity. **This T cell priming effect of the CD40-CD40L pathway might be a useful approach in anti-cancer immunotherapy.**

SELECTED REVIEWS: Cancer immunotherapy: activating innate and adaptive immunity through CD40 agonists: G.L. Beatty, et al.; Expert Rev. Anticancer Ther. 17, 175 (2017) • Multiple effects of CD40-CD40L axis in immunity against infection and cancer: A. Ara, et al.; Immunotargets Ther. 7, 55 (2018)

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
CD40 (human):Fc (human) (rec.)	AG-40B-0083	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
CD40 (human):Fc (human) (rec.)	CHI-HF-210CD40	100 µg	CHO cells	<0.06EU/µg	Hu
CD40 (human)-mulg Fusion Protein	ANC-504-020 *	25 µg	CHO cells	n.d.	Hu
CD40L (human) (multimeric) (rec.)	AG-40B-0010 *	10 µg 3 x 10 µg	CHO cells	<0.01EU/µg	Hu
CD40L (human) (multimeric) (rec.) (Biotin)	AG-40B-0010B *	10 µg 3 x 10 µg	CHO cells	<0.01EU/µg	Hu
CD40L (human):Fc (human) (rec.)	CHI-HF-210CD40L	50 µg	CHO cells	<0.06EU/µg	Hu
CD40L (human)-muCD8 Fusion Protein	ANC-505-020 *	25 µg	CHO cells	n.d.	Hu
CD40L (mouse) (multimeric) (rec.)	AG-40B-0020	10 µg 3 x 10 µg	CHO cells	<0.01EU/µg	Hu, Ms
CD40L (mouse) (multimeric) (rec.) (Biotin)	AG-40B-0020B	10 µg 3 x 10 µg	CHO cells	<0.01EU/µg	Hu, Ms
CD40L (rat) (multimeric) (rec.)	AG-40B-0107	10 µg 3 x 10 µg	CHO cells	<0.02EU/µg	Hu, Ms, Rt
ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
CD40 (human), mAb (BE-1)	ANC-189-020 *	100 µg	Mouse IgG1	FACS, FUNC, IP	Hu
CD40 (human), mAb (EA-5)	ANC-300-020 *	100 µg	Mouse IgG1	FACS, FUNC	Hu, Rt
CD40 (mouse), mAb (FGK45) (PF)	AG-20B-0036PF	100 µg 500 µg	Rat IgG2a	FACS, FUNC	Ms
CD40L (human), mAb (rec.) (blocking) (hu5c8) (PF)	AG-27B-6002PF	100 µg	Human IgG1k	FUNC, WB	Hu, Dog
CD40L (human), mAb (24-31)	ANC-353-020 *	100 µg	Mouse IgG1	FACS, FUNC, IHC, WB	Hu, Primate
ELISA KITS	PID	SIZE	-	-	SPECIES
CD40L (human) ELISA Kit	AG-45B-0018	96 wells	-	-	Hu

LATEST INSIGHT B Cell Expansion

Highly Potent B Cell Activators and T Cell Priming Reagents

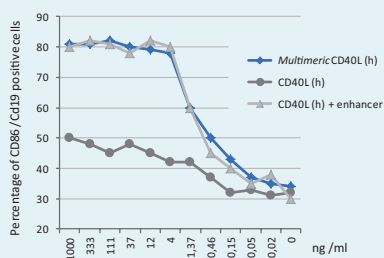
CD40 activation tools can be used to expand B cells (EBCs), which, as antigen-presenting cells (APCs), are as effective as dendritic cells and promises to streamline the generation of antitumor CD8⁺ T cells. **Several studies show that usage of the agonistic anti-CD40 antibody (FGK45) (Prod. No. AG-20B-0036PF) and MultimericCD40L (Prod. No. AG-40B-0010) are strong stimulators of antitumor immunity.**

LIT: R.S. Kornbluth, et al.; Int. Rev. Immunol. 31, 279 (2012) • K.T. Byrne & R.H. Vonderheide; Cell Rep. 15, 2719 (2016)

CD40L (human) (multimeric) (rec.)

AG-40B-0010 10 µg | 3 x 10 µg
AG-40B-0010B Biotin 10 µg | 3 x 10 µg

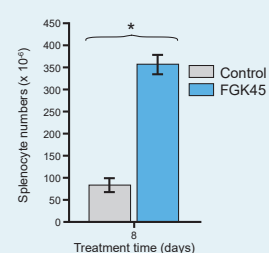
FIGURE: CD40L (human) (multimeric) (rec.) (Prod. No. AG-40B-0010) does not need an enhancer to induce B cell activation.



iv anti-CD40 (mouse), mAb (FGK45)

AG-20B-0036 100 µg | 500 µg
AG-20B-0036PF Preservative Free 100 µg | 500 µg | 5mg

FIGURE: Systemic immune activation by CD40 ligation. Mice were sacrificed on day 8 after daily treatment on day 4-7 with FGK45 or control. FGK45 treatment elevated splenocyte numbers in both groups. *P < 0.005. Data represent mean ± SD for three to four mice per group.



Fas – FasL Pathway

FasL (CD95L; CD178; TNFSF6) binds to Fas (CD95; TNFRSF6), a receptor that transduces the apoptotic signal into cells. It is involved in cytotoxic T cell-mediated apoptosis and in T cell development. The formation of the Fas death-inducing signaling complex (DISC) is the initial step of Fas signaling. Activation of procaspase-8 at the DISC leads to the induction of death receptor (DR)-mediated apoptosis. Stimulation of Fas

has also been reported to trigger non-apoptotic pathways. It has been shown that **membrane-bound FasL is essential for the cytotoxic activity, whereas soluble FasL appears to promote autoimmunity and tumorigenesis** via induction of non-apoptotic pathways, in particular NF- κ B. FasL binds also to decoy receptor 3 (DcR3; TNFRSF6B).

THE STANDARD BULK



THE STANDARDS Fas Antibodies

Widely cited antibodies for *in vivo* application! Induce apoptosis.

PRODUCT NAME	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
Fas (human), mAb (APO-1-3) (PF)	AG-20B-0062PF	50 µg 100 µg 500 µg	Ms IgG3	FACS, FUNC, IP, WB	Hu
Fas (human), mAb (APO-1-1) (PF)	AG-20B-0079PF	100 µg 500 µg	Mouse IgG1	FACS, FUNC, IHC	Hu

BULK

MultimericFasL™ [MegaFasL™]

FasL (human) (multimeric) (rec.)

AG-40B-0130

10 µg | 3 x 10 µg

MultimericFasL™ very effectively simulates the natural membrane-assisted aggregation of FasL *in vivo*.

Source: HEK 293 cells.

Sequence: Human FasL (aa 139-281) is fused at the N-terminus to mouse ACRP30^{headless} (aa 18-111) and a FLAG®-tag.

Specificity: Binds to human and mouse Fas.

Biological Activity: Induces apoptosis of human Jurkat T cells at a concentration of <1ng/ml.

Endotoxin Content: <0.01 EU/µg purified protein (LAL test; Lonza).

