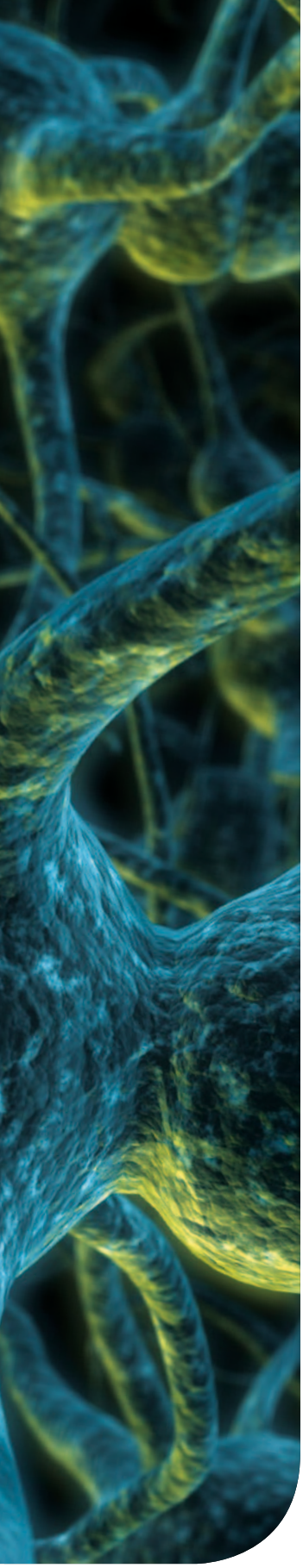

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TRAIL & TRAIL Receptors

Effectors of Apoptotic Signaling

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].

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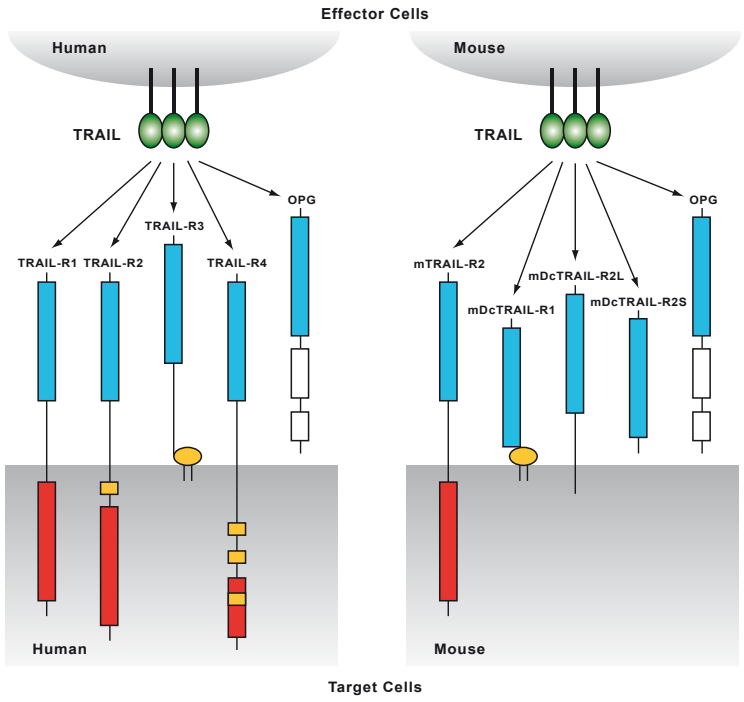

FIGURE 1: Overview on human and mouse TRAIL receptors.
Adapted from: TRAIL and its receptors as targets for cancer therapy: H. Yagita, et al.; Cancer Sci. **95,** 777 (2004) (Review)

What's Inside

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TRAIL Receptors: Effectors of Apoptosis 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.

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 need 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].

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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TRAIL receptor signaling and therapeutics: J. Abdulghani & W.S. El-Deiry; Expert Opin. Ther. Targets **14,** 1091 (2010) (Review)
New insights into apoptosis signaling by Apo2L/TRAIL: F. Gonzalvez & A. Ashkenazi; Oncogene **29,** 4752 (2010) (Review)

