

TRAIL Signaling and Synergy Mechanisms Used in TRAIL-Based Combination Therapies

Christian T. Hellwig^{1,2} and Markus Rehm^{2,3}

Abstract

TRAIL and agonistic antibodies raised against TRAIL death receptors are highly promising new anticancer agents. In this brief review, we describe the recent advances in the molecular understanding of TRAIL signaling and the progress made in using TRAIL or agonistic antibodies clinically in mono- and combination therapies. Synergies have been reported in various scenarios of TRAIL-based multidrug treatments, and these can be used to potentiate the efficacy of therapies targeting TRAIL death receptors. We pay particular attention to structure the current knowledge on the diverse molecular mechanisms that are thought to give rise to these synergies and describe how different signaling features evoking synergies can be associated with distinct classes of drugs used in TRAIL-based combination treatments. *Mol Cancer Ther*; 11(1); 3–13. ©2012 AACR.

Introduction

Since the discovery of members of the TNF family and their so-called death receptors, the possibility of a new avenue for apoptosis-inducing cancer therapies has emerged. The death ligand-dependent extrinsic apoptotic pathway can induce apoptosis independent of the transcription factor p53 and, therefore, can circumvent the apoptosis resistance acquired by many tumors through loss-of-function mutations in this tumor suppressor. However, TNF α and agonistic antibodies activating the Fas receptor were found to be highly cytotoxic toward primary hepatocytes and other nontransformed cells when applied at clinically relevant concentrations (1, 2). In contrast, soluble TRAIL as well as leucine zipper-trimerized TRAIL potently induce apoptosis in a range of cancer cell lines, can inhibit tumor xenografts in mice, and were found to be of very low systemic toxicity in mice and nonhuman primates (3, 4). These findings sparked tremendous interest in exploring the potential of TRAIL as an anticancer therapeutic for a broad range of human malignant neoplasms. In this review, we briefly describe the key signaling events during TRAIL-induced apoptosis

initiation, give a short overview on the status of TRAIL-based clinical research and trials, and describe the mechanisms of signaling processes that evoke synergistic apoptosis responses in TRAIL-based combination treatments.

TRAIL

Endogenous TRAIL is expressed as a 281-amino acid type II *trans*-membrane protein, which is anchored to the plasma membrane and presented on the cell surface. TRAIL was independently identified by Wiley and colleagues and Pitti and colleagues in 1995 and 1996, respectively, and sequence alignments indicated its close relation to other death ligands, with highest sequence similarities reported for Fas ligand (FasL; refs. 5, 6). TRAIL is expressed by natural killer cells, which, following the establishment of cell-cell contacts, can induce TRAIL-dependent apoptosis in target cells (7). Physiologically, the TRAIL-signaling system was shown to be essential for immune surveillance, for shaping the immune system through regulating T-helper cell 1 versus T-helper cell 2 as well as "helpless" CD8⁺ T-cell numbers, and for the suppression of spontaneous tumor formation (8–10).

TRAIL-Induced Apoptosis Initiation: Signaling through Death and Decoy Receptors

TRAIL induces apoptosis through ligation with its cognate death receptors TRAIL-R1 (also known as DR4) and TRAIL-R2 (DR5; Fig. 1; refs. 11–13), and the trimerization of TRAIL around a central zinc atom via cysteine²³⁰ is essential for its apoptotic potential (14, 15). TRAIL binding is followed by receptor trimerization, and groups of trimerized death receptors can further cluster together to form bigger aggregates. These

Authors' Affiliations: ¹Rudolf Boehm Institute of Pharmacology and Toxicology, University of Leipzig, Leipzig, Germany; ²Department of Physiology and Medical Physics, and ³Center for Systems Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

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Corresponding Author: Markus Rehm, Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, RCSI York House, York Street, Dublin 2, Ireland. Phone: 353-1-402-8563; Fax: 353-1-402-2447; E-mail: mrehm@rcsi.ie