LITERATURE REFERENCES: Two adjacent trimeric Fas ligands are required for Fas signaling and formation of a death-inducing signaling complex: N. Holler, et al; Mol. Cell. Biol. 23, 1428 (2003) • A Fas agonist induces high levels of apoptosis in haematological malignancies: P. Greaney, et al; Leuk. Res. 30, 415 (2006)

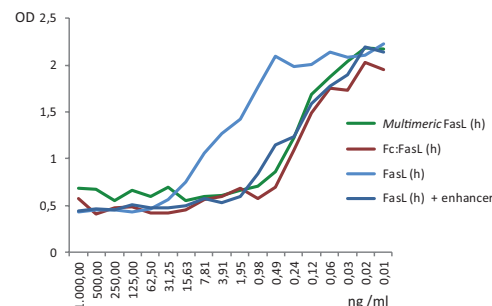


FIGURE: Oligomerisation of FasL (human) efficiently triggers Jurkat cell death.

METHOD: Jurkat cells were treated O/N with the indicated concentrations of FasL (human) (multimeric) (rec.) (AG-40B-0130), Fc (human):FasL (human) (rec.) (AG-40B-0132), FasL (human) (rec.) (AG-40B-0001) or FasL (human) (rec.) + Enhancer (AG-44B-0001) (2 fold-dilutions, first concentration of 1000ng/ml). Cell death was quantified using PMS/MTS. The oligomeric FasL recombinant proteins (FasL (human) (multimeric), Fc (human):FasL (human) and FasL (human) + Enhancer) kill Jurkat cells at IC₅₀ <0.2ng/ml.

Also available

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
Fas (human):Fc (human) (rec.)	AG-40B-0082	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
Fas (human)-hulg Fusion Protein	ANC-506-020 *	25 µg	CHO cells	n.d.	Hu
FasL, Soluble (human) (rec.)	AG-40B-0001	10 µg 3 x 10 µg	HEK 293 cells	<0.05EU/µg	Hu, Ms
Fc (human):FasL, Soluble (human) (rec.)	AG-40B-0132	10 µg 3 x 10 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
EnhancedFasL, Soluble (human) (rec.) Pack	AG-44B-0001	1 Set	HEK 293 cells	<0.05EU/µg	Hu, Ms
DcR3 (human):Fc (human) (rec.)	CHI-HF-210DCR3	100 µg	CHO cells	<0.06EU/µg	Hu
ANTIBODIY	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
CD95 (human), mAb (ANC95.1) *	ANC-316-020 *	100 µg	Mouse IgG1	ELISA, FACS	Hu

LTβ – HVEM – LIGHT – BTLA Network

LTβ (LTα1β2; TNFSF3) binds to the LTβR (TNFRSF3) activating two different NF-κB pathways that lead to distinct patterns of gene induction, including selected chemokines and the cytokine BAFF, which is essential for the survival of mature B lymphocytes. LTβR activates the classical NF-κB (relA/p50) pathway, like the type 1 TNF receptor (TNF-R1), that regulates pro-inflammatory genes and also activates the processing of p100 to form RelB/p52 complexes, which activate genes involved in lymphoid organ formation and lymphocyte survival.

LIGHT (CD258; TNFSF14) binds to LTβR. It activates NF-κB, stimulates the proliferation of T cells and inhibits growth of the adenocarcinoma HT-29. It also binds to decoy receptor 3 (DcR3; TNFRSF6B) and HVEM.

HVEM (CD270; TNFRSF14) is a molecular switch that acts both as an immune system stimulator and as an inhibitor. It is expressed in T cells, B cells, natural killer cells, dendritic cells and endothelial cells. LIGHT is an immune stimulator that contributes to dendritic cell maturation and T cell expansion. The immune suppressor BTLA functions in opposition to LIGHT in suppression of naïve T cell expansion and induction of Treg cells. CD160 acts as an immune suppressor through its interactions with HVEM. The **checkpoint receptors/ligands system HVEM, LIGHT, CD160 and BTLA (CD272)** is part of a complex network of overlapping receptor interactions that function in both immune stimulation and suppression and which is a **potential therapeutic target for treatment of autoimmune diseases and allergies and controlling antitumor responses**.

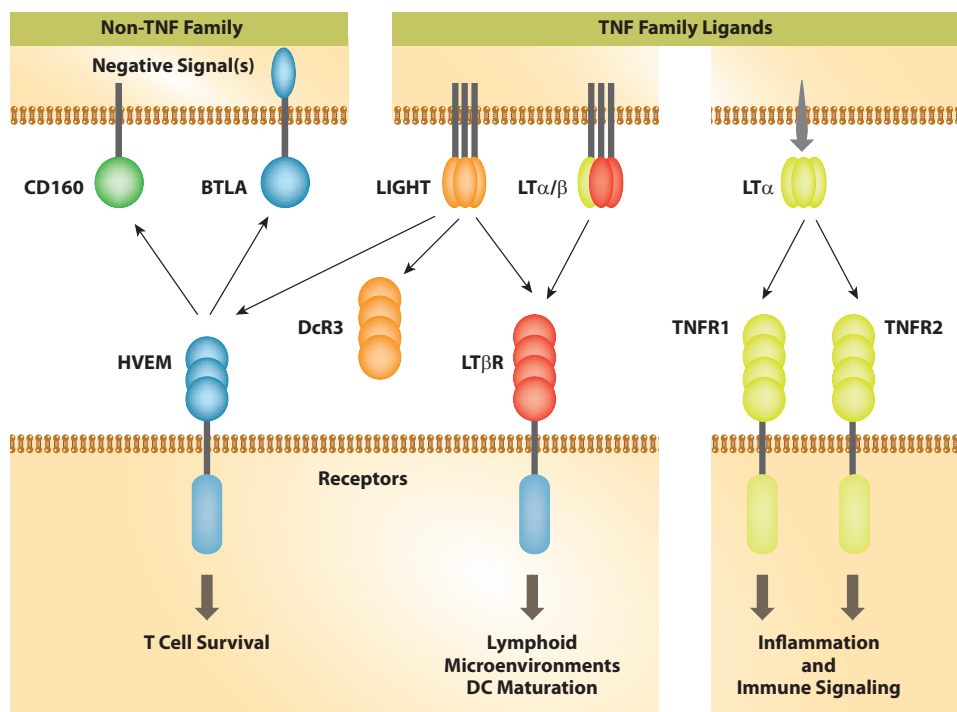


FIGURE: Overview on LTβ – HVEM – LIGHT – BTLA network signaling.

