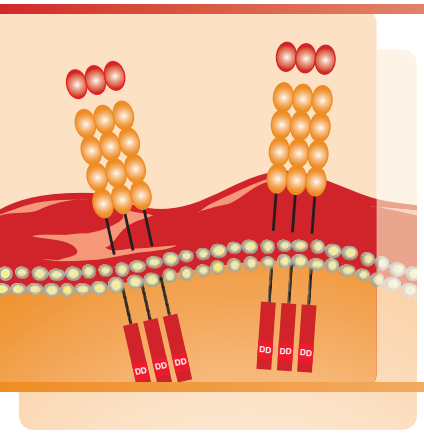

TRAIL & TRAIL Receptors

Effectors of Apoptotic Signaling

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. One important group of cell surface death receptors is called TRAIL receptors (TRAIL-Rs) which are activated by TRAIL.

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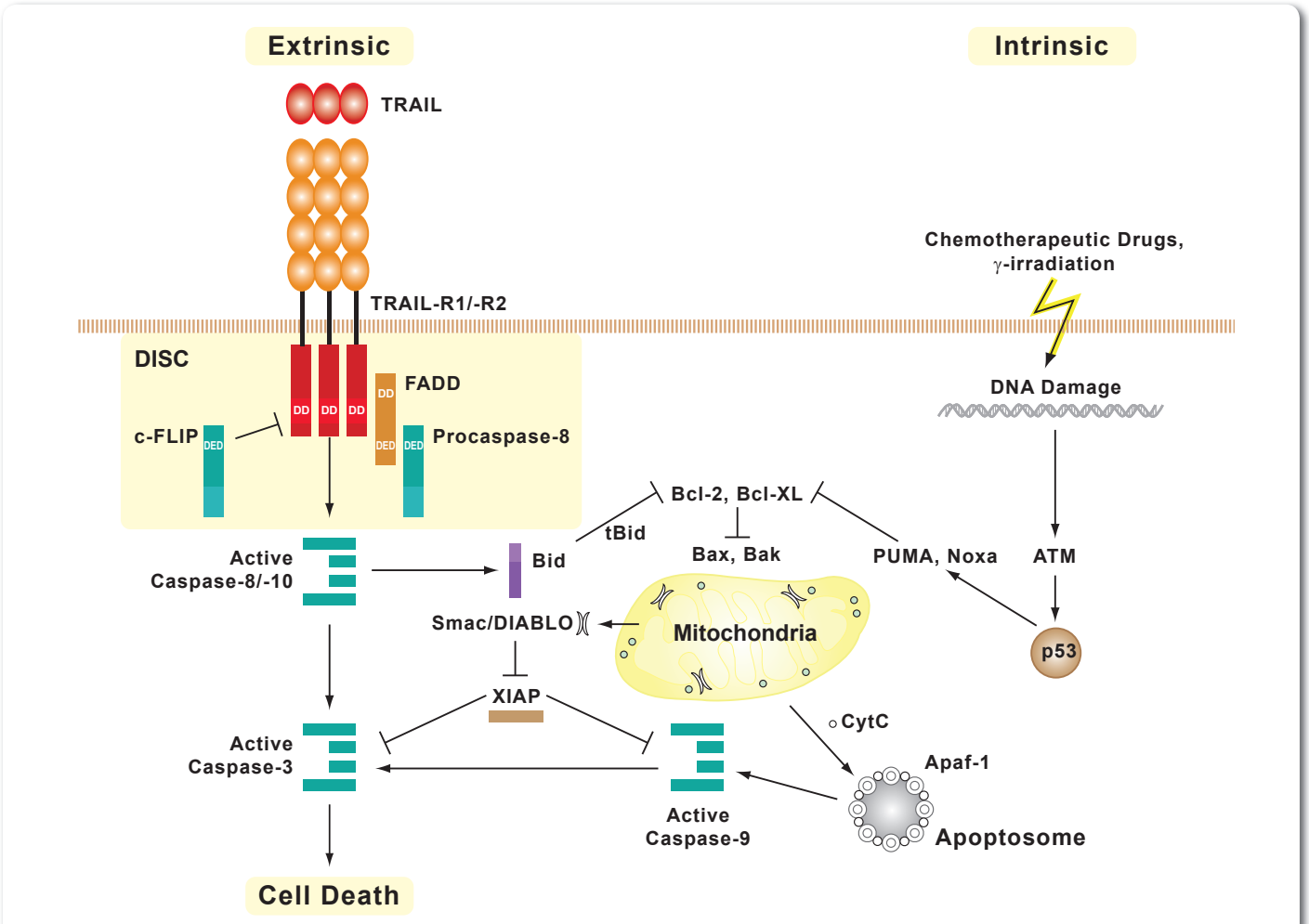

FIGURE 2: The extrinsic and intrinsic apoptosis pathway.

Adapted from: Following TRAIL's path in the immune system: C. Falschlehner, et al.; Immunology ***127,*** *145 (2009) (Review)*

TNF-related apoptosis-inducing ligand (TRAIL; Apo2L; CD253; TNFSF10) is a type II transmembrane protein of about 34kDa, which has been discovered by two groups in 1995/1996 [1, 2]. Like most members of the tumor necrosis factor (TNF) superfamily of cytokines TRAIL can be cleaved at the cell surface by metalloproteases to form a soluble molecule [3]. Active TRAIL forms trimers and specifically binds to five distinct receptors: TRAIL-R1 (DR4; Apo2; CD261; TNFRSF10A) [4], TRAIL-R2 (DR5; KILLER; TRICK2A; TRICK2B; CD262; TNFRSF10B) [5, 6, 7], TRAIL-R3 (DcR1; LIT; TRID; CD263; TNFRSF10C) [5, 6, 8], TRAIL-R4 (DcR2; TRUNDD; CD264; TNFRSF10D) [9], and osteoprotegerin (OPG; OCIF; TNFRSF11B) [10, 11].

TRAIL-R1 and TRAIL-R2 are death domain (DD) containing type I transmembrane proteins which mediate apoptosis. In contrast, neither TRAIL-R3 (which is GPI anchored) nor TRAIL-R4 (which is a type I membrane protein) contain a complete cytoplasmic death domain, and neither can mediate apoptosis upon ligand binding. Based on overexpression studies, it has been proposed that these receptors may act as regulatory or decoy receptors [12]. However, the relevance of these results in physiological conditions compared to the overexpressing systems needs to be shown [13]. Moreover, it seems that TRAIL-R3 inhibits apoptosis by competitive binding of TRAIL whereas the anti-apoptotic effect of TRAIL-R4 is based on the formation of heterocomplexes with TRAIL-R2 [14]. The latter observation is supported by the fact that there is a correlation between the co-expression of TRAIL-R2 and TRAIL-R4 [15].

OPG (osteoprotegerin) is a soluble receptor capable of binding to TRAIL among others [11]. Like TRAIL-R3 and TRAIL-R4, OPG binds to TRAIL without transducing apoptosis.

In mice, only mTRAIL-R2 (MK; mDR5), which is equally homologous to human TRAIL-R1/-R2, possesses apoptosis-inducing properties [16, 17]. Furthermore, two potential decoy receptors, mDcTRAIL-R1 (mDcR1) and mDcTRAIL-R2 (mDcR2), have been identified [17]. The latter one can be expressed as a secreted form (mDcTRAIL-R2S) and as a transmembrane form (mDcTRAIL-R2L), due to alternative splicing [17]. Furthermore, in mice OPG also binds to TRAIL and may act as a soluble decoy receptor [18].

Apoptosis may be accomplished by several pathways of which the extrinsic and intrinsic pathways are the best characterized ones [19]. As its name implies, the intrinsic pathway begins within the cell triggered by stress stimuli such as DNA damage or growth factor deprivation. In contrast, the extrinsic pathway is initiated upon ligation of cell surface TRAIL-R1 and/or TRAIL-R2 by trimerized TRAIL to induce the formation of the so-called multiprotein death-inducing signaling complex (DISC). Multimerized receptor molecules cause the recruitment of the DD containing adapter molecule Fas associated death domain (FADD; MORT1) [19]. Via its second functional domain, the death effector domain (DED), FADD recruits procaspase-8 and procaspase-10 via homotypic interactions to the DISC [20]. Within the DISC procaspases become autoactivated [21]. Active caspase-8/-10 in turn activate downstream effector caspase-3 or caspase-7 which cleave different cellular substrates finally causing the morphological features of apoptosis [22]. Cellular FLICE-like inhibitory protein (cFLIP) can also be recruited to the DISC where it might have a regulatory function by inhibiting the procaspase activation [20].

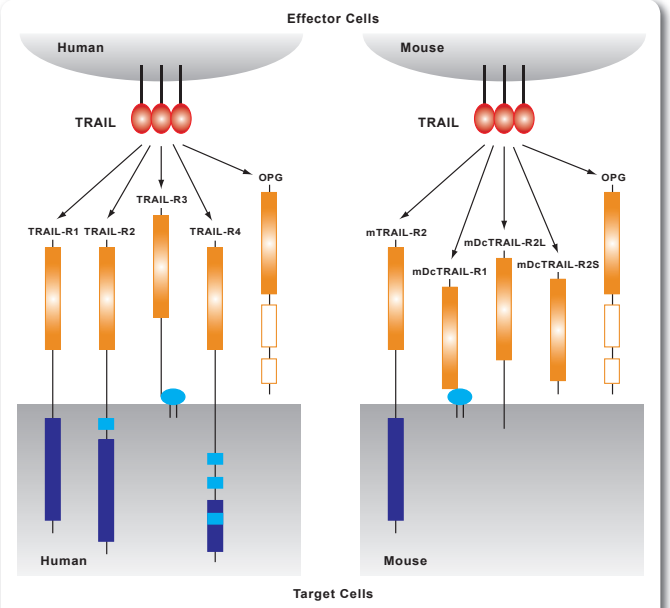

FIGURE 1: Overview on human and mouse TRAIL receptors.
Adapted from: TRAIL and its receptors as targets for cancer therapy: H. Yagita, et al.; Cancer Sci. ***95,*** *777 (2004) (Review)*

In so-called type I cells, the caspase-8/-10 activity is strong enough to directly activate downstream caspases and apoptosis. However, in so-called type II cells, caspase-8/-10 activation is ineffective and the extrinsic apoptosis signal must be amplified through caspase-8/-10 mediated cleavage of the pro-apoptotic Bcl-2 family member Bid to truncated Bid (tBid), which links the extrinsic and intrinsic apoptosis pathway, also known as the mitochondrial pathway [23, 24, 25, 26]. tBid translocates to the mitochondria where it is thought to promote Bax and Bak activation and the release of apoptogenic factors such as cytochrome c, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis (IAP) binding protein with low pI (Smac/DIABLO), and Omi stress-regulated endoprotease/high temperature requirement protein A2 (Omi/HtrA2). Once released, cytochrome c binds apoptotic protease-activating factor 1 (Apaf-1) to form a complex known as the apoptosome in the presence of ATP/dATP. The apoptosome recruits procaspase-9, promoting its autocatalytical activation due to induced proximity. Caspase-9 then in turn activates downstream effector caspase-3 or caspase-7.

Proteins of the IAP family, including X-linked IAP (XIAP), c-IAP1, and c-IAP2, can bind and inhibit the active sites of caspase-3, caspase-7 and caspase-9. When released from mitochondria, Smac/DIABLO and Omi/HtrA2 bind these IAPs and ensure fully activated effector caspases [27, 28, 29].