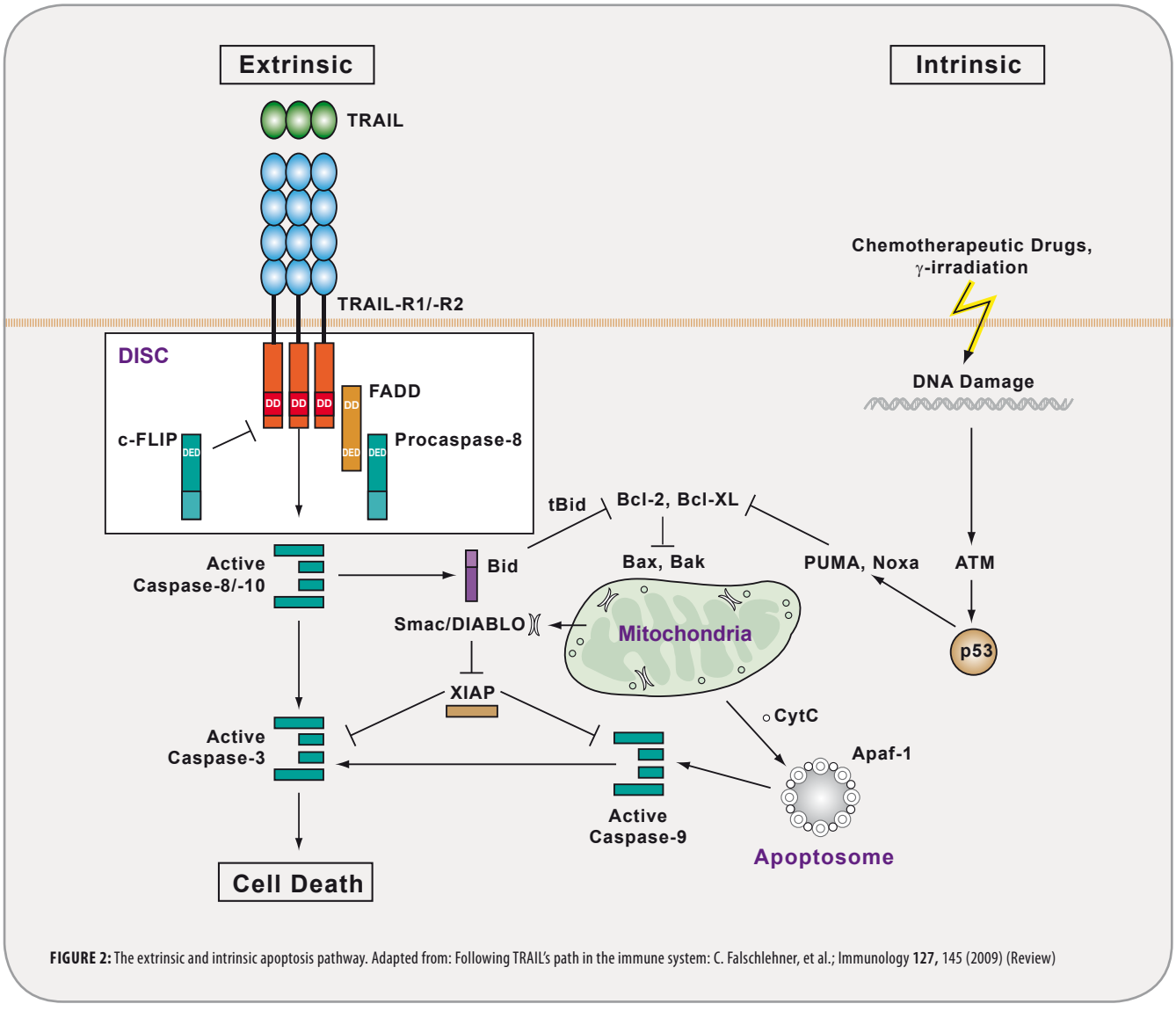

FIGURE 2: The extrinsic and intrinsic apoptosis pathway. Adapted from: Following TRAIL's path in the immune system: C. Falschlehner, et al.; Immunology **127,** 145 (2009) (Review)

TRAIL & TRAIL Receptors: 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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TNF superfamily: a growing saga of kidney injury modulators: M.D. Sanchez-Niño; Mediators Inflamm. **2010:182958** (2010) (Review)

Exploring death receptor pathways as selective targets in cancer therapy: M. Russo; Biochem. Pharmacol. **80,** 674 (2010) (Review)

Decoy receptor 3: a pleiotropic immunomodulator and biomarker for inflammatory diseases, autoimmune diseases and cancer: W.W. Lin & S.L.Hsieh; Biochem Pharmacol. **81,** 838 (2011) (Review)

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

TRAIL Receptors & Ligands

TRAIL, Soluble (human) (rec.)

AG-40B-0003-C010		10 µg
AG-40B-0003-5010	MultiPack	5 x 10 µg
AG-40B-0003AA-C500	BULK	500 µg

Produced in *E. coli*. Human TRAIL (aa 95-281) fused at the N-terminus to a FLAG-tag. **SPECIFICITY:** Binds to human and mouse TRAIL receptors and human osteoprotegerin (OPG).

*Enhanced*TRAIL, Soluble (human) (rec.) Pack

AG-44B-0002-KI01	1 Set

BIOLOGICAL ACTIVITY: Induces apoptosis in a concentration range of 5-50 ng/ ml if applied with the cross-linking enhancer. **SET CONTAINS:** 2 x 10 µg of TRAIL, Soluble (human) (rec.) (Prod. No. AG-40B-0003) 3 x 50 µg of TNF Ligands Enhancer (Prod. No. AG-35B-0001).

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) • 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) • 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)

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

AG-40T-0001-C050		50 µg
AG-40T-0001-3050	MultiPack	3 x 50 µg
AG-40T-0001-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. AG-40T-0002).