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
BTLA (human):Fc (human) (rec.)	CHI-HF-210CD272	100 µg	CHO cells	<0.06EU/µg	Hu
BTLA (human):Fc (mouse) (rec.)	CHI-HF-211CD272	100 µg	CHO cells	<0.06EU/µg	Hu
BTLA (human)-mulg Fusion Protein	ANC-542-020 *	25 µg	CHO cells	N/A	Hu
CD160 (human):Fc (human) (rec.)	CHI-HF-210CD160	100 µg	CHO cells	<0.06EU/µg	Hu
DcR3 (human):Fc (human) (rec.)	CHI-HF-210DcR3	100 µg	CHO cells	<0.06EU/µg	Hu
HVEM (human)-mulg Fusion Protein	ANC-531-020 *	25 µg	CHO cells	n.d.	Hu
LIGHT, Soluble (human) (rec.)	AG-40B-0009	10 µg 3 x 10 µg	CHO cells	<0.01EU/µg	Hu, Ms
LTβR (human):Fc (human) (rec.) (non-lytic)	CHI-HF-220LTBR	100 µg	CHO cells	<0.06EU/µg	Hu
LTβR (human)-mulg Fusion Protein	ANC-536-020 *	25 µg	CHO cells	n.d.	Hu
ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
BTLA (human), mAb (6F4)	AG-20B-0049	100 µg	Rat IgG1	FACS	Hu
BTLA (human), mAb (ANC6E9)	ANC-272-020 *	100 µg	Mouse IgG1κ	FACS, FUNC	Hu
BTLA (human), mAb (ANC5A5)	ANC-372-020 *	100 µg	Mouse IgG1κ	FACS	Hu
HVEM (human), mAb (ANC3B7)	ANC-270-020 *	100 µg	Mouse IgG2aκ	FACS	Hu
LTβR (mouse), mAb (3C8)	AG-20B-0041 *	100 µg	Rt IgG1κ	FUNC (Activation)	Ms
LTβR (mouse), mAb (4H8 WH2)	AG-20B-0008 *	100 µg	Rt IgG2a	FACS, FUNC (Activation)	Ms
LTβR (human), mAb (ANCLTR2/9E2)	ANC-267-020 *	100 µg	Ms IgG1κ	ELISA, FACS	Hu

iv
iv

TNF- α – LT α (TNF- β) – TNF-R's Pathway

Tumor necrosis factor (TNF, cachexin or cachectin and formerly known as tumor necrosis factor- α) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It binds to the receptors TNF-R1 (CD120a; TNFRSF1A) and TNF-R2 (CD120b; TNFRSF1B). TNF-R1 is linked to cytotoxic signaling pathways triggering apoptosis or necroptosis and mainly to pro-inflammatory signaling by activating transcription factors of NF- κ B or kinases of the MAPK family. TNF-R2 has no intrinsic cell death inducing activity but stimulates NF- κ B signaling and activation of various kinases.

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
TNF-α (human) (multimeric) (rec.)	AG-40B-0019	10 μ g 3 x 10 μ g	HEK 293 cells	<0.02EU/ μ g	Hu, Ms
TNF-α, Soluble (human) (rec.)	AG-40B-0006	10 μ g 50 μ g 3 x 50 μ g	E. coli	<0.01EU/ μ g	Hu, Ms
TNF-α (human) (rec.) (His)	CHI-HR-200TNF	10 μ g 50 μ g	E. coli	<0.1EU/ μ g	Hu, Ms
TNF-α (mouse) (multimeric) (rec.)	AG-40B-0021	10 μ g 3 x 10 μ g	HEK 293 cells	<0.02EU/ μ g	Hu, Ms
TNF-R1 (human):Fc (human) (rec.)	AG-40B-0074	50 μ g 3 x 50 μ g	HEK 293 cells	<0.01EU/ μ g	Hu, Ms
TNF-R1 (mouse):Fc (human) (rec.)	CHI-MF-111TNFR1	50 μ g	HEK 293 cells	<0.06EU/ μ g	Ms
TNF-R1 (mouse):Fc (mouse) (rec.)	CHI-MF-110TNFR1	50 μ g	HEK 293 cells	<0.06EU/ μ g	Ms
ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
NEW TNF-α (mouse), mAb (blocking) (V1q) (PF)	AG-20B-0081PF	100 μ g 500 μ g	Rat IgG	FACS, FUNC (Blocking)	Ms
TNF-α (human), mAb (J1D9)	ANC-398-020 *	100 μ g	Mouse IgG1	FACS, FUNC, WB	Hu

CD137 – CD137L Pathway

CD137 (4-1BB; TNFRSF9) is an activating receptor binding to CD137L (4-1BBL; TNFSF9) expressed on activated macrophages, dendritic cells and mature B cells. Because CD137 is expressed on both natural killer (NK) cells and T cells, it can trigger both innate and adaptive immunity. After these cells have been activated by exposure to tumor antigen, CD137 signals stimulate them to reproduce and to generate antitumor activity. CD137 has been shown to play a critical role on T cells in the development of immune memory and the creation of a durable immune response. On lymphocytes, the presence of CD137 appears to be a marker for tumor reactivity. Activation of CD137 signaling can stimulate both cytotoxic T cell and NK cell activity and generate a lasting memory response. In addition, CD137 (4-1BB) and CD137L have been reported to be involved in tumor rejection, apoptosis, antiviral immunity, diabetes, in T and B cell co-stimulation and modulation of the immune response. Cross-linking of CD137 enhances T cell proliferation, IL-2 secretion survival and cytolytic activity.

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
CD137 (human) (rec.) (His)	CHI-HR-200CD137	25 μ g	E. coli	<0.1EU/ μ g	Hu
CD137 (human):Fc (human) (rec.)	AG-40B-0060	50 μ g 3 x 50 μ g	HEK 293 cells	<0.01EU/ μ g	Hu
CD137 (human):Fc (human) (rec.)	CHI-HF-210CD137	100 μ g	CHO cells	<0.06EU/ μ g	Hu
CD137 (human):Fc (mouse) (rec.)	CHI-HF-211CD137	100 μ g	HEK 293 cells	<0.005EU/ μ g	Ms
CD137 (human)-hulg Fusion Protein	ANC-502-020 *	25 μ g	CHO cells	n.d.	Hu
CD137 (mouse):Fc (human) (rec.)	AG-40A-0025	50 μ g	HEK 293 cells	<0.1EU/ μ g	Ms
CD137L, Soluble (human) (rec.)	AG-40A-0198T	50 μ g	HEK 293 cells	<0.06EU/ μ g	Hu
CD137L, Soluble (mouse) (rec.)	AG-40A-0020Y	50 μ g	HEK 293 cells	<0.01EU/ μ g	Ms
Fc (human):CD137L, Soluble (human) (rec.)	AG-40B-0173	10 μ g 3 x 10 μ g	HEK 293 cells	<0.01EU/ μ g	Hu
CD137L (human)-muCD8 Fusion Protein	ANC-503-020 *	25 μ g	CHO cells	n.d.	Hu
ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
CD137 (human), mAb (4B4-1)	ANC-360-020 *	100 μ g	Mouse IgG1 κ	FACS, FUNC	Hu, Mk
CD137 (human), pAb	AG-25A-0018	100 μ g	Rabbit	FACS, WB	Hu
CD137 (mouse), mAb (M4173)	AG-20A-0072	50 μ g	Rat IgG1 κ	FACS, WB	Ms
CD137L (human), mAb (41B436)	AG-20A-0031	50 μ g 100 μ g	Mouse IgG1 κ	FACS, ICC, WB	Hu
CD137L (human), mAb (ANC5D6)	ANC-365-020 *	100 μ g	Mouse IgG2a κ	FACS, WB	Hu