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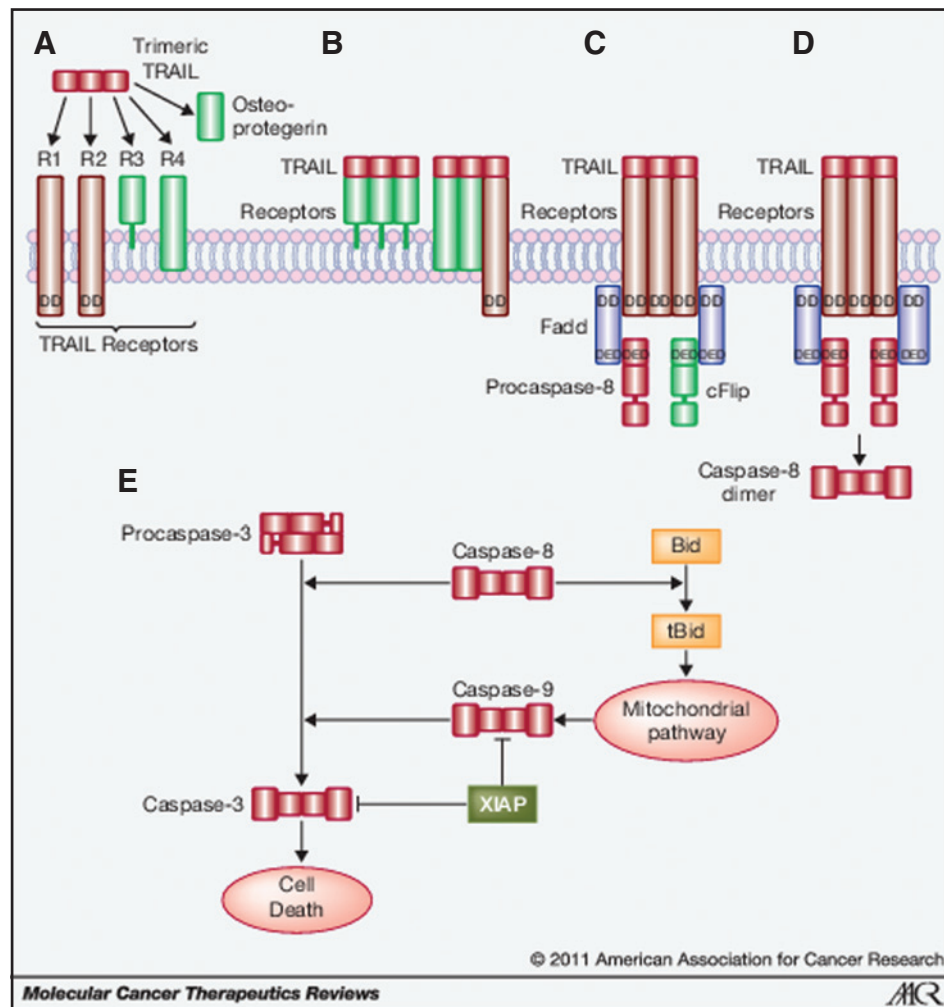


Figure 1. Simplified overview of TRAIL-mediated apoptosis signaling. A, trimers of TRAIL can bind to the extracellular regions of TRAIL-R1, TRAIL-R2, TRAIL-R3, and TRAIL-R4. The proapoptotic TRAIL-R1 and TRAIL-R2 comprise cytosolic regions, including a death domain (DD), which is missing in the antiapoptotic TRAIL-R3 and TRAIL-R4. TRAIL-R3 also lacks the transmembrane region. Osteoprotegerin can serve as a soluble decoy receptor. B, antiapoptotic TRAIL-R3 forms homotrimers following TRAIL binding. TRAIL-R4 can also form heterotrimers with TRAIL-R1 and/or TRAIL-R2. These receptor clusters are unable to transduce the TRAIL signal further and, therefore, act as antiapoptotic TRAIL scavengers. C, TRAIL-R1 or TRAIL-R2 can recruit FADD to form the DISC. Procaspase-8 and cFLIP (a catalytically inactive caspase-8 homolog) compete for FADD binding via their death effector domains (DED). Procaspase-8 consists of a DED-containing prodomain, as well as large and small catalytic subunits. Heterodimers of caspase-8 and the long splice variant cFLIP have a very limited substrate repertoire and, in most cases, cannot transduce the apoptosis signal. The short splice variant of cFLIP (not shown) forms inactive heterodimers with caspase-8. D, induced proximity of 2 procaspase-8 zymogens at the DISC results in autocatalytic cleavage of the linkers between the procaspase-8 subunits and caspase-8 activation. Active caspase-8 either resides at the DISC or, once fully processed, can be released into the cytosol. E, active caspase-8 cleaves Bid, inducing the mitochondrial apoptosis pathway, or directly activates effector caspase-3. XIAP suppresses caspase-9 and caspase-3 activity.