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) • Chronic lymphocytic leukemic cells exhibit apoptotic signaling via TRAIL-R1: M. MacFarlane, et al.; Cell Death Differ. **12,** 773 (2005)

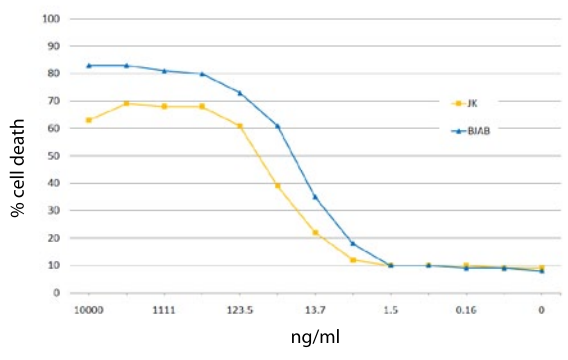

FIGURE: Apoptosis of TRAIL-sensitive cells. Concentration dependence of apoptosis induction in Jurkat and BJAB cells by KillerTRAIL™, Soluble (human) (rec.) (Prod. No. AG-40T-0001) reveals high activity even at concentrations of 10-100ng/ml.

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

AG-40T-0002-C020		20 µg
AG-40T-0002-3020	MultiPack	3 x 20 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: 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)

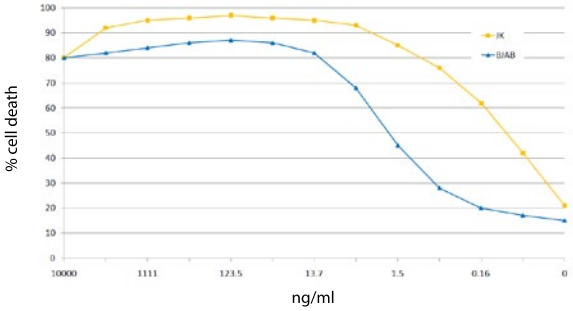

FIGURE: Apoptosis of TRAIL-sensitive cells. Apoptosis induction in BJAB cells by Super*Killer*TRAIL™, Soluble (human) (rec.) (Prod. No. AG-40T-0002) reveals killing activity at concentrations of 10-15ng/ml, on Jurkat cells even as low as 1-10ng/ml.

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

AG-40T-0004-C020		20 µg
AG-40T-0004-3020	MultiPack	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.

NOTE: Does not require a cross-linking enhancer for its potent biological activity.

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

AG-40B-0070-C050		50 µg
AG-40B-0070-3050	MultiPack	3 x 50 µg

Produced in HEK 293 cells. The extracellular domain of human TRAIL-R1 (aa 24-239) is fused to the Fc portion of human IgG1. **SPECIFICITY:** Binds human and mouse TRAIL.

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

AG-40B-0071-C050		50 µg
AG-40B-0071-3050	MultiPack	3 x 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.

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)

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].

Next to tailored recombinant human TRAIL proteins, which show either enhanced anti-tumor potency or selectivity against certain TRAIL-Rs [10], 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 [11–15]. 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* [16, 17]. 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 [18–20]. 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* [21].

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 [18, 22, 23] .

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: [1] Clearing the TRAIL for Cancer Therapy: M. A. Hall & J. L. Cleveland; Cancer Cell **12,** 4 (2007) (Review) • **[2]** Safety and antitumor activity of recombinant soluble Apo2 ligand: A. Ashkenazi, et al.; J. Clin. Invest. **104,** 155 (1999) • **[3]** Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo: H. Walczak, et al.; Nat. Med. **5,** 157 (1999) • **[4]** On the TRAIL toward death receptor-based cancer therapeutics: T. F. Gajewski; J. Clin. Oncol. **25,** 1305 (2007) • **[5]** Multiple mechanisms underlie resistance of leukemia cells to Apo2 Ligand/TRAIL: J. Cheng, et al.; Mol. Cancer Ther. **5,** 1844 (2006) • **[6]** Effect of NF-kappaB, survivin, Bcl-2 and Caspase3 on apoptosis of gastric cancer cells induced by tumor necrosis factor related apoptosis inducing ligand: L. Q. Yang, et al.; World J. Gastroenterol. **10,** 22 (2004) • **[7]** Downregulation of Bcl-2, FLIP or IAPs (XIAP and survivin) by siRNAs sensitizes resistant melanoma cells to Apo2L/TRAIL-induced apoptosis: M. Chawla-Sarkar, et al.; Cell Death Differ. **11,** 915 (2004) • **[8]** The promise of TRAIL--potential and risks of a novel anticancer therapy: R. Koschny, et al.; J. Mol. Med. **85,** 923 (2007) (Review) • **[9]** TRAIL and cancer therapy: F. A. Kruyt; Cancer Lett. **263,** 14 (2008) (Review) • **[10]** Death ligands designed to kill: development and application of targeted cancer therapeutics based on proapoptotic TNF family ligands: J. Gerspach, et al.; Results Probl. Cell Differ. **49,** 241 (2009) • **[11]** DR4-selective tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) variants obtained by structure-based design: V. Tur, et al.; J. Biol. Chem. **283,** 20560 (2008) • **[12]** TRAIL signals to apoptosis in chronic lymphocytic leukaemia cells primarily through TRAIL-R1 whereas cross-linked agonistic TRAIL-R2 antibodies facilitate signalling via TRAIL-R2: A. Natoni, et al.; Br. J. Haematol. **139,** 568 (2007) • **[13]** Designed tumor necrosis factor-related apoptosis-inducing ligand variants initiating apoptosis exclusively via the DR5 receptor: A. M. van der Sloot, et al.; PNAS **103,** 8634 (2006) • **[14]** TRAIL receptor-selective mutants signal to apoptosis via TRAIL-R1 in primary lymphoid malignancies: M. MacFarlane, et al.; Cancer Res. **65,** 11265 (2005) • **[15]** Receptor-selective mutants of apoptosis-inducing ligand 2/tumor necrosis factor-related apoptosis-inducing ligand reveal a greater contribution of death receptor (DR) 5 than DR4 to apoptosis signaling: R. F. Kelley, et al.; J. Biol. Chem. **280,** 2205 (2005) • **[16]** Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions: D. Lawrence, et al.; Nat. Med. **7,** 383 (2001) • **[17]** Is TRAIL hepatotoxic?: G. J. Gores & S. H. Kaufmann; Hepatology **34,** 3 (2001) (Review) • **[18]** Death receptors as targets for anti-cancer therapy: K. Papenfuss, et al.; J. Cell. Mol. Med. **12,** 2566 (2008) (Review) • **[19]** The promise of TRAIL - potential and risks of a novel anticancer therapy: R. Koschny, et al.; J. Mol. Med. **85,** 923 (2007) (Review) • **[20]** 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) • **[21]** Increased hepatotoxicity of tumor necrosis factor-related apoptosis-inducing ligand in diseased human liver: X. Volkmann, et al.; Hepatology **46,** 1498 (2007) • **[22]** TRAIL receptor signalling and modulation: Are we on the right TRAIL?: D. Mahalingam, et al.; Cancer Treat. Rev. **35,** 280 (2009) (Review) • **[23]** Death receptors: Targets for cancer therapy: Z. Mahmood & Y. Shukla; Exp. Cell Res. **316,** 887 (2010)