TRAIL – TRAIL-R Pathway

Among the TNFSF, TRAIL signaling biology is one of the most complex. TNF-related apoptosis-inducing ligand (TRAIL; Apo2L; CD253; TNFSF10) is a type II transmembrane protein of about 34kDa. Active TRAIL specifically binds to five distinct receptors: TRAIL-R1 (DR4; CD261; TNFRSF10A), TRAIL-R2 (DR5; CD262; TNFRSF10B), TRAIL-R3 (DcR1; CD263; TNFRSF10C), TRAIL-R4 (DcR2; CD264; TNFRSF10D) and osteoprotegerin (OPG; TNFRSF11B). Similar to other TNFSF ligands, a trimeric TRAIL ligand binds to three receptor monomers to form the active signaling complex. Unique among TNFSF ligands is that TRAIL contains a Zn ion that is coordinated by a Cys residue (Cys230) from each monomer. The loss of Zn ion can lead to instability and loss of activity of human recombinant TRAIL (hrTRAIL). Trimerized TRAIL triggers apoptosis upon ligation of cell surface TRAIL-R1 and/or TRAIL-R2 by inducing the formation of the so-called multiprotein death-inducing signaling complex (DISC) and its dysregulation has been associated with different cancers. Aside its apoptotic effect and its importance for cancer regulation, TRAIL and TRAIL-Rs seem to be involved in different pathways and regulatory functions such as non-apoptotic, mitogenic and prosurvival pathways including the MAPKs, the protein kinase B (PKB/Akt) and the NF- κ B signaling cascades. They were shown to be involved in bone turnover regulation and angiogenesis. Both recombinant TRAIL and agonistic TRAIL-R antibodies are in various stages of clinical trials for cancer treatment.

Flow Cytometry (FACS)

FACS Analysis

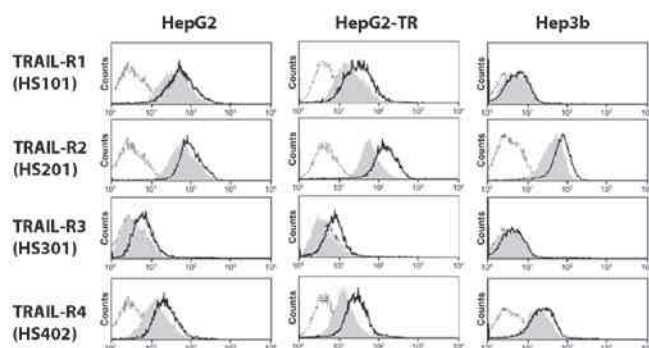


FIGURE: FACS analysis of surface expression of TRAIL-R1 to TRAIL-R4 with (solid bold line) and without (filled line) 5-FU (100 μ g/ml) treatment for 16h, compared to control (dashed line) using TRAIL-R1 mAb (HS101) (Prod. No. AG-20B-0022), TRAIL-R2 mAb (HS201) (Prod. No. AG-20B-0023), TRAIL-R3 mAb (HS301) (Prod. No. AG-20B-0024) and TRAIL-R4 mAb (HS402) (Prod. No. AG-20B-0025).

Immunohistochemistry (IHC)

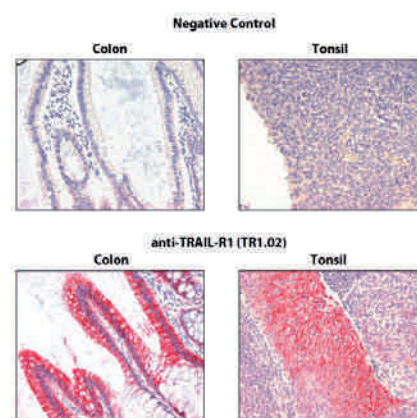


FIGURE: Immunohistochemistry detection of endogenous TRAIL-R1, TRAIL-R2 and TRAIL-R3 in paraffin-embedded human carcinoma tissues (colon, tonsil) using mAb to TRAIL-R1 (TR1.02) (Prod. No. AG-20B-0027), mAb to TRAIL-R2 (TR2.21) (Prod. No. AG-20B-0028) and mAb to TRAIL-R3 (TR3.06) (Prod. No. AG-20B-0029).

Highly Specific Antibodies for FACS and IHC

PRODUCT NAME	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
TRAIL (human), mAb (HS501)	AG-20B-0026	100 μ g	Ms IgG1	WB	Hu
TRAIL-R1 (human), mAb (HS101)	AG-20B-0022 *	100 μ g	Ms IgG1	FACS, ICC, FUNC (Blocking), IP	Hu
TRAIL-R1 (human), mAb (TR1.02)	AG-20B-0027	100 μ g	Ms IgG2b	FACS, IHC, WB	Hu
TRAIL-R2 (human), mAb (HS201)	AG-20B-0023 *	100 μ g	Ms IgG1	FACS, ICC, FUNC (Blocking), IP	Hu
TRAIL-R2 (human), mAb (TR2.21)	AG-20B-0028	100 μ g	Ms IgG1	FACS, IHC, WB	Hu
TRAIL-R3 (human), mAb (HS301)	AG-20B-0024	100 μ g	Ms IgG1	FACS, ICC	Hu
TRAIL-R3 (human), mAb (TR3.06)	AG-20B-0029	100 μ g	Ms IgG1	FACS, IHC, WB	Hu
TRAIL-R4 (human), mAb (HS402)	AG-20B-0025	100 μ g	Ms IgG1	FACS, ICC, IHC, IP	Hu
TRAIL-R1 to -R4 Flow Cytometry Pack	AG-44B-0004	1 Set	Ms IgG1	FACS	Hu

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KillerTRAIL and SuperKillerTRAIL

Oligomerized TRAIL proteins that do not require a cross-linking enhancer for their potent biological activity.

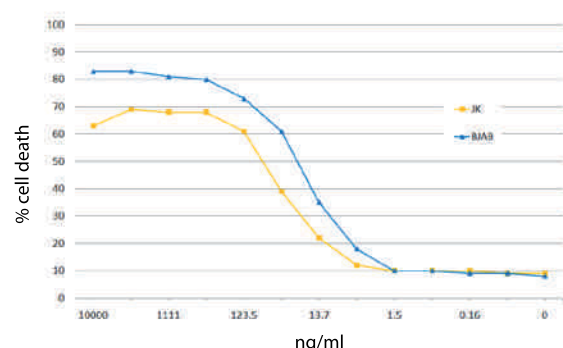


FIGURE: Apoptosis of TRAIL-sensitive cells. Concentration dependence of apoptosis induction in Jurkat and BJAB cells by *KillerTRAIL™*, Soluble (human) (rec.) (Prod. No. AG-40T-0001) reveals high activity even at concentrations of 10-100ng/ml.