aggregates predominantly accumulate in lipid raft microdomains in the plasma membrane (16). The molecular composition of the receptor trimers has been controversially discussed, and it has not been fully resolved whether, at physiologic conditions, exclusively homotypic interactions or also heterocomplexes can be observed (17–20). Following trimerization of TRAIL-R1 or TRAIL-R2, the adaptor protein Fas-associated death domain containing protein (FADD) can bind to the intracellular death domains of the receptors and promote the recruitment and activation of initiator caspase-

8 within the death-inducing signaling complex (DISC; Fig. 1D). In most cells, caspase-8 then initiates apoptosis through Bid cleavage and the mitochondrial apoptosis pathway (Fig. 1E). Both the formation of the caspase-activating DISC, as well as the subsequent signaling network leading to apoptosis execution, can be modulated through a multitude of complex regulatory processes (21, 22).

Additional TRAIL receptors, which are incapable of transducing the death signal because they lack a functional intracellular death domain, have been identified.

These receptors are TRAIL-R3, TRAIL-R4, and osteoprotegerin (22). TRAIL-R3 and TRAIL-R4 are alternatively also referred to as decoy receptors 1 and 2 (DcR1, DcR2). TRAIL-R4 (DcR2) bears huge similarities to TRAIL-R1 and TRAIL-R2; however, it contains only a truncated cytosolic death domain (Fig. 1A; ref. 23). TRAIL-R3 (DcR1) consists of an extracellular cysteine-rich structure that resembles the proapoptotic TRAIL receptors and is associated with the plasma membrane through a COOH-terminal glycosyl-phosphatidylinositol anchor (Fig. 1A; refs. 11, 24). It is still highly debated whether overexpression of TRAIL-R3 or TRAIL-R4 correlates with cellular apoptosis resistance upon TRAIL exposure, and high expression levels are rarely found naturally in isolated cancer cell lines. The exact molecular details of how decoy receptors inhibit TRAIL-induced apoptosis in addition to their obvious role as TRAIL scavengers are not fully resolved and may differ between TRAIL-R3 and TRAIL-R4. For example, it was reported that TRAIL-R3 exclusively forms homotrimers upon TRAIL binding, whereas TRAIL-R4 may also aggregate into heterotrimers with activated TRAIL-R1 and TRAIL-R2 (Fig. 1B; ref. 25). Such heterotrimers are incapable of forming a functional DISC required for apoptosis initiation. Relatively little is known about osteoprotegerin, the fifth receptor capable of TRAIL binding. Osteoprotegerin negatively regulates osteoclastogenesis and is largely secreted as a soluble protein that might act as a scavenger for soluble TRAIL (26). Uncertainties remain about the molecular mechanisms of death and decoy receptor interaction, and it remains to be conclusively shown whether the relative abundance of death versus decoy receptors could serve to indicate cellular susceptibility to TRAIL-induced apoptosis.

Preclinical Evidence for the Anticancer Potential of TRAIL and Its Tolerability

TRAIL treatment strategies largely build on soluble variants of recombinant human TRAIL (rhTRAIL, a 60-kDa homotrimer TRAIL protein without its membrane anchor), as well as on monoclonal agonistic antibodies, which specifically target death receptor TRAIL-R1 or TRAIL-R2, thereby potentially circumventing decoy receptor-mediated resistance, and which in addition have the benefit of a significantly longer plasma half-life (27, 28). Additionally, to increase the local concentration and cancer cell-directed cytotoxicity of TRAIL, fusion proteins have been designed to translocate TRAIL to specific tissues or cells, for example, the epidermal growth factor receptor (EGFR)-selective delivery of soluble TRAIL, combined with an EGFR-targeting antibody fragment (29). Soluble TRAIL fused to a melanoma-associated chondroitin sulfate proteoglycan (MCSP)-specific antibody fragment combines inhibition of MCSP tumorigenic signaling with TRAIL-mediated apoptosis induction in melanoma cells (30). A multitude of studies using models of human tumor xenografts in mice showed a high anticancer

activity of TRAIL or receptor-specific ligands *in vivo*, and TRAIL efficacy can be potentiated in combination treatments with kinase and proteasome inhibitors, genotoxic drugs, histone deacetylase inhibitors, and others drugs (20, 31).