New TRAIL Ligands

*iz*TRAIL is a newly available, 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, ***iz*TRAIL is a potent inducer of apoptosis in many human cancer cells, but not normal human hepatocytes.** In addition, the half-life of *iz*TRAIL is about eight-fold higher than the half-life of shTRAIL. These properties render *iz*TRAIL 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.

new *iz*TRAIL, Soluble (human) (rec.)

AG-40B-0069-C010 10 µg
AG-40B-0069-5010 MultiPack 5 x 10 µg
For BULK please Inquire!

Produced in *E. coli*. Human TRAIL (aa 95-281) fused at the N-terminus to a isoleucine zipper motif. **SPECIFICITY:** Binds to human TRAIL receptors 1-4; does not interact with mouse TRAIL-R. **BIOLOGICAL ACTIVITY:** Induces apoptosis *in vitro* in different human cancer cell lines with an EC_{50} of 5-200 ng/ml, depending on the individual cell line used. Recombinant *iz*TRAIL does not kill 4-day cultures of primary human hepatocytes (PHH) at concentrations of at least up to 1µg/ml (Ganten 2006). Good bioavailability *in vivo*, shows no toxic effects in mice at doses of at least up to 500µg per day (Wissink 2006).

LIT: Preclinical differentiation between apparently safe and potentially hepatotoxic applications of TRAIL either alone or in combination with chemotherapeutic drugs: Ganten, T.M., et al., Clin. Cancer Res. **12,** 2640 (2006) • TRAIL enhances efficacy of radiotherapy in a p53 mutant, Bcl-2 overexpressing lymphoid malignancy: Wissink, E.H., et al., Radiother. Oncol. **80,** 214 (2006) • Bortezomib sensitizes primary human astrocytoma cells of WHO grades I to IV for tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis: Koschny, R., et al., Clin. Cancer Res. **13,** 3403 (2007) • TRAIL/bortezomib cotreatment is potentially hepatotoxic but induces cancer-specific apoptosis within a therapeutic window: Koschny, R., et al., Hepatology **45,** 649 (2007)

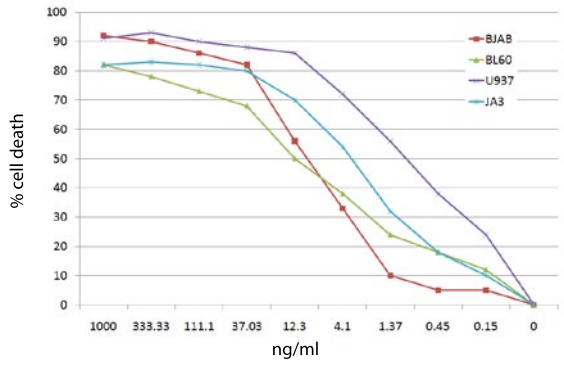

FIGURE: Apoptosis of TRAIL-sensitive tumor cells. Concentration dependence of apoptosis induction in BJAB cells, BL60-cells, U937-cells, and JA3-cells by *iz*TRAIL, Soluble (human) (rec.) (Prod. No. AG-40B-0069) reveals high activity even at concentrations of 10-100ng/ml.

new *Killer*TRAIL™ (R1 specific), Soluble (human) (rec.)