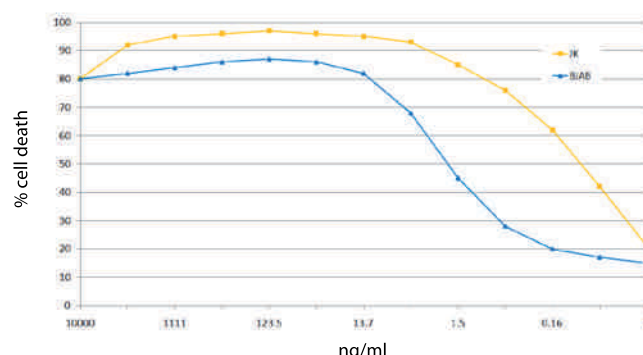


FIGURE: Apoptosis of TRAIL-sensitive cells. Apoptosis induction in BJAB cells by *SuperKillerTRAIL™*, Soluble (human) (rec.) (Prod. No. AG-40T-0002) reveals killing activity at concentrations of 10-15ng/ml on Jurkat cells even as low as 1-10ng/ml.

izTRAIL

Oligomerized TRAIL protein, non-toxic to hepatocytes, suitable for *in vitro* and *in vivo* use.

izTRAIL is a highly active recombinant form of soluble human TRAIL. Due to a trimerizing N-terminal isoleucine zipper (iz) motif the intrinsic trimerization of TRAIL, required for apoptosis-inducing activity of TRAIL, is enhanced when compared to non-tagged soluble human TRAIL (shTRAIL). Therefore, izTRAIL is a potent inducer of apoptosis in many human cancer cells, but not normal human hepatocytes. In addition, the half-life of izTRAIL is about eight-fold higher than the half-life of shTRAIL.

These properties render izTRAIL highly suitable for both, *in vitro* and *in vivo* use, particularly for studies in which investigators plan to transfer their *in vitro* results into an *in vivo* system with human cancer cells in xenotransplant settings examining susceptibility to TRAIL-induced apoptosis.

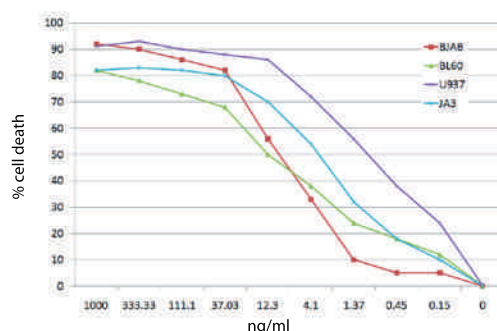


FIGURE: Apoptosis of TRAIL-sensitive tumor cells. Concentration dependence of apoptosis induction in BJAB cells, BL60-cells, U937-cells, and JA3-cells by izTRAIL, Soluble (human) (rec.) (Prod. No. AG-40B-0069) reveals high activity even at concentrations of 10-100ng/ml.

Highly Potent TRAIL Proteins

BULK available

PRODUCT NAME	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
TRAIL, Soluble (human) (rec.)	AG-40B-0003	10 µg 5 x 10 µg	E. coli	<0.01EU/µg	Hu, Ms
EnhancedTRAIL, Soluble (human) (rec.) Pack	AG-44B-0002	1 Set	E. coli	<0.01EU/µg	Hu, Ms
iV izTRAIL, Soluble (human) (rec.)	AG-40B-0069	10 µg 5 x 10 µg	E. coli	<0.1EU/µg	Hu
KillerTRAIL™, Soluble (human) (rec.)	AG-40T-0001	50 µg 3 x 50 µg 500 µg	E. coli	<0.01EU/µg	Hu, Ms
SuperKillerTRAIL™, Soluble (human) (rec.)	AG-40T-0002	20 µg 3 x 20 µg	E. coli	<0.01EU/µg	Hu
iV SuperKillerTRAIL™, Soluble (mouse) (rec.)	AG-40T-0004	20 µg 3 x 20 µg	E. coli	<0.01EU/µg	Ms, (Hu)
KillerTRAIL™ Dilution & Storage Buffer	AG-10T-0001	500 µl		<0.1EU/µg	
TRAIL-R1 (human):Fc (human) (rec.)	AG-40B-0070	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
TRAIL-R2 (human):Fc (human) (rec.)	AG-40B-0071	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms

OX40 – OX40L Pathway

OX40L (CD134L; CD252; TNFSF4) acts as a costimulator through the interaction with OX40 (CD134; TNFRSF4) on T cells, stimulating T cell activation, proliferation and cytokine production. It is expressed on antigen presenting cells including B cells, dendritic cells, mast cells and endothelium. OX40 (CD134; TNFRSF4) is an activating receptor expressed on the surface of activated cytotoxic T cells and regulatory T cells (Tregs). OX40 plays a dual role in the immune response, both activating and amplifying T cell responses. On cytotoxic T cells, OX40 binds to its ligand OX40L (CD252; TNFSF4), resulting in stimulatory signals that promote T cell reproduction, function and survival. OX40/OX40L signaling blocks the ability of Tregs to suppress T cells and reduces Treg generation. By inhibiting the immunosuppressive effect of Tregs and limiting their population, OX40 further amplifies the impact of T cell activation. The recombinant OX40:Fc protein was shown to prevent OX40L from reaching the T cell receptors, thus reducing the T cell response. Experiments in mice have demonstrated that **OX40:Fc can reduce the symptoms associated with the cytokine storm (an immune overreaction) while allowing the immune system to fight off viruses successfully.**

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
OX40 (human) (rec.) (His)	CHI-HR-200CD134	25 µg	E. coli	<0.1EU/µg	Hu
OX40 (human):Fc (human) (rec.)	AG-40B-0014	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
OX40 (human):Fc (human) (rec.)	CHI-HF-210CD134	50 µg	CHO cells	<0.06EU/µg	Hu
OX40 (human):Fc (mouse) (rec.)	CHI-HF-211CD134	100 µg	HEK 293 cells	<0.005EU/µg	Hu
iV OX40 (mouse):Fc (human) (rec.)	CHI-MF-111CD134	100 µg	HEK 293 cells	<0.005EU/µg	Ms
Fc (human):OX40L, Soluble (human) (rec.)	AG-40B-0172	10 µg 3 x 10 µg	HEK 293 cells	<0.01EU/µg	Hu
OX40L (human) (rec.) (His)	CHI-HF-201CD252	50 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
OX40L (human):Fc (mouse) (rec.)	CHI-HF-211CD252	100 µg	HEK 293 cells	<0.005EU/µg	Hu, Ms
OX40L (mouse) (multimeric) (rec.)	AG-40B-0029	10 µg	HEK 293 cells	<0.01EU/µg	Hu, Ms
CD134 [OX40] (human)-mulg Fusion Protein	ANC-513-020 *	25 µg	CHO cells	n.d.	Hu
CD252 [OX40L] (human)-muCD8 Fusion Protein	ANC-512-020 *	25 µg	CHO cells	n.d.	Hu
ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
OX40 (human), mAb (BerAct35)	ANC-355-020 *	100 µg	Mouse IgG1	ELISA, FACS, IHC	Hu
OX40 (human), Rabbit Monoclonal (RM313)	REV-31-1199-00	100 µl	Rabbit IgG	IHC, WB	Hu
OX40L (human), mAb (rec.) (blocking) (R4930) (PF)	AG-27B-6001PF	100 µg	Human IgG1κ	FACS, FUNC	Hu
OX40L (human), mAb (ANC10G1)	ANC-400-020 *	100 µg	Mouse IgG1κ	FACS, FUNC	Hu