It has frequently been debated whether TRAIL can be cytotoxic to untransformed cells *in vivo*. In contrast to the high tolerability of TRAIL found in the seminal studies of Ashkenazi and colleagues and Walczak and colleagues (3, 4), other studies reported toxicities of TRAIL in isolated human hepatocytes (32, 33) and human brain tissue (34). However, significant TRAIL hepatotoxicities seem to be restricted to *ex vivo* cultures of primary liver cells or to highly aggregated TRAIL variants carrying flag or polyhistidine tags (35, 36). In contrast, rhTRAIL, as well as leucine or isoleucine zipper variants of TRAIL, was shown to be well tolerated in animal models. This finding is in stark contrast to the generally high hepatotoxicities that were reported for death ligands FasL and TNF α (3, 4). As a note of caution, however, it was shown that liver steatosis or hepatitis C infection can increase human hepatocyte sensitivity toward TRAIL (37), suggesting that patients from these risk groups should be excluded from TRAIL-based therapies.

TRAIL in Clinical Trials: Monotherapies

Soluble rhTRAIL (also named dulanermin), the TRAIL-R1-targeting monoclonal agonistic antibody mapatumumab, and the TRAIL-R2-targeting monoclonal agonistic antibodies lexatumumab, conatumumab, tigatuzumab, and DAB4 (also named PRO95780) have entered clinical studies. A large number of phase I and phase II clinical trials have been undertaken with these agents by now or are still ongoing, either as monotherapy or in combination with other chemotherapeutic drugs. These trials target a wide range of both solid and nonsolid malignant neoplasms.

Toxicity studies as part of an rhTRAIL phase I trial provided encouraging results. In a dose-escalating monotherapy in patients with advanced cancers, rhTRAIL was well tolerated at serum concentrations that were shown to be effective against cancer cells in preclinical models (38). Similarly, TRAIL-R1-specific mapatumumab seems to be well tolerated, and in a phase II study in patients with relapsed or refractory non-Hodgkin lymphoma, approximately one third of all patients responded to mapatumumab (28, 39, 40). In a phase II clinical trial, mapatumumab was well tolerated in patients with colorectal cancer (41). Lexatumumab, conatumumab, tigatuzumab, PRO95780, and other TRAIL-R2-targeting antibodies, likewise, were investigated in phase I and II clinical trials (42–44). Like mapatumumab, these antibodies only caused low-to-moderate side effects in a small number of patients. Several patients showed partial responses or attenuated disease progression when treated with TRAIL-R2-targeting antibodies (45, 46). In general, TRAIL and TRAIL receptor-binding antibodies, therefore, seem to be

well tolerated, and only a few cases of adverse responses have been documented. Furthermore, promising results in patients with advanced or refractory cancers underline the therapeutic potential of TRAIL receptor–targeting molecules. However, as a caveat, signaling through TRAIL death receptors may also induce antiapoptotic responses and proliferation signaling. This alternative response was shown to require the activation of kinases, such as RIP1, IKK, *c-jun*-NH₂-kinase, or p38 in signaling complexes secondary to the DISC (47) and to induce transcription-dependent prosurvival responses that attenuate apoptosis signaling. Indeed, subpopulations of primary cells isolated from children with acute leukemia showed increased proliferation rates following TRAIL treatment (48). These prosurvival responses may, therefore, limit the usability of TRAIL in monotherapies.