AG-40T-0003-C050 50 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 and contains aa substitutions, which generate a binding specificity towards TRAIL receptor 1 (TRAIL-R1; DR4). **SPECIFICITY:** Binds to human TRAIL-R1 (DR4), but not human TRAIL-R2 (DR5). Binding to TRAIL receptors -R3, -R4 and osteoprotegerin (OPG) not tested. **BIOLOGICAL ACTIVITY:** Induces apoptosis in a concentration range of 20-500ng/ml of TRAIL-R1 (DR4) expressing cell lines (e.g. RAMOS), whereas it is inefficient to kill via TRAIL-R2 (DR5) such as Jurkat.

NOTE: Does not require a cross-linking enhancer for its potent biological activity.

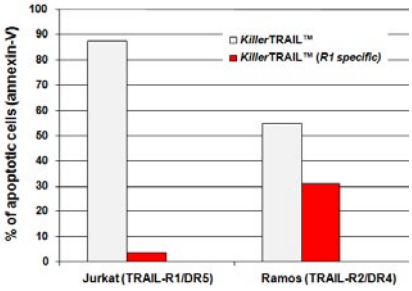

FIGURE: Treatment of Jurkat or RAMOS cells with 200ng/ml *Killer*TRAIL™ (R1 specific) (Prod. No. AG-40T-0003) and *Killer*TRAIL ™, Soluble (human) (rec.) (Prod. No. AG-40T-0001) for 3h at 37°C.

TRAIL Ligands Overview

LIGAND	TRAIL-R SELECTIVITY	POTENCY	ENDOTOXIN CONTENT	BEST CHOICE FOR	MODIFICATION
Enhanced TRAIL Pack	TRAIL-R1, -R2 (DR4/DR5) (incl. Jurkat, U937, K562 cells).	++ ED_{50} 5ng/ml	+ <0.1EU/µg	Highest killing potency on all TRAIL-sensitive cells	FLAG-tagged (trimeric, with suspected liver cell toxicity)
*Killer*TRAIL™	TRAIL-R1 (DR4) (e.g. BJAB, Ramos, but less active on Jurkat, U937, K562)	++ ED_{50} 20ng/ml	+ <0.01EU/µg	Standard killer agent for *in vitro* (assays) if via TRAIL-R1 (DR4)	His-tagged (monomeric)
Super*Killer*-TRAIL™	TRAIL-R1, -R2 (DR4/DR5) (incl. Jurkat, U937, K562 cells).	+++ ED_{50} 0.1ng/ml	+ <0.02EU/µg	Highest killing potency on all TRAIL-sensitive cells	His-tagged (trimeric, with suspected liver cell toxicity)
*Killer*TRAIL™ (R1 specific)	TRAIL-R1 (DR4) (e.g. BJAB, Ramos, but no activity on Jurkat, U937, K562)	++ ED_{50} 20ng/ml	+ <0.01EU/µg	Highest killing potency on TRAIL-R1-sensitive cells	His-tagged (trimeric, with suspected liver cell toxicity)
*iz*TRAIL	TRAIL-R1-4; does not interact with mouse TRAIL-R.	++ ED_{50} 5ng/ml	+ <0.1EU/µg	All TRAIL-sensitive cells. Suitable for *in vitro* and *in vivo* use, particularly to transfer *in vitro* results into an *in vivo* system with human cancer cells in xenotransplant settings.	Isoleucine zipper motif (trimeric, with no liver cell toxicity)

TRAIL & TRAIL Receptors: Effectors 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].

SELECTED REVIEW ARTICLE

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

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)

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

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[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)

Latest Insight

PEDF promotes tumor cell death through TRAIL

The research findings of T.C. Ho, et al. identified a new interaction between TRAIL and PEDF. Their results suggest a novel mechanism for the tumoricidal activity of PEDF, which involves tumor cell killing via PPARγ-mediated TRAIL induction in macrophages.

LIT: PEDF promotes tumor cell death by inducing macrophage membrane TRAIL: T.C. Ho, et al.; J. Biol. Chem. (**Epub ahead of print**) 2011

PEDF (human) (rec.)

AG-40B-0077-C010		10 µg
AG-40B-0077-3010	MultiPack	3 x 10 µg

Produced in CHO cells. Human PEDF (aa 20-418) is fused at the C-terminus of to a FLAG®-tag.

Latest Insight

HIV-induced cell death

Virus-host interactions are characterized by the selection of adaptive mechanisms to evade pathogenic and defense mechanisms, respectively. D.J. Schnepple, et al. have shown that in primary T cells infected with HIV, HIV infection upregulates TNF-Related Apoptosis Inducing Ligand (TRAIL) and death-inducing TRAIL receptors, but blockade of TRAIL/TRAIL receptor interaction does not alter HIV-induced cell death. Instead, HIV infection results in a novel splice variant that they call TRAIL-short (TRAIL-s), which antagonizes TRAIL-R2. In HIV patients, plasma TRAIL-s concentration increases with increasing viral load and renders cells resistant to TRAIL-induced death. They propose that TRAIL-s is a novel adaptive mechanism of apoptosis resistance acquired by HIV infected cells to avoid their elimination by TRAIL-dependent effector mechanisms. Knowledge of these novel responses may allow new strategies aimed at interrupting elements of HIV pathogenesis.