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[13] Following a TRAIL: update on a ligand and its five receptors: F. C. Kimberley & G. R. Screaton; Cell Res. **14,** 359 (2004) (Review)

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[28] A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death: Y. Suzuki, et al.; Mol. Cell **8,** 613 (2001)

[29] Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins: A. M. Verhagen, et al.; Cell **102,** 43 (2000)

Effects in Other Pathways

Aside its apoptotic effect, TRAIL and TRAIL-Rs seem to be involved in different pathways and regulatory functions like:

• Signal Transduction

TRAIL can induce non-apoptotic, mitogenic and prosurvival pathways, including the MAPKs, the protein kinase B (PKB/Akt) and the NF-κB signaling cascade [1, 2, 3, 4].

• Bone Turnover Regulation

TRAIL has a role as a negative modulator in osteoclast differentiation and as an inducer of apoptosis in mature osteoclast [5, 6, 7, 8]. Furthermore, human osteoblasts seem to be resistant to TRAIL induced apoptosis, even though TRAIL is expressed in large quantities by osteoblasts [9]. Therefore, alterations in either TRAIL mediated signaling cascades, or in the ratio of TRAIL to TRAIL receptors might be involved in different bone-related diseases.

• Angiogenesis

TRAIL induces apoptosis in sensitized cerebral endothelial cells and thereby inhibits cerebral angiogenesis, leading to vessel regression [10]. Therefore, TRAIL exerts a potential anti-inflammatory role in the human central nervous system (CNS) [10]. However, other *in vitro* studies have found evidence that TRAIL is rather pro-angiogenetic [11, 12]. The physiological role of TRAIL in angiogenesis, if there is any, needs further investigation.

• Stem Cells

A recent study has shown that human bone marrow derived mesenchymal stem cells (MSCs) can localize to brainstem gliomas with high specificity [13]. This tropism of MSCs in combination with the fact that stem cells can be genetically modified and their cell number rapidly increased *in vitro* is of particular interest for delivering therapeutic agents to most tumor types [13]. Therefore, MSCs were engineered to express TRAIL and their systemic delivery prolonged the survival of brainstem glioma-bearing mice [13]. In another study, it was demonstrated, *in vivo*, that early subcutaneous tumor growth was reduced by TRAIL-expressing MSCs [14]. Furthermore, lung metastases were reduced and could be eliminated upon systemic delivery of TRAIL-expressing MSCs in the corresponding metastasis model [14]. Together, those studies may reveal a new approach for cancer treatment.

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TRAIL Ligands

TRAIL (soluble) (human), (rec.)

BML-SE721-0100 100 µg

Produced in *E. coli*. Human TRAIL (aa 114-281). **SPECIFICITY:** Binds to human and mouse TRAIL receptors and human osteoprotegerin (OPG). **BIOLOGICAL ACTIVITY:** Induces apoptosis in U937 cells at 1 µg/ml, within 5 hours. At 0.5-2.0 µg/ml, induced apoptosis in various thyroid carcinoma lines within 18 hours. **NOTE: This protein does not require a cross-linking enhancer for its potent biological activity.**

LIT: Identification and characterization of a new member of the TNF family that induces apoptosis: S.R. Wiley et al.; Immunity **3,** 673 (1995) ▪ Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors: J. P. Sheridan et al.; Science **277,** 818 (1997) ▪ Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule: N. Holler, et al.; Nat. Immunol. **1,** 489 (2000) ▪ The death domain kinase RIP is essential for TRAIL (Apo2L)-induced activation of IkappaB kinase and c-Jun N-terminal kinase: Y. Lin et al.; Mol. Cell Biol. **20,** 6638 (2000) ▪ Cytoskeleton-mediated death receptor and ligand concentration in lipid rafts forms apoptosis-promoting clusters in cancer chemotherapy: C. Gajate & F. Mollinedo; J. Biol. Chem. **280,** 11641 (2005) ▪ **For a comprehensive bibliography please visit our website.**

TRAIL (soluble) (human), (rec.) set

ALX-850-018-KI01 1 Set

BIOLOGICAL ACTIVITY: Induces apoptosis in a concentration range of 1-100ng/ml if applied with the cross-linking enhancer. **SET CONTAINS:** 10µg of TRAIL (soluble) (human), (recombinant) (Prod. No. ALX-522-003) 2 x 50µg of Enhancer for Ligands (Prod. No. ALX-804-034). **SPECIFIC ACTIVITY:** ED_{50}: 10ng/ml (Jurkat cells).

LIT: Sensitivity to TRAIL/APO-2L-mediated apoptosis in human renal cell carcinomas and its enhancement by topotecan: M. Dejosez, et al.; Cell Death Differ. **7,** 1127 (2000) ▪ The anti-apoptotic protein BAG-3 is overexpressed in pancreatic cancer and induced by heat stress in pancreatic cancer cell lines: Q. Liao, et al.; FEBS Lett. **503,** 151 (2001) ▪ Death ligand TRAIL induces no apoptosis but inhibits activation of human (auto)antigen-specific T cells: J.D. Lunemann, et al.; J. Immunol. **168,** 4881 (2002) ▪ Requirement of BAX for TRAIL/Apo2L-induced apoptosis of colorectal cancers: synergism with sulindac-mediated inhibition of Bcl-x(L): R. Ravi and A. Bedi; Cancer Res. **62,** 1583 (2002) ▪ Chemotherapy enhances TNF-related apoptosis-inducing ligand DISC assembly in HT29 human colon cancer cells: S. Lacour, et al.; Oncogene **22,** 1807 (2003) ▪ Human mast cells undergo TRAIL-induced apoptosis: B. Berent-Maoz, et al.; J. Immunol. **176,** 2272 (2006) ▪ **For a comprehensive bibliography please visit our website.**

TRAIL (soluble) (mouse), (rec.)

BML-SE722-0100 100 µg

Produced in *E.coli*. Soluble mouse TRAIL (aa 118-291). **BIOLOGICAL ACTIVITY:** Induces apoptosis at 0.1 and 1.0µg/ml, in primary cultured mouse hepatocytes, in the presence of cycloheximide (5µg/ml) or actinomycin D (0.5µg/ml). Induces apoptosis in Jurkat cells (a TRAIL-sensitive human cell line) at 1µg/ml, within 3 hours. Inhibits secondary clonal expansion of mouse $CD8^+$ T cells *in vitro* (50ng/ml and greater).

Super*Killer*TRAIL™ (soluble) (human), (rec.)

ALX-201-115-C010 10 µg

ALX-201-115-3010 3 x 10 µg

Produced in *E. coli*. The extracellular domain of human TRAIL (aa 95-281) is fused at the N-terminus to a His-tag and a linker peptide. The active multimeric conformation is stabilized by an inserted mutation allowing an additional CC-bridge. **SPECIFICITY:** Binds to human TRAIL receptors. **BIOLOGICAL ACTIVITY:** Induces apoptosis at concentrations of >1ng/ml. **NOTE: This protein does not require a cross-linking enhancer for its potent biological activity.**

LIT: Synthetic lethal targeting of MYC by activation of the DR5 death receptor pathway: Y. Wang, et al.; Cancer Cell **5,** 501 (2004) ▪ Sulforaphane targets pancreatic tumor-initiating cells by NF-{kappa}B-induced anti-apoptotic signaling: G. Kallifatidis, et al.; Gut **58,** 949 (2009)

Super*Killer*TRAIL™ (soluble) (mouse), (rec.)

ALX-201-130-C020 20 µg

ALX-201-130-3020 3 x 20 µg

Produced in *E. coli*. The extracellular domain of mouse TRAIL (aa 99-291) is fused at the N-terminus to a His-tag and a linker peptide. The active multimeric conformation is stabilized by an inserted mutation allowing an additional CC-bridge. **SPECIFICITY:** Binds to mouse and less potently to human TRAIL receptors. **BIOLOGICAL ACTIVITY:** Induces apoptosis at concentrations of >10ng/ml as tested on human tumor cells. **NOTE: This protein does not require a cross-linking enhancer for its potent biological activity.**

*Killer*TRAIL™ (soluble) (human), (rec.)

ALX-201-073-C020 20 µg

ALX-201-073-3020 3 x 20 µg

ALX-201-123-C500 ***BULK*** **500 µg**

Produced in *E. coli*. The extracellular domain of human TRAIL (aa 95-281) is fused at the N-terminus to a His-tag and a linker peptide. **SPECIFICITY:** Binds to human and mouse TRAIL receptors and osteoprotegerin (OPG). **BIOLOGICAL ACTIVITY:** Induces apoptosis in a concentration range of 10-100ng/ml. **NOTE:** Does not require a cross-linking enhancer for its potent biological activity. For cell lines that require extensive cross-linking of the TRAIL-Rs for killing (e.g. Jurkat) use Super*Killer*TRAIL™ (Prod. No. ALX-201-115).

LIT: The cytokines tumor necrosis factor-α (TNF-α) and TNF-related apoptosis-inducing ligand differentially modulate proliferation and apoptotic pathways in human keratinocytes expressing the human papilloma: J.R. Basile, et al.; J. Biol. Chem. **276,** 22522 (2001) ▪ The anti-apoptotic protein BAG-3 is overexpressed in pancreatic cancer and induced by heat stress in pancreatic cancer cell lines: Q. Liao, et al.; FEBS Lett. **503,** 151 (2001) ▪ TRAIL and its receptors in the colonic epithelium: a putative role in the defense of viral infections: J. Sträter, et. al.; Gastroenterology **122,** 659 (2002) ▪ TRAIL (Apo2L) suppresses growth of primary human leukemia and myelodysplasia progenitors: M. Plasilova, et al.; Leukemia 16, 67 (2002) ▪ Chronic lymphocytic leukemic cells exhibit apoptotic signaling via TRAIL-R1: M. MacFarlane, et al.; Cell Death Differ. **12**, 773 (2005)

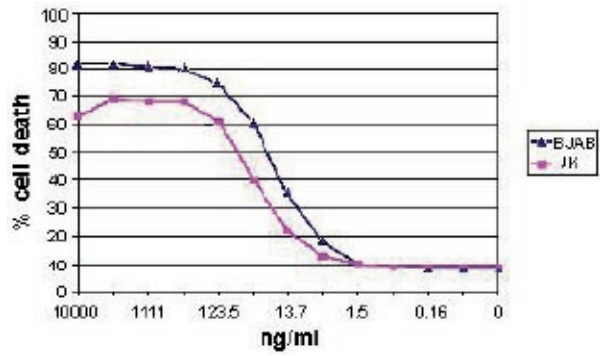


FIGURE: Apoptosis of TRAIL-sensitive cells. Concentration dependence of apoptosis induction in Jurkat and BJAB cells by *Killer*TRAIL™ (soluble) (human), (rec.) (Prod. No. ALX-201-073) reveals high activity even at concentrations of 10-100 ng/ml.