TRAIL in Clinical Trials: Combination Therapies

Cancers like melanoma, leukemia, multiple myeloma, breast, bladder, prostate, renal, colon, and others would be expected to be largely TRAIL sensitive judging on the basis of preclinical drug response data. However, cases of TRAIL resistance are frequently being observed and often could be associated with the overexpression of antiapoptotic proteins or the low expression of TRAIL receptors. An attractive and preclinically successful strategy, therefore, is to identify combination treatments that sensitize otherwise resistant cancers to TRAIL. Although this strategy holds risks because of the potential sensitization of nontransformed cells to the treatment, initial results are very promising. In a phase Ib clinical study to determine safety, pharmacokinetics, and maximum dose tolerance levels, patients with non–small cell lung cancer were treated with rhTRAIL (dulanermin), combined with paclitaxel, carboplatin, and bevacizumab. No dose-limiting toxicity was observed, and the treatment was well tolerated, with 1 complete response and a high percentage of partial responses observed (49). Other early phase Ib trials assessing combination treatments with mapatumumab and paclitaxel or carboplatin, as well as mapatumumab together with gemcitabine or cisplatin, in patients with advanced solid tumors reported high tolerability of mapatumumab (50, 51). By now, many more additional phase I and II clinical trials have been conducted or are still ongoing in other cancer types and using additional U.S. Food and Drug Administration–approved drugs, such as kinase inhibitors, proteasome inhibitors, histone deacetylase inhibitors, or genotoxic drugs, in combination with TRAIL (Table 1; ref. 45). Even though cases of adverse effects and toxicities were reported, overall, TRAIL combination therapies seem to be remarkably well tolerated. Still, outstanding results from ongoing phase II clinical trials will soon allow assessment of whether patients may benefit from such alternative treatment regimes and whether patient responses are indicative of multidrug synergies that were reported *in vitro*. The mechanisms bringing about synergistic multidrug

responses are very complex. However, significant progress in understanding the underlying signaling cross-talk has been made in recent years, as outlined in the following discussion.

Drug Classes and Their Synergy Mechanisms in TRAIL Combination Treatments

Synergistic effects of chemotherapeutics in combination with TRAIL have been reported for various classes of drugs. In general, synergies arise from nonlinear cross-potentialiation of drug effects. In the framework of apoptosis signaling, such potentiation may arise from the (often p53-dependent) transcriptional upregulation of proapoptotic proteins, from their accumulation due to impaired protein degradation, from alterations in regulatory posttranslational protein modifications, or from combinations of these processes. In the following paragraphs, we discuss the synergy mechanisms exerted by different drug classes. Related to this discussion, an overview of clinical trials aiming to use drug synergies in TRAIL-based combination treatments is provided in Table 1. Information on additional novel and promising cotreatment strategies that are still at the preclinical stage is provided in Supplementary Table S1.

Different genotoxic drugs have been suggested to enhance TRAIL signaling and its anticancer potential, with 5-fluorouracil and cisplatin having been studied most intensely. A typical stress response to genotoxic monotherapies is the transcriptional induction of BH3-only proteins, such as Puma, which is a potent inducer of apoptosis through the mitochondrial pathway (52). Such responses may synergistically complement the BH3-only protein Bid, which is cleaved and activated by caspase-8 in response to TRAIL. In this respect, similar synergy mechanisms would be relevant in cotreatment scenarios based on synthetic BH3-only protein mimetics, such as AT-101 (a gossypol derivate), ABT-263, ABT-737, and GX-15-070 (obatoclax; ref. 53). These novel drugs are already undergoing evaluation in monotherapy trials and may hold great potential for future combination treatments. However, synergies achieved with genotoxic drugs may also arise from upstream modulation of TRAIL signaling. For example, 5-fluorouracil can prime TRAIL-resistant hepatocellular carcinoma cells to apoptosis, at least partially, through the downregulation of the inactive caspase-8 homolog cFlip (Fig. 1C) and through the parallel upregulation of TRAIL-R2 at physiologically relevant doses (54). Importantly, cytotoxicity to normal cells seems to be limited to acceptable levels in this scenario (54). Similar scenarios of death receptor upregulation or cFlip downregulation were also reported in response to other genotoxic drugs, as well as to ionizing irradiation and epigenetic inhibitors, such as histone deacetylase inhibitors (55, 56). Also IFNs, which are frequently used in hematologic cancer therapy, were shown to potently promote TRAIL-induced apoptosis through evoking transcriptional responses. For example, IFN- α exposure can increase

Table 1. Modulation of TRAIL-mediated apoptosis through combination treatments: clinical trials