LIT: Isolation of a TRAIL antagonist from the serum of HIV infected patients: D.J. Schnepple, et al.; J. Biol. Chem. (**Epub ahead of print**) 2011

Role of TRAIL & Osteoprotegerin (OPG) 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 soluble 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 two-fold 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].

LITERATURE REFERENCES:

[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)

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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)

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[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)

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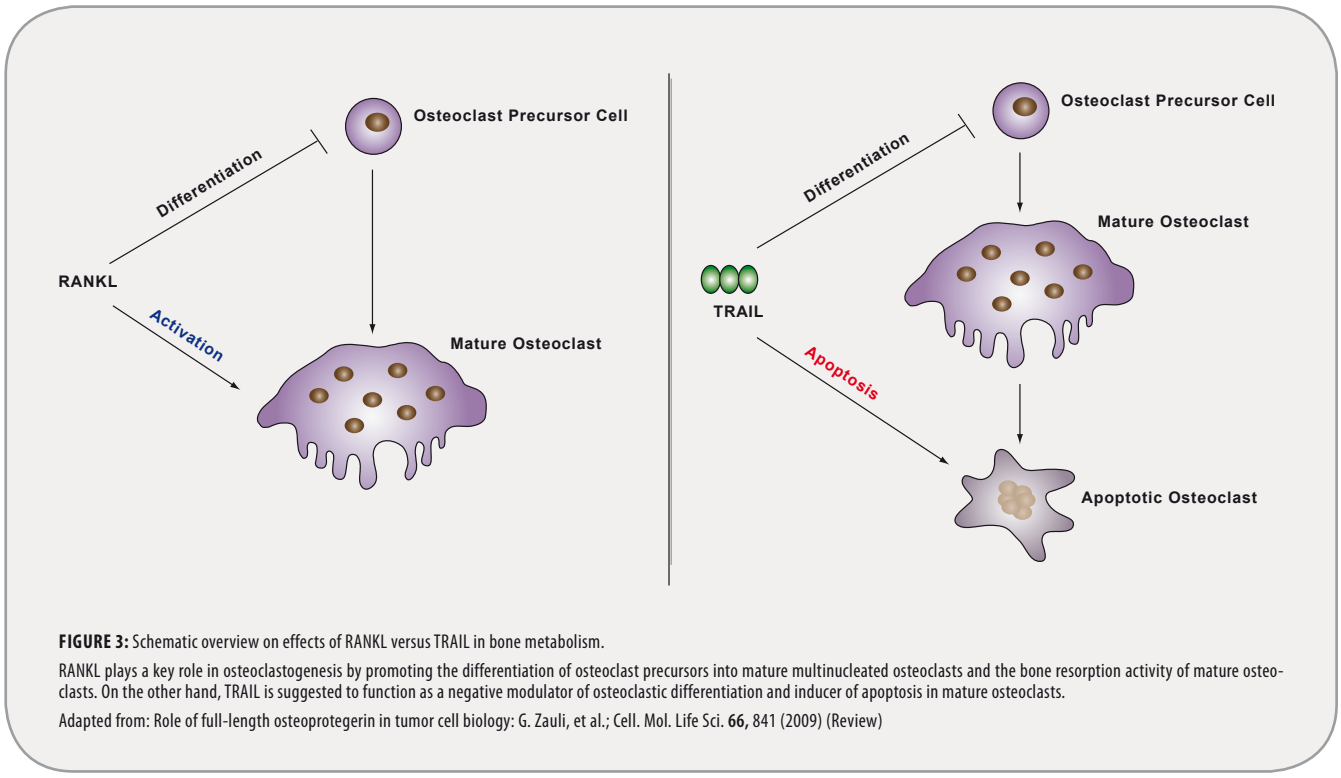

FIGURE 3: Schematic overview on effects of RANKL versus TRAIL in bone metabolism.

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)

Monoclonal Antibodies to TRAIL & TRAIL Receptors

anti-TRAIL-R1 (human), mAb (HS101)

AG-20B-0022-C100	Purified	100 µg
AG-20B-0022PF-C100	PF	100 µg
AG-20B-0022F-T100	FITC	100 tests
AG-20B-0022TD-T100	ATTO 488	100 tests
AG-20B-0022TS-T100	ATTO 647N	100 tests

CLONE: HS101. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human TRAIL-R1. **SPECIFICITY:** Recognizes human TRAIL-R1. Does not cross-react with human TRAIL-R2, -R3 or-R4. **APPLICATION:** FACS, ICC, IP, FUNC (blocking).

anti-TRAIL-R2 (human), mAb (HS201)

AG-20B-0023-C100	Purified	100 µg
AG-20B-0023PF-C100	PF	100 µg
AG-20B-0023F-T100	FITC	100 tests
AG-20B-0023TD-T100	ATTO 488	100 tests
AG-20B-0023TS-T100	ATTO 647N	100 tests