GITR – GITRL Pathway

Glucocorticoid-induced TNFR-related protein (GITR; CD357; TNFRSF18) is an activating receptor on the surface of T cells and other immune cells, binding to its ligand GITRL (TNFSF18). Once exposure to tumor antigen activates a T cell, the number of GITR receptors on its surface increases. On the activated T cell, GITR acts as a costimulatory receptor, meaning that it is a receptor whose signaling enhances cell reproduction and the generation of cancer-killing activity. Activation of GITR signaling can also help to enhance immunity through the activation of cytotoxic T cells and inhibition of Treg activity.

SELECTED REVIEWS: Modulation of GITR for cancer immunotherapy: D.A. Schaer, et al.; Curr. Opin. Immunol. **24**, 217 (2012) | Rationale for anti-GITR cancer immunotherapy: D.A. Knee, et al.; Eur. J. Cancer **67**, 1 (2016)

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
GITR (human):Fc (human) (rec.)	AG-40B-0028	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Hu
iV GITR (mouse):Fc (human) (rec.)	AG-40B-0002	50 µg 3 x 50 µg	HEK 293 cells	<0.01EU/µg	Ms
GITRL, Soluble (human) (rec.)	CHI-AG-40A-0019	50 µg	HEK 293 cells	<0.06EU/µg	Hu
GITRL, Soluble (human) (rec.) (His)	AG-40A-0024T	10 µg 50 µg	HEK 293 cells	<0.06EU/µg	Hu
iV GITRL, Soluble (mouse) (rec.)	AG-40A-0008	50 µg	HEK 293 cells	<0.1EU/µg	Ms
ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
GITR (human), mAb (ANC7D6)	ANC-268-020 *	100 µg	Mouse IgMκ	FACS	Hu
GITR (human), mAb (ANC5E3)	ANC-368-020 *	100 µg	Mouse IgG3κ	FACS	Hu
GITR (human), mAb (AIT 158D)	AG-20A-0017	50 µg 100 µg	Rat IgG2aκ	FACS	Hu
GITR (human), pAb	AG-25A-0017	100 µg	Rat	FACS	Hu
GITRL (human), pAb	AG-25A-0023	100 µg	Rabbit	IHC, WB	Hu

iV For in vivo Studies

* Different Formats available!

APPLICATIONS: FACS: Flow Cytometry; FUNC: Functional Application; ICC: Immunocytochemistry; IHC: Immunohistochemistry IP: Immunoprecipitation; WB: Western blot

FORMULATION: PF = Preservative free
SPECIES: Hu = Human; Ms = Mouse; Rt = Rat; Rb = Rabbit; Prm = Primate

RANK – RANKL/OPG Pathway

Receptor activator of nuclear factor κ B (RANK; TRANCE receptor; TNFRSF11A) is a member of the TNF receptor superfamily. RANKL (TRANCE; CD254; TNFSF11) is an osteoclast differentiation and activation factor and is involved in osteoclastogenesis, binding to RANK (CD265; TNFRSF11A). RANKL augments the ability of dendritic cells to stimulate naïve T cell proliferation. RANKL is an important regulator of interactions between T cells and dendritic cells and is involved in the regulation of the T cell-dependent immune response. It also plays an important role in the progression of breast cancer. The RANK/RANKL/OPG signaling pathway is associated with bone remodeling and repair, immune cell function, lymph node development, thermal regulation and mammary gland development. Osteoprotegerin (OPG) is a decoy receptor for RANKL and regulates the stimulation of the RANK signaling pathway by competing for RANKL. The cytoplasmic domain of RANK binds TRAFs 1, 2, 3, 5, and 6 which transmit signals to downstream targets such as NF- κ B and JNK. Most therapies that target the RANK/RANKL/OPG axis aim to either down-regulate expression of RANKL or upregulate the expression of the decoy receptor OPG.

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
RANK (human):Fc (human) (rec.)	AG-40B-0018	50 μ g 3 x 50 μ g	HEK 293 cells	<0.01 EU/ μ g	Hu, Ms
iV RANK (mouse):Fc (human) (rec.)	AG-40B-0092	50 μ g 3 x 50 μ g	HEK 293 cells	<0.01 EU/ μ g	Hu, Ms
RANKL, Soluble (human) (rec.)	AG-40B-0008	10 μ g 3 x 10 μ g	HEK 293 cells	<0.01 EU/ μ g	Hu, Ms
iV Fc (human):RANKL, Soluble (mouse) (rec.)	AG-40B-0059	10 μ g	HEK 293 cells	<0.01 EU/ μ g	Ms
ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
RANK (ectodomain) (human), pAb	AG-25A-0021	50 μ g	Rb	FACS, WB	Hu
RANKL (human), pAb	AG-25A-0016	100 μ g	Rb	ELISA, FACS, WB	Hu

Other TNF Superfamily Members or Related Ligands

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES	TNF NR
CD27 (human) (rec.) (His)	CHI-HR-200CD27	50 μ g	E. coli	<0.1 EU/ μ g	Hu	TNFRSF7
CD27 (human):Fc (human) (rec.)	CHI-HF-210CD27	100 μ g	CHO cells	<0.06 EU/ μ g	Hu	TNFRSF7
CD27 (human)-mulg Fusion Protein	ANC-543-020 *	25 μ g	CHO cells	n.d.	Hu	TNFRSF7
CD70 (human)-muCD8 Fusion Protein	ANC-537-020 *	25 μ g	CHO cells	N/A	Hu	TNFSF7
NGFR (human)-mulg Fusion Protein	ANC-527-020 *	25 μ g	CHO cells	N/A	Hu	TNFRSF16
DR3 (human)-mulg Fusion Protein	ANC-528-020 *	25 μ g	CHO cells	N/A	Hu	TNFRSF25
DR6 (human):Fc (human) (rec.)	AG-40B-0011	50 μ g 3 x 50 μ g	HEK 293 cells	<0.01 EU/ μ g	Hu	TNFRSF21
iV DR6 (mouse):Fc (human) (rec.)	AG-40B-0062	50 μ g 3 x 50 μ g	HEK 293 cells	<0.01 EU/ μ g	Ms	TNFRSF21
EDA-A1, Soluble (human) (rec.)	AG-40B-0106	10 μ g 3 x 10 μ g	E. coli	<0.01 EU/ μ g	Hu, Ms	N/A
EDAR (human):Fc (human) (rec.)	AG-40B-0116	50 μ g 3 x 50 μ g	CHO cells	<0.01 EU/ μ g	Hu, Ms	N/A
Fn14 (human):Fc (human) (rec.)	AG-40B-0034	50 μ g 3 x 50 μ g	HEK 293 cells	<0.01 EU/ μ g	Hu, Ms	TNFRSF12A
ANTIBODIES	PID	SIZE	ISOTYPE	APPLICATION	SPECIES	TNF NR
CD27 (human), mAb (M-T271)	ANC-176-020 *	100 μ g	Ms IgG1	ELISA, FACS	Hu	TNFRSF7
CD30 (human), mAb (AC10)	ANC-179-020 *	100 μ g	Ms IgG2b κ	FACS	Hu	TNFRSF8
CD70 (human), mAb (BU69)	ANC-222-020 *	100 μ g	Ms IgG1	ELISA, FACS, FUNC (Inhibition), ICC, IHC	Hu, Prm	TNFSF7
NGFR (human), mAb (ANC271/3D7)	ANC-271-020 *	100 μ g	Ms IgG1 κ	ELISA, FACS	Hu	TNFRSF16
DR3 (human), mAb (ANC2D12)	ANC-250-020 *	100 μ g	Ms IgG1 κ	ELISA	Hu	TNFRSF25