Overview of Different TRAIL Reagents

Product	Trail-R Selectivity	Potency	Endotoxin Content	Best Choice for	Modification
rhsTRAIL Set ALX-850-018	TRAIL-R1, -R2 (DR4/DR5) (incl. Jurkat, U937, K562 cells).	++ ED_{50} 10 ng/ml	+ <0.1EU/µg	Highest killing potency on all TRAIL-sensitive cells	Flag-tagged (trimeric, with suspected liver cell toxicity)
***Killer*TRAIL™ ALX-201-073 / ALX-201-123**	TRAIL-R1 (DR4) (e.g. BJAB, Ramos, but less active on Jurkat, U937, K562)	++ ED_{50} 20 ng/ml	+/- ≤0.004 EU/µg	Standard killer agent for *in vitro* (assays) if via TRAIL-R1 (DR4)	His-tagged (monomeric)
Super*Killer*TRAIL™ ALX-201-115	TRAIL-R1, -R2 (DR4/DR5) (incl. Jurkat, U937, K562 cells).	+++ ED_{50} 0.1 ng/ml	+/- ≤0.03 EU/µg	Highest killing potency on all TRAIL-sensitive cells	His-tagged (trimeric, with suspected liver cell toxicity)

TRAIL Receptors

TRAIL-R1 (human):Fc (human), (rec.)

ALX-522-004-C050 50 µg

Produced in HEK 293 cells. The extracellular domain of human TRAIL-R1 (DR4) (aa 24-239) is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds human and mouse TRAIL. **BIOLOGICAL ACTIVITY:** Inhibits human soluble TRAIL-mediated apoptosis in a concentration range of 2-10µg/ml. Use TRAIL-R2 (human):Fc (human), (rec.) (Prod. No. ALX-522-005) for the detection of human surface TRAIL by FC.

LIT: The lymphotoxin-β receptor is necessary and sufficient for LIGHT-mediated apoptosis of tumor cells: I.A. Rooney, et al.; J. Biol. Chem. **275,** 14307 (2000) ▪ The tumor necrosis factor-related apoptosis-inducing ligand receptors TRAIL-R1 and TRAIL-R2 have distinct cross-linking requirements for initiation of apoptosis and are non-redundant in JNK activation: F. Mühlenbeck et al.; J. Biol. Chem. **275,** 32208 (2000) ▪ T cell costimulation by the TNF ligand BAFF: B. Huard, et al.; J. Immunol. **167,** 6225 (2001)

TRAIL-R2 (human):Fc (human), (rec.)

ALX-522-005-C050 50 µg

ALX-522-005F-C050 FITC 50 µg

Produced in HEK 293 cells. The extracellular domain of human TRAIL-R2 (DR5) (aa 52-212) is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds human and mouse TRAIL. **BIOLOGICAL ACTIVITY:** Inhibits human soluble TRAIL-induced apoptosis in a concentration range of 0.5-10µg/ml.

LIT: TRAIL/Apo-2 ligand induces primary plasma cell apoptosis: J. Ursini-Siegel, et al.; J. Immunol. **169,** 5505 (2002) ▪ 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) ▪ **For a comprehensive bibliography please visit our website.**

TRAIL-R3 (human):Fc (human), (rec.)

ALX-522-006-C050 50 µg

Produced in HEK 293 cells. The extracellular domain of human TRAIL-R3 (DcR1) (aa 25-240) is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds human and mouse TRAIL. **BIOLOGICAL ACTIVITY:** Inhibits human soluble TRAIL-mediated apoptosis in a concentration range of 50-100ng/ml. Use TRAIL-R2 (human):Fc (human) (rec.) (Prod. No. ALX-522-005) for the detection of surface TRAIL by FC.

TRAIL-R4 (human):Fc (human), (rec.)

ALX-522-011-C050 50 µg

Produced in HEK 293 cells. The extracellular domain of human TRAIL-R4 (DcR2) (aa 56-212) is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds human and mouse TRAIL. **BIOLOGICAL ACTIVITY:** Inhibits human TRAIL-mediated apoptosis in a concentration range of 2-10µg/ml. Use TRAIL-R2 (human):Fc (human) (rec.) (Prod. No. ALX-522-005) for the detection of surface human TRAIL by FC.

TRAIL-receptor-1 to -4 set

ALX-850-062-KI02 1 Set

SET CONTAINS:

TRAIL-R1 (human):Fc (human), (rec.) (Prod. No. ALX-522-004)
TRAIL-R2 (human):Fc (human), (rec.) (Prod. No. ALX-522-005)
TRAIL-R3 (human):Fc (human), (rec.) (Prod. No. ALX-522-006)
TRAIL-R4 (human):Fc (human), (rec.) (Prod. No. ALX-522-011)

TRAIL-R2 (mouse):Fc (human), (rec.)

ALX-522-067-C050 50 µg

Produced in HEK 293 cells. The extracellular domain of mouse TRAIL-R2 (DR5) (aa 1-166) is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds mouse and less potently human soluble TRAIL. **BIOLOGICAL ACTIVITY:** Inhibits mouse soluble TRAIL-induced apoptosis assessed in human B cells (BJAB) in a concentration range of 1-10µg/ml.

DcTRAIL-R1 (mouse):Fc (human), (rec.)

ALX-522-066-C050 50 µg

Produced in HEK 293 cells. The extracellular domain of mouse DcTRAIL-R1 (aa 1-160) is fused to the Fc portion of human IgG1. **SPECIFICITY:** rmDcTRAIL-R1:Fc binds mouse soluble TRAIL in an ELISA assay. Does not bind human soluble TRAIL. **BIOLOGICAL ACTIVITY:** Inhibits mouse soluble TRAIL-induced apoptosis assessed in human Jurkat T-lymphoma cells and human B cells myeloma (BJAB) in a concentration range of 500-1'000ng/ml.

LIT: Identification of a new murine tumor necrosis factor receptor locus that contains two novel murine receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) : P. Schneider, et al.; J. Biol. Chem. **278,** 5444 (2003)

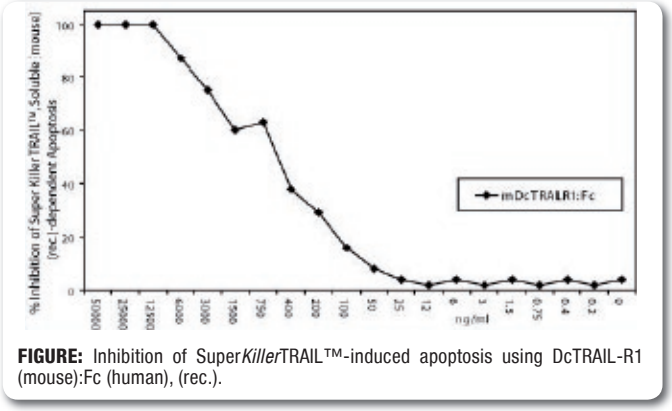


FIGURE: Inhibition of Super*Killer*TRAIL™-induced apoptosis using DcTRAIL-R1 (mouse):Fc (human), (rec.).

DcTRAIL-R2 (mouse):Fc (human), (rec.)

ALX-522-068-C050 50 µg

Produced in HEK 293 cells. The extracellular domain of mouse DcTRAIL-R2 (aa 1-170), of the long splice variant of mouse DcTRAIL-R2, is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds mouse soluble TRAIL and less potently human soluble TRAIL in an ELISA assay. **BIOLOGICAL ACTIVITY:** Inhibits mouse soluble TRAIL-induced apoptosis assessed in human Jurkat T-lymphoma cells and human B cells myeloma (BJAB) in a concentration range of 50-500µg/ml.

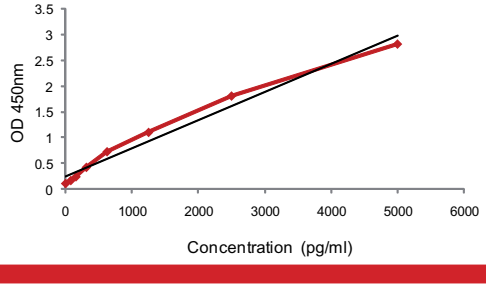
LIT: Identification of a new murine tumor necrosis factor receptor locus that contains two novel murine receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL): P. Schneider, et al.; J. Biol. Chem. **278,** 5444 (2003)

TRAIL-R2 ELISA kit

TRAIL-R2 ELISA kit (human)

BML-AK123-0001 1 Kit

Allows quantitative measurement of human TRAIL-R2 (DR5) in an ELISA format. DR5 binds the death ligand, TRAIL, and is expressed in most human tissue. Formation of a complex between TRAIL and TRAIL-R1 (DR4) and DR5 triggers apoptosis by inducing the oligomerization of intracellular death domains.



TRAIL & TRAIL Receptor Antibodies

Product	Source/Host/ Isotype	Specificity	Application	Prod. No.	Size
TRAIL (human), mAb (2E5)	Mouse IgG1	Human	FC, Neutralizing	ALX-804-296-C100	100 µg
TRAIL (human), mAb (HS501)	Mouse IgG1	Human	WB (excellent)	ALX-804-300-C100	100 µg
TRAIL (human), mAb (III6F)	Mouse IgG2b	Human	FC, IHC (FS), IP, WB	ALX-804-326-C100	100 µg
TRAIL (human), mAb (VI10E)	Mouse IgG2b	Human	FC, IP	ALX-804-325-C100	100 µg
TRAIL (human), pAb	From rabbit	Human	FC, ICC, IHC (FS), WB	ALX-210-732-R100	100 µl
TRAIL (human), pAb	From rabbit	Human	WB	BML-SA259-0100	100 µg
TRAIL, pAb	From rabbit	Human	IHC, WB	ADI-AAP-470-C ADI-AAP-470-E	25 µg 100 µg

Product	Source/Host/ Isotype	Specificity	Application	Prod. No.	Format	Size
TRAIL-R1 (human), mAb (HS101)	Mouse IgG1	Human	FC, ICC, IP, FUNC	ALX-804-297-C100 ALX-804-297A-C100 ALX-804-297F-T100 ALX-804-297TS-T100	PF Purified FITC ATTO 647N	100 µg 100 µg 100 tests 100 tests
TRAIL-R1, pAb	From rabbit	Human	WB	ADI-AAP-420-C ADI-AAP-420-E		25 µg 100 µg
TRAIL-R1, pAb	From rabbit	Human	WB	BML-SA225-0100		100 µg
TRAIL-R2 (human), mAb (HS201)	Mouse IgG1	Human	FC, ICC, IP, FUNC	ALX-804-298-C100 ALX-804-298A-C100 ALX-804-298F-T100	PF Purified FITC	100 µg 100 µg 100 tests
TRAIL-R2, pAb	From goat	Human, mouse	FC, IHC (PS), ICC, WB	ALX-210-743-C200		200 µg
TRAIL-R3 (human), mAb (HS301)	Mouse IgG1	Human	FC, ICC	ALX-804-344A-C100		100 µg
TRAIL-R3 (human), mAb (LEIA)	Rat IgG2a	Human	FC, WB (over-expressed)	ALX-804-136-C100		100 µg
TRAIL-R3 (human), pAb	From goat	Human	FC, IHC (PS), ICC, WB	ALX-210-744-C200		200 µg
TRAIL-R3 (ED), pAb	From rabbit	Human, mouse, rat	ICC, WB	ADI-905-216-100		100 µg
TRAIL-R3, pAb	From rabbit	Human, mouse, rat	WB	BML-SA233-0100		100 µg
TRAIL-R4 (human), mAb (HS402)	Mouse IgG1	Human	FC, IHC (FS), ICC, IP	ALX-804-299A-C100		100 µg
TRAIL-R4, pAb	From rabbit	Human	ICC, WB	ADI-AAP-371-C ADI-AAP-371-E		25 µg 100 µg
DcTRAIL-R2 (mouse), mAb (Lucy-1)	Rat IgG2a	Mouse	WB	ALX-804-817-C100		100 µg
anti-TRAIL receptor-1 to -4 flow cytometry set		Human	FC	ALX-850-273-KI01		1 Set

Latest Insight

S. Araki, et al. may have found potential biomarkers for the sensitivity of solid cancers to TRAIL-R1 mAb and other TRAIL-agonistic drugs. They identified four genes (*STK17B, SP140L, CASP8* and *AIM1*) whose expression pattern significantly differs between TRAIL-R1 mAb sensitive and resistant cells. Their results may be useful for evaluating the best strategy of a potential TRAIL-R1 mAb or agonistic drugs based anticancer therapy. However, the validation of the method's accuracy in a clinical trial is awaited.