CLONE: HS201. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human TRAIL-R2. **SPECIFICITY:** Recognizes human TRAIL-R2. Does not cross-react with human TRAIL-R1, -R3 and -R4. **APPLICATION:** FACS, ICC, IP, FUNC (blocking).

anti-TRAIL-R3 (human), mAb (HS301)

AG-20B-0024-C100 100 µg

CLONE: HS301. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human TRAIL-R3. **SPECIFICITY:** Recognizes human TRAIL-R3. Does not cross-react with human TRAIL-R1, -R2 and -R4. **APPLICATION:** FACS, ICC.

anti-TRAIL-R4 (human), mAb (HS402)

AG-20B-0025-C100 100 µg

CLONE: HS402. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human TRAIL-R4. **SPECIFICITY:** Recognizes human TRAIL-R4. Does not cross-react with human TRAIL-R1, -R2 and -R3. **APPLICATION:** FACS, IHC (FS), ICC, IP.

TRAIL-R1 to -R4 Flow Cytometry Pack

AG-44B-0004-KI01 1 Set

ISOTYPE: Mouse IgG1. **SPECIFICITY:** Recognizes human TRAIL-R1 to -R4. **APPLICATION:** FACS.

LITERATURE REFERENCES: TNF-Related Apoptosis-Inducing Ligand Mediates Tumoricidal Activity of Human Monocytes Stimulated by Newcastle Disease Virus: B. Washburn, et al.; J. Immunol. **170,** 1814 (2003) • 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)

FACS Analysis

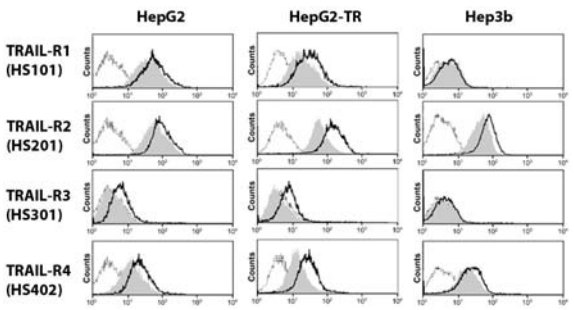

FIGURE: FACS analysis of surface expression of TRAIL-R1 to TRAIL-R4 with (solid bold line) and without (filled line) 5-FU (100µg/ml) treatment for 16h, compared to control (dashed line) using TRAIL-R1 mAb (HS101) (Prod. No. AG-20B-0022), TRAIL-R2 mAb (HS201) (Prod. No. AG-20B-0023), TRAIL-R3 mAb (HS301)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

anti-TRAIL (human), mAb (HS501)

AG-20B-0026-C100 100 µg

CLONE: HS501. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human soluble TRAIL. **SPECIFICITY:** Recognizes human TRAIL. **APPLICATION:** WB (excellent).

LIT: TNF-Related Apoptosis-Inducing Ligand Mediates Tumoricidal Activity of Human Monocytes Stimulated by Newcastle Disease Virus: B. Washburn, et al.; J. Immunol. **170,** 1814 (2003)

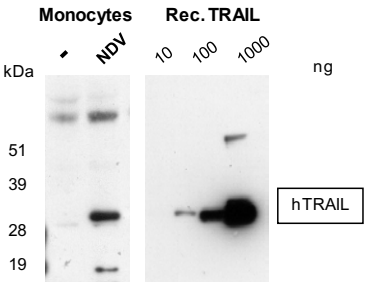

FIGURE: Western blots of human primary monocytes, either control treated or treated with NDV and recombinant TRAIL were perfomed using anti-TRAIL, mAb (HS501) (Prod. No. AG-20B-0026).

PRODUCT	SOURCE / HOST / ISOTYPE	SPECIFICITY	APPLICATION	PROD. NO.	FORMAT	SIZE
TRAIL (human), mAb (HS501)	Mouse IgG1	Human	WB (excellent)	AG-20B-0026-C100	Purified	100 µg
TRAIL-R1 (human), mAb (HS101)	Mouse IgG1	Human	FACS, ICC, IP, FUNC	AG-20B-0022-C100 AG-20B-0022PF-C100 AG-20B-0022F-T100 AG-20B-0022TD-T100 AG-20B-0022TS-T100	Purified PF FITC ATTO 488 ATTO 647N	100 µg 100 µg 100 tests 100 tests 100 tests
TRAIL-R2 (human), mAb (HS201)	Mouse IgG1	Human	FACS, ICC, IP, FUNC	AG-20B-0023-C100 AG-20B-0023PF-C100 AG-20B-0023F-T100 AG-20B-0023TD-T100 AG-20B-0023TS-T100	Purified PF FITC ATTO 488 ATTO 647N	100 µg 100 µg 100 tests 100 tests 100 tests
TRAIL-R3 (human), mAb (HS301)	Mouse IgG1	Human	FACS, ICC	AG-20B-0024-C100	Purified	100 µg
TRAIL-R4 (human), mAb (HS402)	Mouse IgG1	Human	FACS, IHC (FS), ICC, IP	AG-20B-0025-C100	Purified	100 µg
TRAIL-R1 to -R4 Flow Cytometry Pack	Mouse IgG1	Human	FACS,	AG-44B-0004-KI01	Purified	1 Set

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

NEW TRAIL Receptors 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.