Drugs in clinical trials with TRAIL-R ligand	TRAIL-R ligand	Additional combination	Type of cancer	Clinical trial phase	Reference	Mode of action and/or target	Consequence and/or modulation of the TRAIL pathway
Proteasome inhibitor							
Bortezomib	Conatumumab	—	Lymphoma	Ib, susp. ^a	NCT00791011	Inhibition of proteasomal degradation	Stabilization of TRAIL-R, active caspase-8, -3, BH3-only proteins, p53, IκBα. BH3-only protein and p53 expression
Genotoxic and/or mitosis stress-inducing drugs							
5-FU	Mapatumumab Conatumumab	— FOLFOX6, bevacizumab	Multiple myeloma CRC	II, compl. Ib, II	NCT00315757 NCT00625651	Nucleoside replacement by analogs	p53-mediated transcriptional upregulation of BH3-only proteins and TRAIL-R2; p53-mediated downregulation of antiapoptotic Bcl-2 family proteins and cFlip
Capecitabine (5-FU prodrug)	Conatumumab	Gemcitabine, radiation	Pancreatic	I, II	NCT01017822		
Gemcitabine	Conatumumab Mapatumumab	— Cisplatin	Pancreatic Solid tumors	Ib, II I, compl.	NCT00630552 (60)		
Cisplatin	Mapatumumab	Radiotherapy	Cervical cancer	II	NCT01088347	DNA alkylation	
Carboplatin	Mapatumumab	Paclitaxel	NSCLC	II, compl.	NCT00583830	DNA	
Doxorubicin	Conatumumab	—	Soft tissue sarcoma	II	NCT00626704	DNA intercalation	
Paclitaxel	Dulanermin	Carboplatin, bevacizumab	NSCLC	Ib, compl.	(58) NCT00508625	Tubulin disassembly inhibition	Prolonged activation of the mitotic checkpoint, p53 signaling
Irinotecan (OPT-11, Camtothecin-11)	Conatumumab Mapatumumab Mapatumumab Tigatuzumab Dulanermin PRO95780	Carboplatin Carboplatin Carboplatin — Cetuximab Cetuximab	NSCLC Solid tumors NSCLC Breast cancer CRC CRC	Ib, II, compl. I, compl. II, compl. II Ib Ib, compl.	NCT00534027 (59) NCT00583830 NCT01307891 NCT00671372 NCT00497497	Topoisomerase I inhibition	

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Table 1. Modulation of TRAIL-mediated apoptosis through combination treatments: clinical trials (Cont'd)

Drugs in clinical trials with TRAIL-R ligand	TRAIL-R ligand	Additional combination	Type of cancer	Clinical trial phase	Reference	Mode of action and/or target	Consequence and/or modulation of the TRAIL pathway
Epigenetic inhibitors							
Vorinostat	Conatumumab	—	Lymphoma	Ib, susp. ^a	NCT00791011	Histone deacetylase inhibition	CDK-inhibitor 1 (p21) activation, cell cycle arrest, altered activity of NF-κB, p53
Irradiation							
Radiotherapy	Mapatumumab	Cisplatin	Cervical cancer	II	NCT01088347	Alteration of gene transcription, radical generation	Induction of BH3-only proteins, ROS production
3D conformal radiation therapy	Conatumumab	Gemcitabine, capecitabine	Pancreatic	I, II	NCT01017822		
Growth factor receptor and/or receptor tyrosine kinase inhibitors							
Cetuximab	Dulanermin	Irinotecan	CRC	Ib	NCT00671372	Inhibition of the epidermal growth factor receptor	Inhibition of PI3K/Akt prosurvival and proliferation signaling
Panitumumab (mAB)	Conatumumab	—	CRC	Ib, II	NCT00630786		
Bevacizumab (mAB)	Dulanermin	Carboplatin, paclitaxel	NSCLC	II	NCT00508625	Inhibition of vascular endothelial growth factor receptor tyrosine kinases	Inhibition of PI3K/Akt signaling: decrease of cFlip and XIAP expression, decrease of NF-κB activation, dephosphorylation of the BH3-only protein Bad and its release from 14-3-3 sequestration
	Dulanermin	FOLFOX	CRC	I	NCT00873756		
	Dulanermin	FOLFIRI	CRC	I	NCT00671372		
	Conatumumab	FOLFOX6	CRC	Ib, II	NCT00625651		
	PRO95780	Carboplatin, paclitaxel	NSCLC	II, compl.	NCT00480831		
	PRO95780	FOLFIRI	CRC	Ib, compl.	NCT00497497		
	PRO95780	FOLFOX	CRC	Ib, compl.	NCT00851136		