LIT: Biomarkers for predicting the sensitivity of cancer cells to TRAIL-R1 agonistic monoclonal antibody, S. Araki, et al.: Cancer Letters, **Epub ahead of print**

NEW TRAIL Receptor Antibodies for IHC

Specially developed for the immunohistochemical detection of TRAIL receptors in paraffin embedded tissue. Detect TRAIL receptors expressed at endogenous levels in paraffin-embedded tissue in different mammary carcinoma tissues.

TRAIL-R1 (human), mAb (TR1.02)

ALX-804-665-C100 100 µg

CLONE: TR1.02. **ISOTYPE:** Mouse IgG2b. **IMMUNOGEN:** Recombinant human TRAIL-R1 (DR4). **SPECIFICITY:** Recognizes human TRAIL-R1. Does not cross-react with human TRAIL-R2, -R3 or-R4. **APPLICATION:** FC, IHC (PS), WB.

LIT: Prognostic significance of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor expression in patients with breast cancer: T. M. Ganten, et al.; J. Mol. Med **87,** 995 (2009)

TRAIL-R2 (human), mAb (TR2.21)

ALX-804-666-C100 100 µg

CLONE: TR2.21. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human TRAIL-R2 (DR5). **SPECIFICITY:** Recognizes human TRAIL-R2. Does not cross-react with human TRAIL-R1, -R3 and -R4. **APPLICATION:** FC, IHC (PS), WB.

TRAIL-R3 (human), mAb (TR3.06)

ALX-804-667-C100 100 µg

CLONE: TR3.06. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human TRAIL-R3 (DcR1). **SPECIFICITY:** Recognizes human TRAIL-R3. Does not cross-react with human TRAIL-R1, -R2 and -R4. **APPLICATION:** FC, IHC (PS), WB.

Selected Review Article

Specific Human TRAIL Receptors Monoclonal Antibodies for Immunohistochemistry

T. M. Ganten and colleagues [1] set out to determine the expression pattern of all surface-bound TRAIL receptors and their prognostic clinical value, and investigated tumor samples of 311 patients with breast cancer by immunohistochemistry. TRAIL receptor expression profiles were correlated with clinico-pathological data, disease-free survival and overall survival. TRAIL-R1 was more strongly expressed in better differentiated tumors, and correlated positively with surrogate markers of a better prognosis (hormone receptor status, Bcl-2, negative nodal status), but negatively with the expression of Her2/neu and the proliferation marker Ki67. In contrast, TRAIL-R2 and TRAIL-R4 expression correlated with higher tumor grades, higher Ki67 index, higher Her2/neu expression and a positive nodal status at the time of diagnosis, but with lower expression of Bcl-2. Thus, the TRAIL receptor expression pattern was predictive of nodal status. In this study they also reviewed the newly developed four specific human TRAIL-Rs mAbs for paraffin-embedded tissue immunohistochemical detection. In addition, the publication provides technical information and procedures.

TRAIL-R	Clone	Isotype	Specificity	Additional Literature
TRAIL-R1	TR1.02	mIgG2b	Recognizes human TRAIL-R1. Does not cross-react with human TRAIL-R2, -R3 or -R4.	[2], [3], [4]
TRAIL-R2	TR2.21	mIgG1	Recognizes human TRAIL-R2. Does not cross-react with human TRAIL-R1, -R3 or -R4.	[2], [3], [4]
TRAIL-R3	TR3.06	mIgG1	Recognizes human TRAIL-R3. Does not cross-react with human TRAIL-R1, -R2 or -R4.	[2], [4]
TRAIL-R4	TR4.18	mIgG1	Recognizes human TRAIL-R4. Does not cross-react with human TRAIL-R1, -R2 or -R3.	[2], [4]

LITERATURE REFERENCES:

[1] Prognostic significance of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor expression in patients with breast cancer: T. M. Ganten, J. Sykora, R. Koschny, E. Batke, S. Aulmann, U. Mansmann, W. Stremmel, H. P. Sinn, H. Walczak; J. Mol. Med. **87,** 995 (2009)

[2] Differential expression of the TRAIL/TRAIL-receptor system in patients with inflammatory bowel disease: S. Brost, et al.; Pathol. Res. Pract. **206,** 43 (2010)

[3] TRAIL/bortezomib cotreatment is potentially hepatotoxic but induces cancer-specific apoptosis within a therapeutic window: R. Koschny, et al.; Hepatology **45,** 649 (2007)

[4] Preclinical differentiation between apparently safe and potentially hepatotoxic applications of TRAIL either alone or in combination with chemotherapeutic drugs: T. M. Ganten, et al.; Clin. Cancer Res. **12,** 2640 (2006)

Purified (PF) = Purified (Preservative free); FC = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

DISC Complex Components

FADD

FADD, mAb (1F7)

ADI-AAM-212-E 100 µg

CLONE: 1F7. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human FADD protein. **SPECIFICITY:** Recognizes human and mouse FADD. **APPLICATION:** IP, WB.

FADD, pAb

ADI-AAP-210-D 50 µg

ADI-AAP-210-F 200 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide derived from the sequence of human FADD. **SPECIFICITY:** Recognizes human and monkey FADD. **APPLICATION:** WB.

FLIP

FLIP, mAb (Dave-2)

ALX-804-127-C100 100 µg

CLONE: Dave-2. **ISOTYPE:** Rat IgG2a. **IMMUNOGEN:** Recombinant human FLIP (aa 1-480). **SPECIFICITY:** Recognizes an epitope (aa 1-200) present in both short ($FLIP_S$) and long ($FLIP_L$) splice variants of human and mouse FLIP. **APPLICATION:** IP, WB.

LIT: Fas engagement induces the maturation of dendritic cells (DCs), the release of interleukin (IL)-1beta, and the production of interferon gamma in the absence of IL-12 during DC-T cell cognate interaction: a new role for Fas ligand in inf: M. Rescigno, et al.; J. Exp. Med. **192,** 1661 (2000) ▪ Fas-associated protein with death domain (FADD)-independent recruitment of c-FLIPL to death receptor 5: T.G. Jin, et al.; J. Biol. Chem. **279,** 55594 (2004) ▪ The E3 ubiquitin ligase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP(L) turnover: L. Chang, et al.; Cell **124,** 601 (2006) ▪ A Protective Role for the Human SMG-1 Kinase against Tumor Necrosis Factor-α-induced Apoptosis: V. Oliveira, et al.; J. Biol. Chem. **283,** 13174 (2008) ▪ **For a comprehensive bibliography please visit our website.**

FLIP (human), mAb (NF6)

ALX-804-428-C050 50 µg

ALX-804-428-C100 100 µg

CLONE: NF6. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human FLIP (aa 1-194). **SPECIFICITY:** Recognizes short ($FLIP_S$) and long ($FLIP_L$) splice variants of human FLIP. **APPLICATION:** WB.

LIT: An inducible pathway for degradation of FLIP protein sensitizes tumor cells to TRAIL-induced apoptosis: Y. Kim, et al.; J. Biol. Chem. **277,** 22320 (2002) ▪ Suramin inhibits death receptor-induced apoptosis in vitro and fulminant apoptotic liver damage in mice: S.T. Eichhorst, et al.; Nature Med. **10,** 602 (2004) ▪ Elevated NF-κB responses and FLIP levels in leukemic but not normal lymphocytes: reduction by salicylate allows TNF-induced apoptosis: C. Rae, et al.; PNAS **104,** 12790 (2007) ▪ A Protective Role for the Human SMG-1 Kinase against Tumor Necrosis Factor-α-induced Apoptosis: V. Oliveira, et al.; J. Biol. Chem. **283,** 13174 (2008) ▪ Stx2 Causes Apoptosis In Human Brain Microvascular Endothelial Cells Via CHOP: J. Fujii, et al.; Infect. Immun. **76,** 3679 (2008) ▪ **For a comprehensive bibliography please visit our website.**

FLIP γ/δ (CT), pAb

ADI-905-226-100 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide derived from sequence near the C-terminus of human FLIP δ/FLIP short form. **SPECIFICITY:** Recognizes human and monkey FLIP gamma/δ. **APPLICATION:** IHC, WB.

c-FLIP, pAb

ADI-AAP-440-C 25 µg

ADI-AAP-440-E 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide derived from the sequence of human c-FLIP6; sequence conserved among all c-FLIP variants. **SPECIFICITY:** Recognizes human, mouse and rat c-FLIP. **APPLICATION:** ICC, WB.

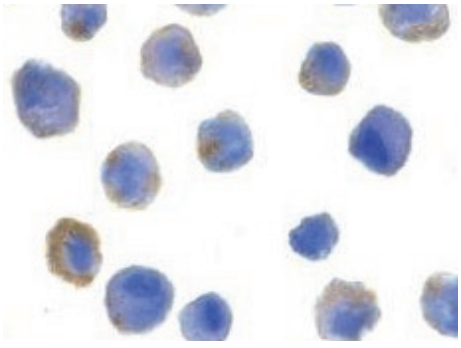


FIGURE: Immunocytochemistry of HeLa cells with c-FLIP polyclonal antibody at 5µg/ml.

Caspase-10

Caspase-10, pAb

ADI-AAP-110-C 25 µg

ADI-AAP-110-E 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide derived from the sequence of human Caspase-10. **SPECIFICITY:** Recognizes human, mouse, rat and bovine Caspase-10. **APPLICATION:** WB.

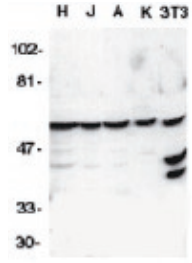


FIGURE: Western blot analysis of HeLa (H), Jurkat (J), A431 (A), K562 (K), and NIH3T3 (3T3) whole cell lysates probed with caspase-10 polyclonal antibody at 1:1000 dilution.

Product	Source/Host	Prod. No.	Size
Caspase-10 (human), (rec.)	*E coli*	BML-SE174-5000	5000 U
Caspase-10/a (human), (rec.) (active)	*E coli*	ALX-201-091-U025 ALX-201-091-U100	25 U 100 U
Caspase-10/a (human), (rec.) (active) (high stability)	*E coli*	ALX-201-193-U050	50 U
Caspase-10/b (human), (rec.) (active)	*E coli*	ALX-201-092-U025 ALX-201-092-U100	25 U 100 U
Caspase-10/b (human), (rec.) (active) (high stability)	*E coli*	ALX-201-194-U050	50 U

Caspase-8

Caspase-8 (human), (rec.)
BML-SE172-5000 5000 U

Produced in *E. coli*.