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

AG-20B-0027-C100 100 µg

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

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

AG-20B-0028-C100 100 µg

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

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

AG-20B-0029-C100 100 µg

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

anti-TRAIL-R4 (human), mAb (HS402)

AG-20B-0025-C100 100 µg

CLONE: HS402. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human TRAIL-R4. **SPECIFICITY:** Recognizes human TRAIL-R4. Does not cross-react with human TRAIL-R1, -R2 and -R3. **APPLICATION:** FACS, IHC (FS), ICC, IP.

Technical Note

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	Mouse IgG2b	Recognizes human TRAIL-R1. Does not cross-react with human TRAIL-R2, -R3 or -R4.	[2], [3], [4]
TRAIL-R2	TR2.21	Mouse IgG1	Recognizes human TRAIL-R2. Does not cross-react with human TRAIL-R1, -R3 or -R4.	[2], [3], [4]
TRAIL-R3	TR3.06	Mouse IgG1	Recognizes human TRAIL-R3. Does not cross-react with human TRAIL-R1, -R2 or -R4.	[2], [4]
TRAIL-R4	HS402	Mouse IgG1	Recognizes human TRAIL-R4. Does not cross-react with human TRAIL-R1, -R2 or -R3.	

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)

NEW TRAIL Receptors 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.

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

AG-20B-0027-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:** FACS, IHC (PS), WB.

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

AG-20B-0028-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:** FACS, IHC (PS), WB.

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

AG-20B-0029-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:** FACS, IHC (PS), WB.

LITERATURE REFERENCES:

- 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)
- TRAIL/bortezomib cotreatment is potentially hepatotoxic but induces cancer-specific apoptosis within a therapeutic window: R. Koschny, et al.; Hepatology **45,** 649 (2007)
- 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)
- Differential expression of the TRAIL/TRAIL-receptor system in patients with inflammatory bowel disease: S. Brost, et al.; Pathol. Res. Pract. **206,** 43 (2010)

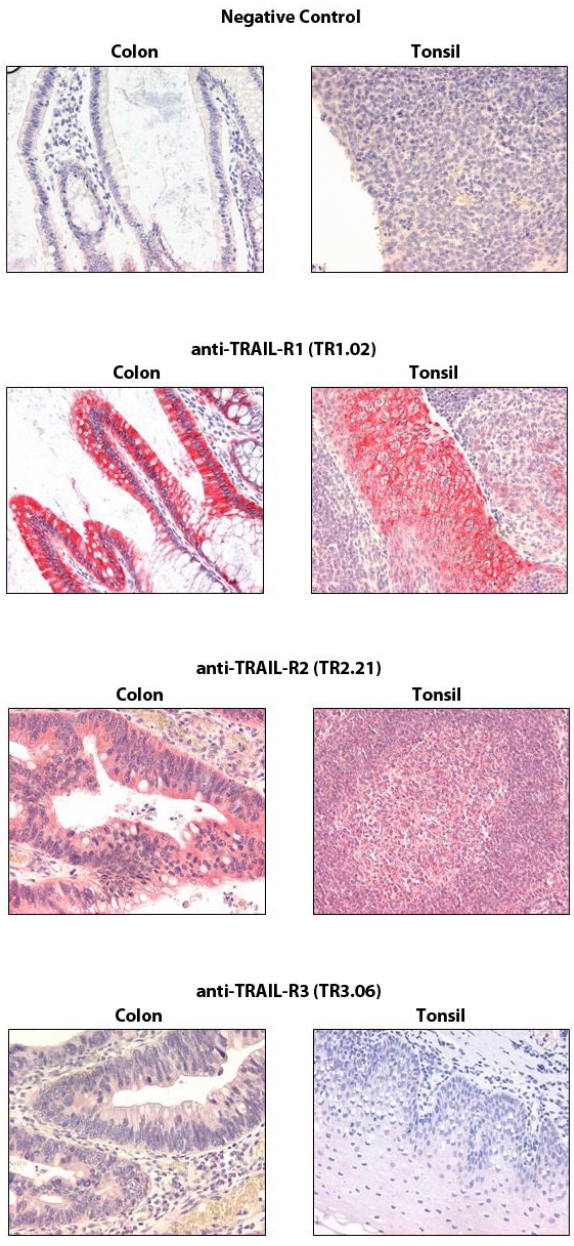

FIGURE: Immunohistochemistry detection of endogenous TRAIL-R1, TRAIL-R2 and TRAIL-R3 in paraffin-embedded human carcinoma tissues (colon, tonsil) using mAb to TRAIL-R1 (TR1.02) (Prod. No. AG-20B-0027), mAb to TRAIL-R2 (TR2.21) (Prod. No. AG-20B-0028) and mAb to TRAIL-R3 (TR3.06) (Prod. No. AG-20B-0029).

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