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Table 1. Modulation of TRAIL-mediated apoptosis through combination treatments: clinical trials (Cont'd)

Drugs in clinical trials with TRAIL-R ligand	TRAIL-R ligand	Additional combination	Type of cancer	Clinical trial phase	Reference	Mode of action and/or target	Consequence and/or modulation of the TRAIL pathway
Ganitumab (mAb)	Conatumumab	—	NSCLC, CRC, pancreatic, ovarian, sarcoma	Ib, II	NCT00819169	Inhibition of the insulin-like growth factor I receptor	Downregulation of levels of active Akt, ERK1/2, and MAPK p38
Kinase inhibitors	Conatumumab	FOLFIRI	KRAS-mutant CRC	II	NCT00813605	RAF/MEK/ERK pathway inhibition. Vascular endothelial/platelet-derived growth factor receptor tyrosine kinases inhibition in vasculature.	Downregulation of phospho-STAT3, Mcl-1, survivin, and cyclin D1. Downregulation of Mcl-1, cIAP2, cFlip. Inhibition of NF-κB activation and tumor vascularization.
	Conatumumab	FOLFOX6, bevacizumab	Solid tumors, CRC, lymphoma, NSCLC	II	NCT01327612		
Sorafenib	Mapatumumab	—	Hepatocellular carcinoma	Ib	NCT00712855		
Cytokines	Mapatumumab	—	Hepatocellular carcinoma	II	NCT01258608		
	IFN-γ	Lexatumumab	Pediatric solid tumors	I	NCT00428272	Alteration of gene transcription	Transcriptional downregulation of cFlip
CD20 antibody	Rituximab	Dulanermin	Non-Hodgkin lymphoma	Ib/II, compl.	NCT00400764	Inhibition of the B-cell surface protein CD20	Inhibition of NF-κB, downregulation of Bcl-XL, ADCC and CDC induction
	PRO95780	—	Non-Hodgkin lymphoma	II, compl.	NCT00517049		

NOTE: Drugs listed are currently being investigated in clinical trials in combination with rhTRAIL or TRAIL-receptor ligands. Dulanermin is recombinant human Apo2L/TRAIL; mapatumumab (HGS1012) is a monoclonal antibody directed against TRAIL-R1; lexatumumab (HGS-ETR2), conatumumab, tigatuzumab, and PRO95780 (apomab) are monoclonal antibodies directed against TRAIL-R2. FOLFOX and FOLFIRI are combination chemotherapies including folinic acid and 5-fluorouracil, plus oxaliplatin or irinotecan, respectively. Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; compl., completed; CRC, colorectal cancer; ERK, extracellular signal-regulated kinase; 5-FU, 5-fluorouracil; mAb, monoclonal antibody; MEK, MAP/ERK kinase; NSCLC, non-small cell lung cancer; ROS, reactive oxygen species; susp., suspended. ^aThe clinical trial NCT00791011 was suspended because of a lack of mantle cell lymphoma subjects who were bortezomib naive. Trial information was sourced from <http://www.clinicaltrials.gov> and additional trial-specific information can be accessed online using the respective reference numbers.

TRAIL and TRAIL-R2 expression in human hepatocellular carcinoma cells (57, 58), whereas IFN- γ can reduce cFlip protein levels (59).

Prolonged proteasome inhibition induces the expression and accumulation of BH3-only proteins, such as Bim, Bik, Puma, and Noxa (60), which leads to synergy mechanisms like those described above for genotoxic drugs. Furthermore, TRAIL-induced NF- κ B activation and prosurvival signaling can be limited by proteasome inhibition because the degradation of NF- κ B inhibitor I κ B α is prevented (23, 61). Maybe more importantly, proteasome inhibition can lead to the direct accumulation of proteins that promote TRAIL-induced apoptosis. An accumulation of both TRAIL-R1 and TRAIL-R2 was described in response to proteasome inhibition in various cancers (62–64), potentially resulting in more efficient DISC formation and caspase activation upon TRAIL addition. The consequences arising from a general inhibition of protein degradation are a naturally complex, due to the global disturbance of relative protein abundances in addition to active transcriptional stress responses. Attributing synergies in TRAIL and/or proteasome inhibitor cotreatments to the modulation of single proteins or processes, as proposed in several studies, therefore likely presents an oversimplification of the underlying molecular mechanisms that enhance TRAIL responsiveness. Nevertheless, the fact that proteasome inhibition can promote proapoptotic signaling, at least partially, independent of protein neosynthesis makes proteasome inhibitors particularly attractive for the treatment of cancers that present with deficiencies in transcriptional proapoptotic responses, as can arise from loss-of-function mutations in tumor suppressor p53.