Caspase-8 (mouse), (rec.) (active)
ALX-201-163-C020 20 µg

Produced in *E. coli*.

Caspase-8, mAb (5F7)
ADI-AAM-118-E 100 µg

CLONE: 5F7. **ISOTYPE:** Mouse IgG2b. **IMMUNOGEN:** Recombinant human Caspase-8 protein (carboxy-terminal fragment). **SPECIFICITY:** Recognizes human Caspase-8. **APPLICATION:** WB.

Caspase-8 (human), pAb
BML-SA322-0100 100 µl

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 427-441 of human caspase-8. **SPECIFICITY:** Recognizes human proenzyme (55 kDa) and small (11 kDa) subunit of mature caspase-8. Does not cross-react with other caspases (caspases 1-7, -9, -10, -14). **APPLICATION:** WB.

Caspase-8 (mouse), mAb (1G12)
ALX-804-447-C100 100 µg

CLONE: 1G12. **ISOTYPE:** Rat IgG1. **IMMUNOGEN:** Recombinant mouse caspase-8 p18 subunit. **SPECIFICITY:** Recognizes the p18 subunit of mouse caspase-8. Detects bands of ~55kDa (full-length caspase-8) and ~18kDa (apoptosis-induced cleavage fragment) by Western blot. Does not cross-react with human caspase-8. **APPLICATION:** ELISA, FC, ICC, WB.

LIT: Modifications and intracellular trafficking of FADD/MORT1 and caspase-8 after stimulation of T lymphocytes: L.A. O'Reilly; Cell Death Differ. **11,** 724 (2004) ▪ Caspase-8 serves both apoptotic and nonapoptotic roles: T.B. Kang, et al.; J. Immunol. **173,** 2976 (2004) ▪ Apaf-1 and caspase-9 are required for cytokine withdrawal-induced apoptosis of mast cells but dispensable for their functional and clonogenic death: V.S. Marsden, et al.; Blood **107,** 1872 (2006) ▪ Caspase-8 promotes cell motility and calpain activity under nonapoptotic conditions: B. Helfer, et al.; Cancer Res. **66,** 4273 (2006) ▪ FLIP(L) protects neurons against in vivo ischemia and in vitro glucose deprivation-induced cell death: E. Taoufik, et al.; J. Neurosci. **27,** 6633 (2007)

Caspase-8 (mouse), mAb (3B10)
ALX-804-448-C100 100 µg

CLONE: 3B10. **ISOTYPE:** Rat IgG1. **IMMUNOGEN:** Recombinant mouse caspase-8 p18 subunit. **SPECIFICITY:** Recognizes mouse caspase-8. Detects bands of ~55kDa (full-length caspase-8) and ~18kDa (apoptosis-induced cleavage fragment) by Western blot. Does not cross-react with human caspase-8. **APPLICATION:** ELISA, FC, ICC, WB.

LIT: Modifications and intracellular trafficking of FADD/MORT1 and caspase-8 after stimulation of T lymphocytes: L.A. O'Reilly; Cell Death Differ. **11,** 724 (2004) ▪ Impaired lactation in mice expressing dominant-negative FADD in mammary epithelium.: M. Shackleton, et al.; Dev. Dyn. **238,** 1010 (2009) ▪ Membrane-bound Fas Ligand Only is Essential for Fas-Induced Apoptosis.: L.A. O' Reilly, et al.; Nature **461,** 659 (2009)

Caspase-8 assay kit for drug discovery
BML-AK715-0001 1 Kit

Complete assay system designed to measure protease activity of caspase-8. It contains both a colorimetric substrate (IETD-pNA) and a fluorogenic substrate (IETDAMC). The assays are performed in a convenient, 96-well microtiterplate format. The kit is useful to screen inhibitors of caspase-8, a potential therapeutic target. An inhibitor, IETD-CHO (aldehyde), is included for use as a control. **QUANTITY:** 96 assays.

Our Gold Standards!

Caspase-8 (human), mAb (12F5)
ALX-804-242-C100 100 µg

CLONE: 12F5. **ISOTYPE:** Mouse IgG2b. **IMMUNOGEN:** Recombinant human caspase-8. **SPECIFICITY:** Recognizes human procaspase-8 and active human caspase-8. Detects procaspase-8 (p55/54), the intermediate cleavage products (p43/41) and the p18 active subunit of caspase-8 by Western blot. Does not recognize mouse caspase-8. **APPLICATION:** IP, WB.

LIT: Differential regulation and ATP requirement for caspase-8 and caspase-3 activation during CD95- and anticancer drug-induced apoptosis: D. Ferrari, et al.; J. Exp. Med. **188,** 979 (1998) ▪ Fas-associated death domain protein (FADD) and caspase-8 mediate up-regulation of c-Fos by Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) via a FLICE inhibitory protein (FLIP)-regulated pathway: D. Siegmund, et al.; J. Biol. Chem. **276,** 32585 (2001) ▪ An inducible pathway for degradation of FLIP protein sensitizes tumor cells to TRAIL-induced apoptosis: Y. Kim, et al.; J. Biol. Chem. **277,** 22320 (2002) ▪ A Protective Role for the Human SMG-1 Kinase against Tumor Necrosis Factor-α-induced Apoptosis: V. Oliveira, et al.; J. Biol. Chem. **283,** 13174 (2008) ▪ **For a comprehensive bibliography please visit our website.**

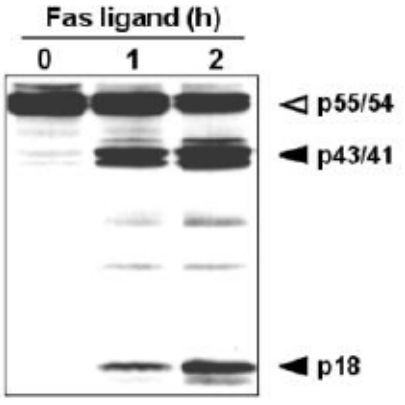


FIGURE: Detection of Fas ligand-induced caspase-8 processing and activation in human Jurkat cells.

Caspase-8 (human), mAb (C15)
ALX-804-429-C050 50 µg

ALX-804-429-C100 100 µg

CLONE: C15. **ISOTYPE:** Mouse IgG2b. **IMMUNOGEN:** Recombinant human caspase-8 (aa 181-478). **SPECIFICITY:** Recognizes the p18 subunit of human caspase-8. **APPLICATION:** ICC, IP, WB.

LIT: FLICE is predominantly expressed as two functionally active isoforms, caspase-8/a and caspase-8/b: C. Scaffidi, et al.; J. Biol. Chem. **272,** 26953 (1997) ▪ Enhanced caspase-8 recruitment to and activation at the DISC is critical for sensitisation of human hepatocellular carcinoma cells to TRAIL-induced apoptosis by chemotherapeutic drugs: T.M. Ganten, et al.; Cell Death Differ. **11 Suppl 1,** S86 (2004) ▪ cFLIPL inhibits tumor necrosis factor-related apoptosis-inducing ligand-mediated NF-kappaB activation at the death-inducing signaling complex in human keratinocytes: T. Wachter, et al.; J. Biol. Chem. **279,** 52824 (2004) ▪ **For a comprehensive bibliography please visit our website.**

TRAIL as Effector of the Immune System

TRAIL is expressed upon induction in different immune cells and is thought to influence the innate and adaptive immune system and to contribute to autoimmune diseases [1].

- Lipopolysaccharide (LPS) and IFN-β stimulation leads to the up-regulation of TRAIL on monocytes and macrophages [2, 3].
- IFN-γ can induce TRAIL expression on monocytes, dendritic cells (DCs) and natural killer (NK) cells [4, 5]. Surface bound TRAIL is one of the effectors of NK cells [6].
- TRAIL-R1/-R2 are upregulated upon virus infection on the corresponding cells. Furthermore, IFN-α and IFN-β are produced. Together with the autocrinly produced IFN-γ of activated cytotoxic lymphocytes (CTLs), those IFNs cause the upregulation of TRAIL on the activated CTLs. This in turn kills the virus infected cell via TRAIL induced apoptosis [7, 8, 9, 10, 11].
- TRAIL is important in IFN-γ dependent suppression of tumor cell growth mediated by NK cells [12].
- The role of TRAIL on autoimmunity was initially thought to be based on its presumed role in thymic negative selection. However, TRAIL is neither expressed on thymic DCs nor on epithelial cells [13, 14]. Additional experiments have further challenged the role of TRAIL in thymic negative selection under physiological conditions [15, 16].

LITERATURE REFERENCES:

[1] TRAIL: a multifunctional cytokine: U. Schaefer, et al.; Front. Biosci. **12,** 3813 (2007) (Review)

[2] Regulation of soluble and surface-bound TRAIL in human T cells, B cells, and monocytes: S. Ehrlich, et al.; Cytokine **24,** 244 (2003)

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[4] Human dendritic cells mediate cellular apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL): N. A. Fanger, et al.; J. Exp. Med. **190,** 1155 (1999)

[5] Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL: T. S. Griffit, et al.; J. Exp. Med. **189,** 1343 (1999)

[6] Expression and function of TNF-related apoptosis-inducing ligand on murine activated NK cells: N. Kayagaki, et al.; J. Immunol. **163,** 1906 (1999)

[7] IFN-gamma mediates a novel antiviral activity through dynamic modulation of TRAIL and TRAIL receptor expression: L. M. Sedger, et al.; J. Immunol. **163,** 920 (1999)

[8] Antiviral response by natural killer cells through TRAIL gene induction by IFN-alpha/beta: K. Sato, et al.; Eur. J. Immunol. **31,** 3138 (2001)

[9] Impaired TRAIL-dependent cytotoxicity of CD1c-positive dendritic cells in chronic hepatitis C virus infection: S. Ciesek, et al.; J. Viral Hepat. **15,** 200 (2008)

[10] Role of Fas ligand in apoptosis induced by hepatitis C virus infection: E. Mita, et al.; BBRC **204,** 468 (1994)

[11] Involvement of tumor necrosis factor-related apoptosis-inducing ligand and tumor necrosis factor-related apoptosis-inducing ligand receptors in viral hepatic diseases: Y. Saitou, et al.; Hum. Pathol. **36,** 1066 (2005)

[12] Involvement of tumor necrosis factor-related apoptosis-inducing ligand in NK cell-mediated and IFN-gamma-dependent suppression of subcutaneous tumor growth: K. Takeda, et al.; Cell. Immunol. **214,** 194 (2001)

[13] Intrathymic and extrathymic clonal deletion of T cells: J. Sprent & S. R. Webb; Curr. Opin. Immunol. **7,** 196 (1995) (Review)

[14] In vitro negative selection of alpha beta T cell receptor transgenic thymocytes by conditionally immortalized thymic cortical epithelial cell lines and dendritic cells: Y. Tanaka, et al.; Eur. J. Immunol. **23,** 2614 (1993)

[15] Normal thymocyte negative selection in TRAIL-deficient mice: E. Cretney, et al.; J. Exp. Med. **198,** 491 (2003)

[16] TRAIL-R as a negative regulator of innate immune cell responses: G. E. Diehl, et al.; Immunity **21,** 877 (2004)

Selected Review Article

Following TRAIL's path in the immune system: C. Falschlehner, et al.; Immunology **127,** 145 (2009)

TRAIL & Anticancer Therapy

Since several years TRAIL has been investigated as a potential anticancer biotherapeutic. Successful cancer therapeutics require high selective and potent antitumor activities and should cause low side effects [1]. TRAIL is capable of inducing apoptosis in a wide range of tumor cells resistant to conventional chemo- and radiotherapy, while not affecting most normal cells [2, 3]. However, after the first findings about its antitumor activity it became clear that many primary tumor cells are not TRAIL sensitive, despite the expression of apoptosis inducing TRAIL receptors. This TRAIL resistance is very likely caused by multiple mechanisms and may include Bcl-2 family proteins overexpression, enhanced XIAP, survivin or FLIP expression, upregulated protein kinase B (PKB/Akt) and NF-κB signaling, deletion of the Bax gene and caspase mutations, among many others [4, 5, 6, 7]. Therefore, the underlying mechanisms of TRAIL resistance are still under investigation and efforts have been made to sensitize tumor cells with other antitumor agents towards TRAIL combination therapies [8, 9]. In addition a recent study has shown that apoptosis-inducing TRAIL-R deficiency in mice enhances lymph node metastasis without affecting primary tumor development, thus TRAIL-R may act as a metastasis suppressor [10].