TNF Ligands Enhancer – Facilitates the Oligomerization

PRODUCT NAME	PID	SIZE	ENDOTOXIN	SPECIES
TNF Ligands Enhancer	AG-35B-0001	50 μ g	<0.01 EU/ μ g	All

TNF Superfamily and Cell Death

Programmed cell death is of fundamental importance for the development of multicellular organisms and homeostasis of their tissues. Aberrant cell death can lead to many human diseases including cancer, autoimmune, neurodegenerative and immunodeficiency disorders. One type of programmed cell death is apoptosis, which has always been recognized to be a pathway of highly orchestrated signaling events. It is characterized by morphological features such as membrane blebbing, cell shrinkage, chromatin condensation, nucleosomal fragmentation and apoptotic bodies. Cell surface death receptors such as TRAIL-Rs (see Page 10–11) and Fas (see Page 7), are death domain (DD) containing transmembrane proteins which mediate apoptosis.

AdipoGen Life Sciences offers a broad range of antibodies and recombinant proteins for Apoptosis and other types of cell death, such as Pyroptosis, Necroptosis and Necrosis.

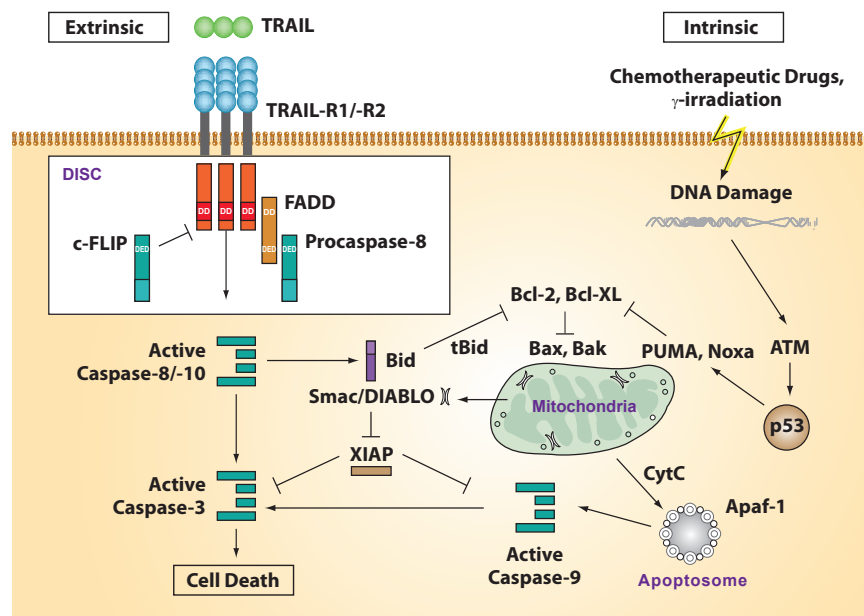


FIGURE: The extrinsic and intrinsic apoptosis pathway. Adapted from: Following TRAIL's path in the immune system: C. Falschlehner, et al.; Immunology 127, 145 (2009) (Review)

Reagents for Apoptosis Cell Death Downstream Signaling Research

ANTIBODIES	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
Apaf-1 (human), mAb (2E12)	AG-20T-0132	100 µg	Rat IgG2ak	ELISA, ICC, IHC, IP, WB	Hu
Apaf-1 (mouse/rat), mAb (13F11)	AG-20T-0133	100 µg	Rat IgG2ak	ELISA, ICC, IP, WB	Ms, Rt
Apaf-1, mAb (18H2)	AG-20T-0134	100 µg	Rat IgG2ak	ELISA, ICC, IP, WB	Hu, Ms
Bcl-2 (human), mAb (Bcl-2/100)	ANC-357-020	100 µg	Mouse IgG1	FACS, WB	Hu
BimS/EL/L, mAb (3C5)	AG-20T-0142	100 µg	Rat IgG2ak	ELISA, FACS, ICC, IHC, IP, WB	Hu, Ms, Rt, Mk, Dg
BimS/EL/L, mAb (10B12)	AG-20T-0143	100 µg	Rat IgG2ak	ELISA, FACS, ICC, IHC, IP, WB	Hu, Ms, Rt, Mk, Dg
Bmf, mAb (9G10)	AG-20T-0130	100 µg	Rat IgG2ak	FACS, IP, WB	Hu, Ms
Bmf (mouse/rat), mAb (17A9)	AG-20T-0131	100 µg	Rat IgG2ak	ELISA, FACS, ICC, IHC, IP, WB	Ms, Rt
Caspase-2, mAb (10C6)	AG-20T-0135	100 µg	Rat IgG2ak	ELISA, FACS, ICC, IHC	Hu, Ms, Rt, Mk, Dg
Caspase-2, mAb (11B4)	AG-20T-0136	100 µg	Rat IgG2ak	IP, WB	Hu, Ms, Rt, Mk, Dg
Caspase-3 (human), Rabbit MAb (RM250)	REV-31-1130-00	100 µl	Rabbit IgG	IHC, WB	Hu
Caspase-8 (human), mAb (C15)	AG-20B-0057	50 ug 100 µg	Mouse IgG2b	ICC, IP, WB	Hu
Caspase-8 (mouse), mAb (1G12)	AG-20T-0137	100 µg	Rat IgG1κ	ELISA, FACS, ICC, WB	Ms
Caspase-8 (mouse), mAb (3B10)	AG-20T-0138	100 µg	Rat IgG1κ	ELISA, FACS, ICC, IHC, WB	Ms
Caspase-12 (mouse), mAb (12G6)	AG-20T-0141	100 µg	Rat IgG1κ	ELISA, FACS, WB	Ms
FADD (human), mAb (1C4)	AG-20B-0080	100 µg	Mouse IgG1	ELISA, IP, WB	Hu
FLIP (human), mAb (NF6)	AG-20B-0056	50 ug 100 µg	Mouse IgG1	ICC, IHC, WB	Hu
FLIP, mAb (Dave-2)	AG-20B-0005	100 µg	Rat IgG2a	IP, WB	Hu, Ms
p53 (human), mAb (Pab240)	ANC-227-020	100 µg	Mouse IgG1κ	IHC, FACS, WB	Hu
p53 (human), Rabbit mAb (RM387)	REV-31-1273-00	100 µl	Rabbit IgG	IHC, WB	Hu
Smac/Diablo (human), Rabbit mAb (RM271)	REV-31-1152-00	100 µl	Rabbit IgG	IHC, WB	Hu

TECHNICAL NOTE TNF Family Member Reactivities & Cross-Reactivities

TNFSF and TNFRSF members are implicated in the development, maintenance, and function of immune cells and secondary lymphoid organs and participate in other functions, such as bone homeostasis. Several TNFSF and TNFRSF members are being used or evaluated as drug targets for the treatment of immune dysfunctions, cancer and other diseases.