Transcription- or p53-independent synergisms of chemotherapeutics and TRAIL can also be achieved by targeting posttranslational protein modifications such as phosphorylation patterns. For example, inhibiting protein kinase CK2 impairs Bid phosphorylation and enhances Bid cleavage by caspase-8 (65, 66) and may be an attractive cotreatment strategy if outstanding safety tests yield satisfying results. Procaspase-8 itself can be phosphorylated by Src kinase, which impairs its binding to and activation at the DISC (67). In addition, formation of the DISC can be modulated upstream of caspase-8 recruitment through mitogen-activated protein kinases (MAPK; ref. 68). Furthermore, protein kinase C activity limits FADD recruitment into the DISC (69). Kinase inhibition can also directly or indirectly induce transcriptional responses, adding further complexity. For example, inhibiting phosphoinositide 3-kinase (PI3K)/AKT signaling by perifosine can restore TRAIL sensitivity in acute myelogenous leukemia through a p53-independent TRAIL-R2 upregulation and a concomitant cFlip and X-linked inhibitor-of-apoptosis protein (XIAP) downregulation (70). Multitarget kinase inhibitors, such as sorafenib, may therefore hold great potential in enhancing cancer cell responsiveness

and are currently being investigated in combination treatments with TRAIL in phase I clinical trials (45). Furthermore, additional posttranslational modifications of the death receptors, such as palmitoylation, nitrosylation, and glycosylation, have been reported, and these modifications can influence cellular sensitivity toward TRAIL (22). Whether these modifications can be therapeutically targeted and used is currently unknown.

Antagonists of inhibitor-of-apoptosis proteins, such as synthetic Smac peptides and Smac mimetics, were shown to sensitize various cancer cell lines to TRAIL-induced apoptosis. In particular, antagonizing XIAP, the most potent inhibitor of executioner caspases, promotes the direct activation of caspase-3 by caspase-8 and results in efficient apoptosis execution (71, 72). Furthermore, Smac mimetics also bind to cellular IAP (cIAP)-1 and cIAP-2. As a consequence, cIAPs are rapidly degraded and cells may respond with secreting TNF α (73, 74). TNF α then may further enhance apoptosis by activating a parallel extrinsic apoptosis pathway that leads to caspase-8 activation. IAP inhibitors have been shown to synergistically kill pancreatic cancer cells in combination with the TRAIL-R1-targeting antibody mapatumumab, whereas the combined treatment with the TRAIL-R2-specific antibody lexatumumab was less efficient, indicating receptor-specific differences in synergy and susceptibility of certain cancers (75). Besides the requirement to better understand and use synergy mechanisms, means to predetermine differential sensitivity to either TRAIL-R1- or TRAIL-R2-transduced apoptosis will be paramount to case specifically identify the most effective treatment strategies in the future.

Conclusions

Experimental preclinical as well as clinical evidence shows that TRAIL has a high potential as a novel anticancer drug, both in monotherapies and in combination treatments (Table 1 and Supplementary Table S1). Given the comprehensive research activities currently focusing on identifying and deciphering the complex intracellular signaling cross-talk that evokes synergies in TRAIL combination treatments, we will soon better understand which conditions need to be fulfilled to more efficiently use the potential of different TRAIL-based treatment strategies. At this stage, TRAIL signaling is already one of the best-characterized apoptotic signaling pathways. In the coming years, this information may greatly assist in identifying potential biomarkers that will allow researchers to rationally predict tumor responsiveness to TRAIL-based therapies. This important, but still outstanding milestone, will need to be achieved before patient benefits can be maximized through appropriately tailored drug administration.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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