Next to tailored recombinant human TRAIL proteins, which show either enhanced anti-tumor potency or selectivity against certain TRAIL-Rs [11], also TRAIL receptor agonistic antibodies are being tested in different studies for their therapeutic potential. Use of TRAIL-R1 or TRAIL-R2-selective variants could permit better tumor-specific therapies through escape from the decoy receptor-mediated antagonism, resulting in a higher efficacy with possibly less side effects as compared with wtTRAIL [12, 13, 14, 15, 16]. For an overview see Table 1.

Recombinant versions of TRAIL with tags such as polyhistidine, FLAG, leucine zippers (LZ) and isoleucine zipper (iz), as well as non-tagged versions have been generated and tested for efficacy and selectivity in different models. While non-tagged versions appeared to possess high selectivity towards cancer cells but lower efficacy, TRAIL-tagged versions showed higher efficacy due to higher-order complexes, but also exhibited a somewhat higher toxicity towards normal cells such as human hepatocytes *in vitro* [17, 18]. LZ-TRAIL and iz-TRAIL represent an intermediate state, whereas they have comparable efficacy as FLAG/His-TRAIL *in vitro*, but are not that highly toxic [19, 20, 21]. Initially, aggregated forms of TRAIL were described to be hepatotoxic [8]. Further studies revealed controversial results and the effect of TRAIL to the liver is under continuous investigation. It remains unclear whether observed *in vitro* hepatotoxicity of the different TRAIL variants would also occur *in vivo* [22].

The use of agonistic antibodies as therapeutics is currently emerging. Several potent antibodies against TRAIL-R1 (Mapatumumab) or TRAIL-R2 (Lexatumumab; HGS-TR2J; Apomab; AMG 655; LBY135; CS-1008 (humanized version of TRA-8)) are currently in clinical trials [19, 23, 24].

Molecule Tested	Alternative Name/ Comments	Targeted Receptor
Agonistic Ab currently in clinical trials		
Mapatumumab	HGS-ETR1	TRAIL-R1
Lexatumumab	HGS-ETR2	TRAIL-R2
HGS-TR2J	-	TRAIL-R2
Apomab	-	TRAIL-R2
AMG 655	-	TRAIL-R2
LBY135	-	TRAIL-R2
CS-1008	humanized TRA-8	TRAIL-R2
Agonistic Ab		
M271	-	TRAIL-R2
M413	-	TRAIL-R1
4HG, 4G7	-	TRAIL-R2
2E12	-	TRAIL-R2
Recombinant human TRAIL (rhTRAIL)		
His-TRAIL	-	TRAIL-R1/TRAIL-R2/TRAIL-R3/TRAIL-R4/OPG
LZ-TRAIL	-	TRAIL-R1/TRAIL-R2/TRAIL-R3/TRAIL-R4/OPG
FLAG-TRAIL	-	TRAIL-R1/TRAIL-R2/TRAIL-R3/TRAIL-R4/OPG
rhTRAIL	-	TRAIL-R1/TRAIL-R2/TRAIL-R3/TRAIL-R4/OPG
DR5-TRAIL	E195R/D269H	TRAIL-R2/TRAIL-R4(reduced)
Apo2L.DR5-8	-	TRAIL-R2/TRAIL-R4(?)
TRAIL-CD19	rhTRAIL fusion proteins	TRAIL-R1/TRAIL-R2/TRAIL-R3/TRAIL-R4/OPG with CD19
TRAIL-EGFR	rhTRAIL fusion proteins	TRAIL-R1/TRAIL-R2/TRAIL-R3/TRAIL-R4/OPG with EGFR
TRAIL-R1-5	-	TRAIL-R2/TRAIL-R3(?)/TRAIL-R4(?)/OPG(?)

TABLE 1: Overview on recombinant human TRAIL variants and agonistic TRAIL-R1 or TRAIL-R2 specific antibodies.

Adapted from: TRAIL receptor signalling and modulation: Are we on the right TRAIL?: D. Mahalingam, et al.; Cancer Treat. Rev. ***35,*** *280 (2009) (Review)*

LITERATURE REFERENCES:

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TRAIL Sensitizer

HDAC Inhibitors

M344

ALX-270-297-M001 1 mg
ALX-270-297-M005 5 mg

Potent inhibitor of histone deacetylases (HDACs) (IC50≤1µM).

LIT: Amide analogues of trichostatin A as inhibitors of histone deacetylase and inducers of terminal cell differentiation: M. Jung, et al.; J. Med. Chem. **42,** 4669 (1999)

Scriptaid

BML-GR326-0001 1 mg
BML-GR326-0005 5 mg

Inhibitor of histone deacetylase (HDAC) (HDAC1 and 3: IC_{50}~0.6µM; HDAC8: IC_{50}~1µM) with lower toxicity than trichostatin A (Prod. No. BML-GR309). Optimal concentration 2-2.5µg/ml (6-8µM). A negative control is available (Prod. No. BML-GR327).

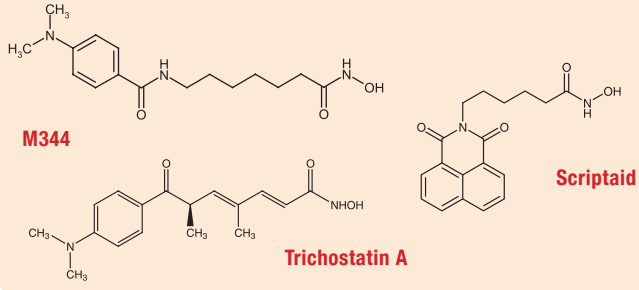
LIT: A novel histone deacetylase inhibitor identified by high-throughput transcriptional screening of a compound library: G.H. Su, et al.; Cancer Res. **60,** 3137 (2000) ▪ Identification of novel isoform-selective inhibitors within class I histone deacetylases: E. Hu, et al.; J. Pharmacol. Exp. Ther. **307,** 720 (2003) ▪ A novel histone deacetylase inhibitor, scriptaid, enhances expression of functional estrogen receptor alpha (ER) in ER negative human breast cancer cells in combination with 5-aza 2'-deoxycytidine: J.C. Keen, et al.; Breast Cancer Res. Treat. **81,** 177 (2003)

Trichostatin A

BML-GR309-0001 1 mg
BML-GR309-0005 5 mg

Isolated from *Streptomyces hygroscopicus*. Potent and reversible inhibitor of histone deacetylases. Induces cell growth arrest at both G1 and G2/M phases. May induce apoptosis in some cases.

LIT: A new antifungal antibiotic, trichostatin: N. Tsuji et al.; J. Antibiot. (Tokyo) **29,** 1 (1976) ▪ Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A: M. Yoshida et al.; J. Biol. Chem. **265,** 17174 (1990) ▪ A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p: J. Taunton et al; Science Science, **408** (1996) ▪ Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo: D.M. Vigushin; Clin. Cancer Res. **7,** 971 (2001) ▪ Chromatin remodeling agent trichostatin A: a key-factor in the hepatic differentiation of human mesenchymal stem cells derived of adult bone marrow: S. Snykers et al.; BMC Dev. Biol. **7,** 24 (2007) ▪ **For a comprehensive bibliography please visit our website.**



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ALX-440-045-M500 500 mg
ALX-440-045-G001 1 g

SN50

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BML-T104-0025 25 mg
BML-T104-0250 250 mg

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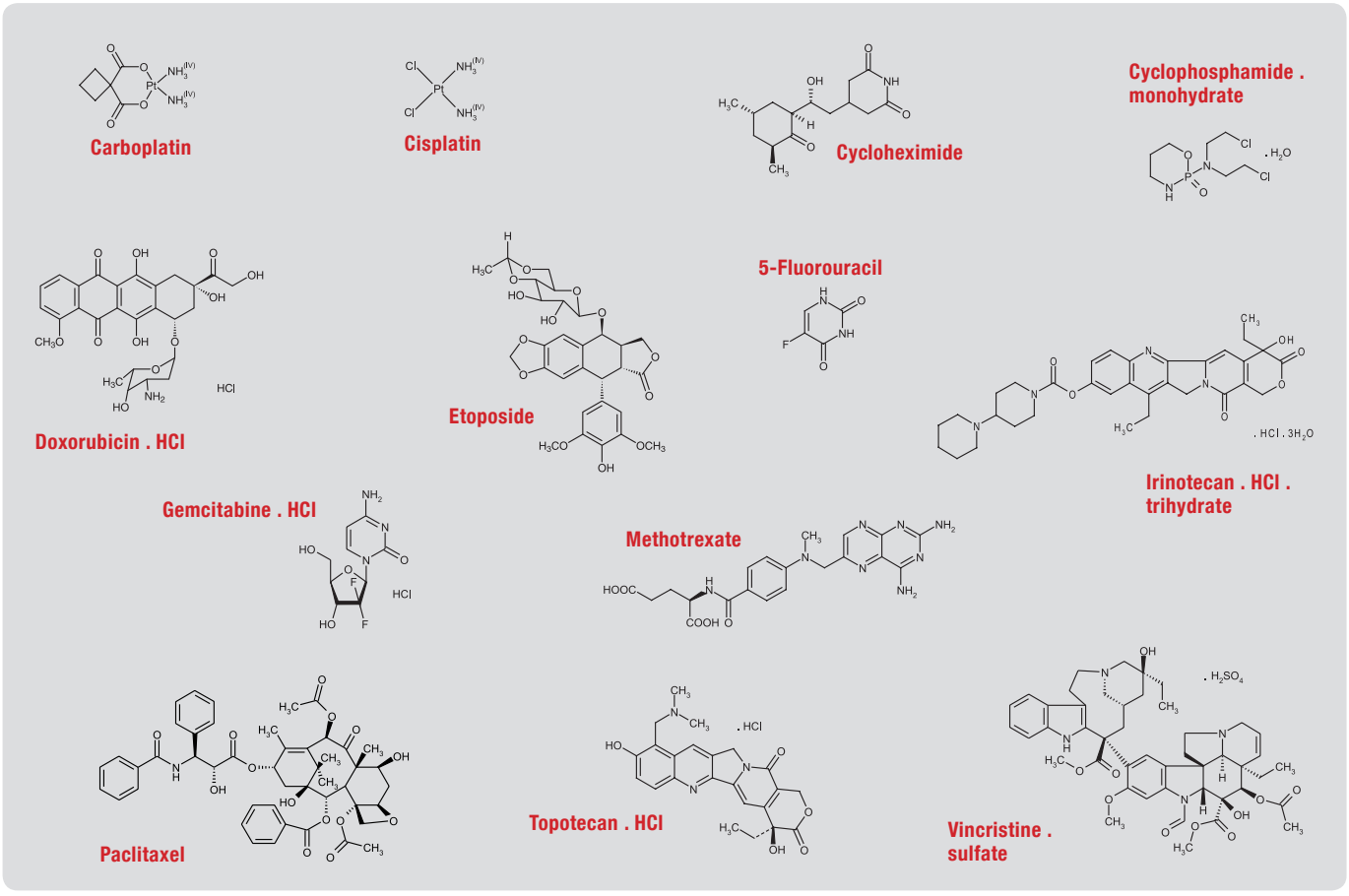
ALX-350-133-M005 5 mg
ALX-350-133-M025 25 mg