Ligands of the tumor necrosis factor superfamily (TNFSF) bind members of the TNF receptor superfamily (TNFRSF). Generally, one trimeric ligand engages three monomeric receptors, a key event for the activation of intracellular signaling pathways. In 2006, the laboratory of Prof. Jürg Tschopp performed a comprehensive survey of ligand-receptor interactions using a flow cytometry-based assay. They have conducted a systematic survey of TNFSF-TNFRSF interactions and report reactivities and cross-reactivities of human and mouse proteins (see figure below).

LIT: Interactions of Tumor Necrosis Factor (TNF) and TNF Receptor Family Members in the Mouse and Human: C. Bossen, et al.; J. Biol. Chem. 281, 13964 (2006)

SPECIES CROSS-REACTIVITIES		TNF LIGANDS																																LIGANDS															
		TRAIL [Apo-L2]		FasL [CD95L]		TNF- α		LT α [TNF- β]		TL1A [VEGI]		TWEAK		LIGHT		LT β [LT α 1 β]		BTLA		RANKL [TRANCE]		CD27L [CD70]		CD30L [CD153]		CD40L [CD154]		CD137L [4-1BBL]		OX40L [CD134L]		GITRL [AITRL]		APRIL		BAFF		EDA-A2		EDA-A1		NGF		N-APP		IGFLs			
		hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms	hu	ms		
TNF RECEPTORS	TRAIL-R1 [DR4]	hu	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TRAIL-R2 [DR5]	hu	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TRAIL-R3 [DcR1] DcTRAIL-R1	hu	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TRAIL-R4 [DcR2] DcTRAIL-R2	ms	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Fas [CD95]	hu	-	-	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TNF-R1	ms	-	-	✓	✓	✓	✓	✓	✓	-	-	-	-	-	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	TNF-R2	hu	-	-	-	✓	✓	✓	✓	✓	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	DR3 [TRAMP]	ms	-	-	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Fn14 [TWEAK-R]	hu	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	DcR3	ms	-	-	✓	✓	-	-	-	✓	✓	✓	-	-	-	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	LT β R	hu	-	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	HVEM	ms	-	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	OPG	hu	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	RANK	ms	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CD27	hu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CD30 [Ki-1]	ms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CD40	hu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	CD137 [4-1BB]	ms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	OX40 [CD134]	hu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	GITR [AITR]	ms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BCMA	hu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	TACI	ms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	BAFF-R [BR3]	hu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	XEDAR	ms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EDAR	hu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NGFR [p75]	ms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																									

— = no interaction ± = weak interaction ✓ = strong interaction

IGFLR1 – A Novel Member of the TNFRSF

Insulin-growth factor-like gene family is a new family of proteins consisting of four proteins in humans (IGFL1 to 4) and one in mice (mIGFL). mIGFL is expressed in normal skin in mice and further upregulated during inflammation responses in skin or after skin wounding. In human only IGFL1 expression is increased in psoriatic skin samples. mIGFL and human IGFL1 and IGFL3 interact with specificity and high affinity to a novel receptor named IGF-like family receptor 1 (formerly TMEM-149). Analysis of the amino acid sequence of IGFLR1 indicated that this receptor is likely a novel member of the TNF-R family. IGFLR1 is expressed most abundantly on mouse T cells, suggesting that mIGFL and IGFL1 produced in the skin may potentially exert regulatory functions on T cell responses.

LIT: Murine insulin growth factor-like (IGFL) and human IGFL1 proteins are induced in inflammatory skin conditions and bind to a novel tumor necrosis factor receptor family member, IGFLR1: A.A. Lobito, et al.; J. Biol. Chem. **286**, 18969 (2011)

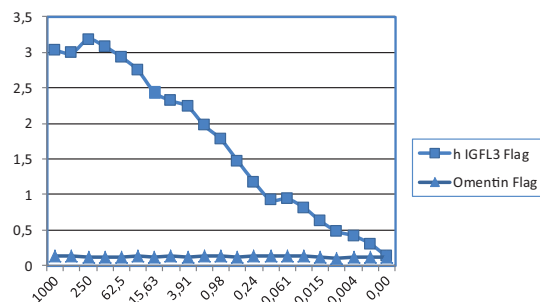


FIGURE: IGFLR1 (human):Fc (human) (Prod. No. AG-40B-0087) binds to its ligand IGFL3 (human) (Prod. No. AG-40B-0090).

METHOD: IGFLR1 (human):Fc was coated on an ELISA plate at 1µg/ml overnight at room temperature. IGFL3 (human) or a negative control, Omentin (human) (Prod. No. AG-40B-0042), were added (starting at 8µg/ml with a twofold serial dilution) during one hour and then detected using an anti-FLAG® antibody (HRP).

PROTEINS	PID	SIZE	SOURCE	ENDOTOXIN	SPECIES
IGFL (mouse) (rec.)	AG-40B-0091	10 µg	HEK 293 cells	<0.1EU/µg	Ms
IGFL3 (human) (rec.)	AG-40B-0090	10 µg	HEK 293 cells	<0.5EU/µg	Hu
IGFLR1 (human):Fc (human) (rec.)	AG-40B-0087	50 µg	HEK 293 cells	<0.05EU/µg	Hu
ANTIBODY	PID	SIZE	ISOTYPE	APPLICATION	SPECIES
IGFLR1 (human), pAb (IN101)	AG-25B-0026	100 µg	Rb	FACS, WB	Hu

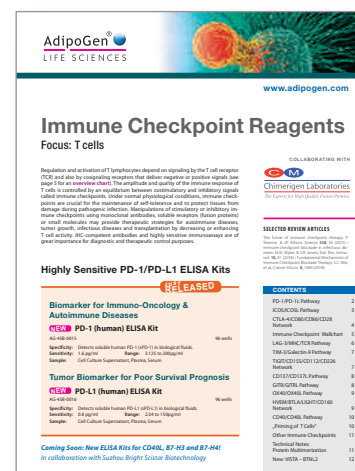
Other Research Fields



Inflammasome Research



Innate Immunity Research



Immune Checkpoint Research