Vincristine . sulfate

BML-T117-0005 5 mg
BML-T117-0025 25 mg



Preclinical Studies of Sensitization of Primary Tumor Cells for TRAIL-induced Apoptosis

Primary tumor cells	TRAIL in combination with	Proposed mechanism	Reference
AML	HDAC inhibitors		[1]
ALL	Vincristin		[2]
CLL	HDAC inhibitors (Depsipeptide, valproic acid)	Signal via TRAIL-R1	[3, 4]
B-CLL	Cycloheximide		[5]
MM	NF-κB inhibitor SN50	$cFLIP_L$ downregulation	[6]
Erythroleukemic cells	Irradiation	Downregulation of Bcl-2, Bfl-1, IAPs up-regulation of Bax	[7]
Colon cancer	Irinotecan, 5-FU	TRAIL-R1 upregulation	[8]
Pancreatic cancer	Gemcitabine	TRAIL-R2 upregulation	[9]
Soft tissue sarcoma	Doxorubicin, cisplatin, etoposide, methotrexate, cyclophosphamide		[10]

TABLE: *Adapted from: The promise of TRAIL-potential and risks of a novel anticancer therapy: R. Koschny, et al.; J. Mol. Med.* ***85,*** *923 (2007)*

LIT: [1] Tumor-selective action of HDAC inhibitors involves TRAIL induction in acute myeloid leukemia cells: A. Nebbioso, et al.; Nat. Med. **11,** 77 (2005) ▪ **[2]** Target cell-restricted apoptosis induction of acute leukemic T cells by a recombinant tumor necrosis factor-related apoptosis-inducing ligand fusion protein with specificity for human CD7: E. Bremer, et al.; Cancer Res. **65,** 3380 (2005) ▪ **[3]** Histone deacetylase inhibitors potentiate TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in lymphoid malignancies: S. Inoue, et al.; Cell Death Differ. 11 Suppl **2,** S193 (2004) ▪ **[4]** Chronic lymphocytic leukemic cells exhibit apoptotic signaling via TRAIL-R1: M. MacFarlane, et al.; Cell Death Differ. **12,** 773 (2005) ▪ **[5]** Sensitization to TRAIL-induced apoptosis and modulation of FLICE-inhibitory protein in B chronic lymphocytic leukemia by actinomycin D: A. Olsson, et al.; Leukemia **15,** 1868 (2001) ▪ **[6]** Biologic sequelae of nuclear factor-kappaB blockade in multiple myeloma: therapeutic applications: N. Mitsiades, et al.; Blood **99,** 4079 (2002) ▪ **[7]** Ionizing radiation sensitizes erythroleukemic cells but not normal erythroblasts to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)--mediated cytotoxicity by selective up-regulation of TRAIL-R1: R. Di Pietro, et al.; Blood **97,** 2596 (2001) ▪ **[8]** Effects of tumor necrosis factor-related apoptosis-inducing ligand alone and in combination with chemotherapeutic agents on patients' colon tumors grown in SCID mice: T. Naka, et al.; Cancer Res. **62,** 5800 (2002) ▪ **[9]** The anti-tumor effect of Apo2L/TRAIL on patient pancreatic adenocarcinomas grown as xenografts in SCID mice: B. L. Hylander, et al.; J. Transl. Med. **3,** 22 (2005) ▪ **[10]** Enhanced apoptosis of soft tissue sarcoma cells with chemotherapy: A potential new approach using TRAIL: M. Clayer, et al.; J. Orthop. Surg. (Hong Kong) **9,** 19 (2001)

Role of TRAIL in Bone Metabolism

There are two key regulators in bone turnover, osteoblasts, which are involved in bone formation, and osteoclasts, which are responsible for bone resorption [1].

OPG, produced by osteoblasts, is a solube decoy receptor for several ligands. One of them is the receptor activator of NF-κB ligand (RANKL; OPGL; ODF; TRANCE), which has been identified independently by four research groups [2, 3, 4, 5]. Binding of RANKL to the receptor RANK mediates osteoclastogenesis [6].

Beside RANKL, OPG can also bind to TRAIL [7]. A recent study has shown that the affinity of native full-length OPG is approximately only twofold lower for TRAIL than for RANKL [8]. Several studies have suggested that TRAIL is an inducer of apoptosis in mature osteoclasts and functions as a negative modulator of osteoclastic differentiation [9, 10, 11, 12]. In addition, it has been demonstrated that, *in vitro,* endogenously expressed and released TRAIL by end-stage osteoclasts promote osteoclastic apoptosis in an autocrine/paracrine manner [11]. TRAIL mediated apoptosis was not observed in preosteoclasts, probably due to the overexpression of the TRAIL decoy receptor TRAIL-R4 [12].

Further investigations are required to clearly demonstrate the exact role of TRAIL in osteoclastogenesis and maybe to uncover a potential role of TRAIL or TRAIL receptors alternations in bone-related diseases [13].

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[1] Pathophysiological roles of osteoprotegerin (OPG): P. Reid & I. Holen; Eur. J. Cell Biol. **88,** 1 (2008) (Review)

[2] Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation: D. L. Lacey, et al.; Cell **93,** 165 (1998)

[3] Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL, Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL: H. Yasuda, et al.; PNAS **95,** 3597 (1998)

[4] A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function: D. M. Anderson, et al.; Nature **390,** 175 (1997)

[5] TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells: B. R. Wong, et al.; J. Biol. Chem. **272,** 25190 (1997)

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[7] Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL: J. G. Emery, et al.; J. Biol. Chem. **273,** 14363 (1998)

[8] Investigating the interaction between osteoprotegerin and receptor activator of NF-kappaB or tumor necrosis factor-related apoptosis-inducing ligand: evidence for a pivotal role for osteoprotegerin in regulating two distinct pathways: S. Vitovski, et al.; J. Biol. Chem. **282,** 31601 (2007)

[9] TNF-related apoptosis-inducing ligand (TRAIL) blocks osteoclastic differentiation induced by RANKL plus M-CSF: G. Zauli, et al.; Blood **104,** 2044 (2004)

[10] TRAIL inhibits osteoclastic differentiation by counteracting RANKL-dependent p27Kip1 accumulation in pre-osteoclast precursors: G. Zauli, et al.; J. Cell. Physiol. **214,** 117 (2008)

[11] Osteoprotegerin decreases human osteoclast apoptosis by inhibiting the TRAIL pathway: E. Chamoux, et al.; J. Cell. Physiol. **216,** 536 (2008)

[12] The death receptor DR5 is involved in TRAIL-mediated human osteoclast apoptosis: S. Colucci, et al.; Apoptosis **12,** 1623 (2007)

[13] Role of full-length osteoprotegerin in tumor cell biology: G. Zauli, et al.; Cell. Mol. Life Sci. **66,** 841 (2009) (Review)

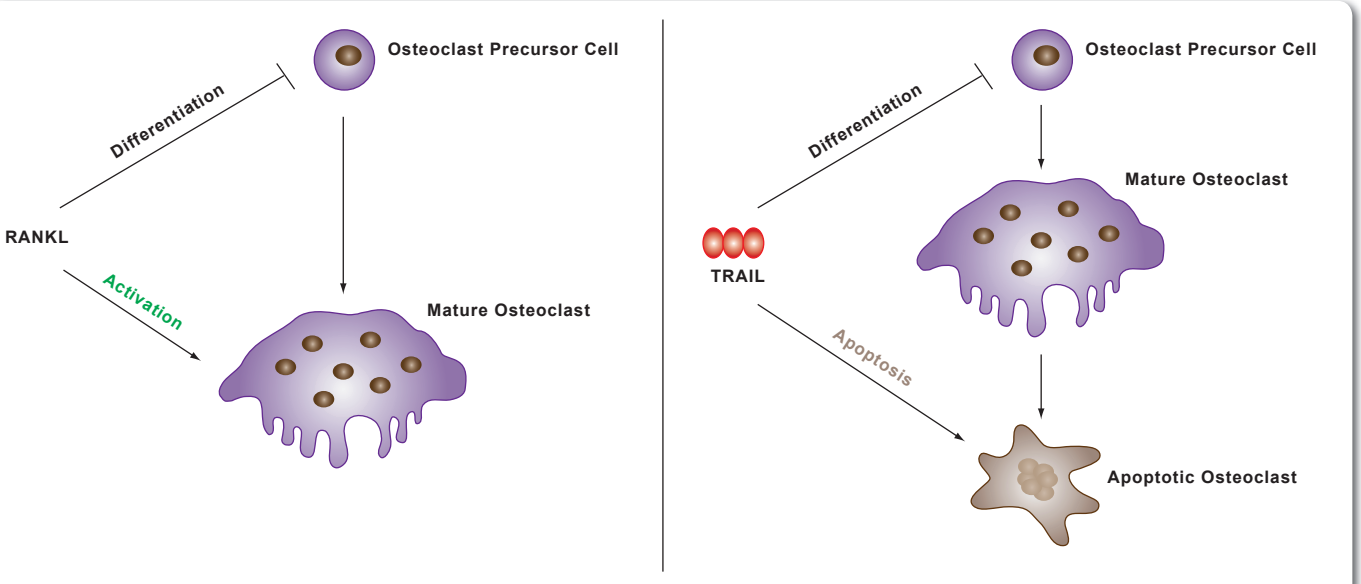


FIGURE 3: Schematic overview on effects of RANKL versus TRAIL in bone metabolism.

RANKL plays a key role in osteoclastogenesis by promoting the differentiation of osteoclast precursors into mature multinucleated osteoclasts and the bone resorption activity of mature osteoclasts. On the other hand, TRAIL is suggested to function as a negative modulator of osteoclastic differentiation and inducer of apoptosis in mature osteoclasts.

Adapted from: Role of full-length osteoprotegerin in tumor cell biology: G. Zauli, et al.; Cell. Mol. Life Sci. ***66,*** *841 (2009) (Review)*

Osteoprotegerin [OPG; TNFRSF 11B]

Osteoprotegerin (human):Fc (human), (rec.)

ALX-522-007-C050 50 µg

Produced in HEK 293 cells. The cysteine-rich region of human OPG (osteoprotegerin) (aa 22-202) is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds human and mouse RANKL and TRAIL. **BIOLOGICAL ACTIVITY:** Inhibits rhsTRAIL-mediated apoptosis at a concentration range of 5-20µg/ml, as well as rhsRANKL-induced osteoclastogenesis and stimulation of dendritic cells. Concentrations of rhOPG:Fc required to inhibit may vary depending on the cell type studied and on the concentration of rhsTRAIL used to kill the cells.

LIT: Receptor activator of NF-κB and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis: P. Collin-Osdoby, et al.; J. Biol. Chem. **276,** 20659 (2001) ▪ A CD40-CD95L fusion protein interferes with CD40L-induced prosurvival signaling and allows membrane CD40L-restricted activation of CD95: C. Assohou-Luty, et al.; J. Mol. Med. **84,** 785 (2006)

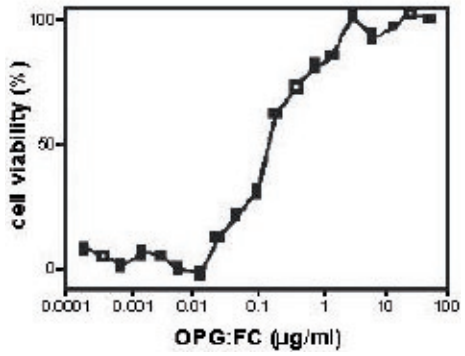


FIGURE: Inhibition of TRAIL (Prod. No. ALX-850-018) mediated apoptosis: Osteoprotegerin (human):Fc (human) (rec.) (Prod. No. ALX-522-007) fusion protein exerts its inhibitory activity in a concentration range of 5-20µg/ml.

Osteoprotegerin (human), mAb (Bony-1)

ALX-804-813-C100 100 µg

ALX-804-813B-C100 Biotin 100 µg

CLONE: Bony-1. **ISOTYPE:** Rat IgG2a. **IMMUNOGEN:** Recombinant human OPG (osteoprotegerin) cysteine-rich region (aa 22-202) (Prod. No. ALX-522-007). **SPECIFICITY:** Recognizes human OPG. **APPLICATION:** ELISA, FC, IP.

Osteoprotegerin (human) (NT), mAb (OPG-13)

ALX-804-207-C100 100 µg

CLONE: OPG-13. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Synthetic peptide corresponding to N-terminal aa 21-39 of human OPG (osteoprotegerin). **SPECIFICITY:** Recognizes human OPG. **APPLICATION:** ELISA, WB.

Osteoprotegerin (human), mAb (OPG-01)

ALX-804-532-C100 100 µg

CLONE: OPG-01. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human OPG (osteoprotegerin). **SPECIFICITY:** Recognizes human OPG. **APPLICATION:** ELISA, WB.

Osteoprotegerin, mAb (98A1071)

ADI-AAM-020-E 100 µg

CLONE: 98A1071. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Synthetic peptide derived from sequence near the amino-terrminus of human OPG. **SPECIFICITY:** Recognizes human OPG. **APPLICATION:** IHC, WB.

Osteoprotegerin (human), pAb (AT121)

ALX-210-945-C100 100 µg

ALX-210-945B-C100 Biotin 100 µg

From rabbit. **IMMUNOGEN:** Recombinant human OPG (osteoprotegerin) cysteine-rich region (aa 22-202) (Prod. No. ALX-522-007). **SPECIFICITY:** Recognizes human OPG. **APPLICATION:** ELISA, IP, WB.

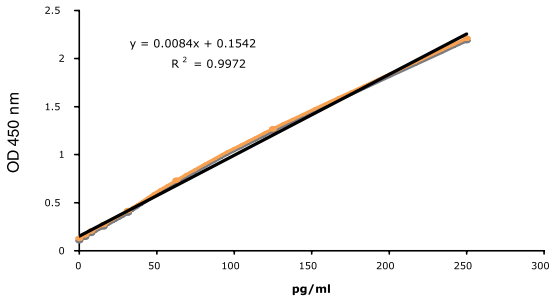
Osteoprotegerin ELISA Kits

Osteoprotegerin (human) ELISA kit

ALX-850-280-KI01 1 Kit

For the quantitative determination of human OPG (osteoprotegerin) in serum, plasma and urine. **QUANTITY:** 96 wells (~80 tests). **SENSITIVITY:** 2.8pg/ml.

LIT: An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction: P. Secchiero, et al.; Am. J. Pathol. **169,** 2236 (2006) ▪ Osteoprotegerin increases leukocyte adhesion to endothelial cells both in vitro and in vivo: G. Zauli, et al.; Blood **110,** 536 (2007) ▪ Activation of the p53 pathway down-regulates the osteoprotegerin (OPG) expression and release by vascular endothelial cells: P. Secchiero, et al.; Blood **111,** 1287 (2008)



Free Osteoprotegerin (human) detection set [for ELISA application]

APO-54N-028-KI01 1 Set

For the quantitative determination of human free osteoprotegerin (not bound to RANKL or TRAIL) in cell culture supernatant, plasma and serum. **QUANTITY:** 5 x 96 wells. **SENSITIVITY:** 2pg/ml (range 0 to 250 pg/ml).

Free Osteoprotegerin (human) ELISA kit

APO-54N-042-KI01 1 Kit

For the quantitative determination of free human OPG (not complexed to RANKL or TRAIL) in biological fluids (serum, plasma and cell culture supernatant). **QUANTITY:** For 96 wells (~80 tests). **SENSITIVITY:** 20pg/ml (range 0.031 to 2ng/ml). **APPLICATION:** ELISA.

RANKL

RANKL (soluble) (human), (rec.)

ALX-522-012-C010 10 µg

Produced in HEK 293 cells. The extracellular domain of human RANKL (aa 152-317) is fused at the N-terminus to a linker peptide (6 aa) and a FLAG®-tag. **SPECIFICITY:** Binds to human and mouse RANK. **BIOLOGICAL ACTIVITY:** Supports the survival of dendritic cells and osteoclasts.

LIT: Receptor activator of NF-κB and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis: P. Collin-Osdoby, et al.; J. Biol. Chem. **276,** 20659 (2001) ▪ Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines: P. Fiumara, et al.; Blood **98,** 2784 (2001) ▪ Suppression of IL-12 Production by Soluble CD40 Ligand: Evidence for Involvement of the p44/42 Mitogen-Activated Protein Kinase Pathway: M. Wittmann, et al.; J. Immunol. **168,** 3793 (2002) ▪ Induction of Cell Cycle Arrest and Apoptosis by the Proteasome Inhibitor PS-341 in Hodgkin Disease Cell Lines Is Independent of Inhibitor of Nuclear Factor-kappaB Mutations or Activation of the CD30, CD40, and RANK Receptors: B. Zheng, et al.; Clin. Cancer Res. **10,** 3207 (2004) ▪ A novel in vivo role for osteoprotegerin ligand in activation of monocyte effector function and inflammatory response: D. Seshasayee, et al.; J. Biol. Chem. **279,** 30202 (2004)

Fc (human):RANKL (soluble) (mouse), (rec.)

ALX-522-131-C010 10 µg

Produced in HEK 293 cells. The extracellular domain of mouse RANKL (aa 157-316) is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds to mouse RANK.

RANKL, mAb (12A380)

ALX-804-244-C100 100 µg

CLONE: 12A380. **ISOTYPE:** Mouse IgG1. IMMUNOGEN: Recombinant mouse RANKL (aa 1-317). **SPECIFICITY:** Recognizes human and mouse RANKL. Detects a band of ~40kDa by Western blot. **APPLICATION:** FC, IHC (FS), WB.

LIT: Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines: P. Fiumara, et al.; Blood **98,** 2784 (2001) ▪ Estrogen deficiency accelerates murine autoimmune arthritis associated with receptor activator of nuclear factor-kappa B ligand-mediated osteoclastogenesis: T. Yoneda, et al.; Endocrinology **145,** 2384 (2004) ▪ Expression of bone-regulatory proteins in human valve allografts: R. Shetty, et al.; Heart **92,** 1303 (2006)

RANKL, mAb (12A668)

ALX-804-243-C100 100 µg

CLONE: 12A668. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant mouse RANKL (aa 1-317). **SPECIFICITY:** Recognizes human and mouse RANKL. Detects a band of ~35-40kDa by Western blot. **APPLICATION:** IHC (PS), WB.

LIT: Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression: R.N. Pearse, et al.; PNAS **98,** 11581 (2001) ▪ Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines: P. Fiumara, et al.; Blood **98,** 2784 (2001) ▪ Gene expression of osteoclast differentiation factor is induced by lipopolysaccharide in mouse osteoblasts via Toll-like receptors: T. Kikuchi, et al.; J. Immunol. **166,** 3574 (2001) ▪ Gamma-glutamyltranspeptidase stimulates receptor activator of nuclear factor-kappaB ligand expression independent of its enzymatic activity and serves as a pathological bone-resorbing factor: S. Niida, et al.; J. Biol. Chem. **279,** 5752 (2004)

RANKL (human), mAb (Ranky-1)

ALX-804-830-C100 100 µg

CLONE: Ranky-1. **ISOTYPE:** Rat IgG1. **SOURCE/HOST:** Purified from concentrated hybridoma tissue culture supernatant. **IMMUNOGEN:** Recombinant human soluble RANKL extracellular domain (aa 151-316) (Prod. No. ALX-522-012). **SPECIFICITY:** Recognizes human RANKL. **APPLICATION:** ELISA, IHC (PS), WB.

RANKL (human), pAb

ALX-210-396-C100 100 µg

From rabbit. **IMMUNOGEN:** Recombinant human RANKL. **SPECIFICITY:** Recognizes human RANKL. Detects a band of ~35kDa band by Western blot. **APPLICATION:** ELISA, FC, WB.

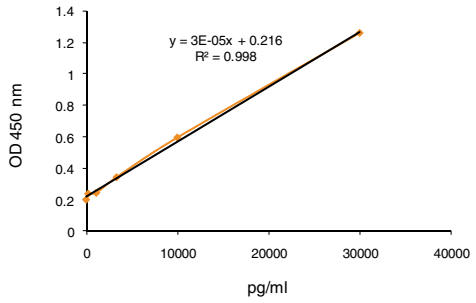
ELISA Kit

totalRANKL (soluble) (human), ELISA kit

APO-54N-016/1-KI01 1 Kit

For the quantitative determination of free and OPG-bound human soluble RANKL from serum and plasma. Not recommended for cell culture supernatant. **SENSITIVITY:** ~1.5pg/ml (range 0 to 3,600pg/ml). **QUANTITY:** For 96 wells (~80 tests).

LIT: Effects of oral contraceptives on circulating osteoprotegerin and soluble RANK ligand serum levels in healthy young women: L.C. Hofbauert, et al.; Clin. Endocrinol. **80,** 214 (2004) ▪ An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction: P. Secchiero, et al.; Am. J. Pathol. **169,** 2236 (2006) ▪ Osteoclasts are active in bone forming metastases of prostate cancer patients: I. Roato, et al.; PLoS ONE **3,** e3627 (2008)



Technical Note

Why measure total RANKL instead of free RANKL?

- Total RANKL corresponds to free RANKL + RANKL complexed to its decoy receptor osteoprotegerin (OPG).
- RANKL complexed to OPG represents almost all RANKL in serum (>99%).
- Free RANKL in serum is present at low concentrations making routine detection difficult (picogram range).
- Determining total RANKL gives values in the nanogram range, 1,000 fold greater than free RANKL alone, making detection easier.
- Measuring total RANKL in serum correlates to free RANKL levels.

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