Expert Opinion

TRAIL receptor signaling and therapeutics

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Importance of the field: TNF-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family of cytokines, which can induce apoptotic cell death in a variety of tumor cells by engaging specific death receptors, TRAIL-R1 and TRAIL-R2, while having low toxicity towards normal cells. There is interest in cancer therapy inducing cell death by activation of the death-receptor-mediated apoptotic pathway while avoiding decoy-receptor-mediated neutralization of the signal. This has led to the development of a number of receptor-specific TRAIL-variants and agonistic antibodies. Some of these soluble recombinant TRAIL and agonist antibodies targeting TRAIL-R1 and/or TRAIL-R2 are progressing in clinical trials. In addition, TRAIL-resistant tumors can be sensitized to TRAIL by a combination of TRAIL or agonistic antibodies with chemotherapeutic agents, targeted small molecules or irradiation.

Areas covered in this review: Recent advances in developing TRAIL or its agonist receptor antibodies in cancer therapy. We also discuss combination therapies in overcoming TRAIL resistance in cancer cells.

What the reader will gain: Knowledge of current clinical trials, the promise and obstacles in the future development of therapies affecting TRAIL signaling pathways.

Take home message: Cancer therapeutics targeting the TRAIL/TRAIL receptor signaling pathway hold great promise for molecularly targeted pro-apoptotic anti-cancer therapy.

Keywords: apoptosis, cancer, cell death, combination resistance, TRAIL, TRAIL receptor, TRAIL receptor agonistic antibodies

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1. Introduction

Apoptosis or programmed cell death is a highly regulated process crucial for embryonic development, tissue remodeling, organ homeostasis and regulation of the immune response. There is a delicate balance between cell death and cell renewal in healthy tissues maintained by growth factors, cell damage and hormones. Disruption of this balance may lead to the development of malignant tumors. Tumor cells are characterized by their ability to acquire resistance to the cell death process and replicate in an uncontrolled fashion even though their DNA is damaged. Surgery, radiation and chemotherapy have been the mainstay of cancer treatment with removal of the cancer without damage to the rest of the body as the goal of treatment. Sometimes this can be accomplished by surgery alone, but the cancers tend to invade adjacent tissue or to spread to distant sites by microscopic metastasis often limiting effectiveness of this therapy. Radiation therapy and many chemotherapeutic agents used in treatment trigger double-stranded breaks of nuclear DNA causing apoptosis (programmed cell death). These therapies are not tumor-specific and

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Article highlights.

- Ongoing attempts to improve chemotherapy efficacy are now entering a new era with rationally designed targets. The process of selectively targeting the cell death pathways in cancer cells while sparing the normal cells is of interest in cancer therapy. Members of the TNF superfamily including Fas ligand, TNF and TNF-related apoptosis-inducing ligand (TRAIL) have been identified as targets for cancer biotherapy.
- About half of tumor cell lines may be TRAIL-resistant and this resistance may vary in primary human tumor cells.
- While soluble recombinant TRAIL may bind to all TRAIL-receptors, including TRAIL-R3 and -R4, antibodies targeting TRAIL-R1 or -R2 only bind to their respective apoptosis-inducing receptor. Thus, if tumor cells were overcoming TRAIL sensitivity by expression of TRAIL-R3 (DcR1) and -R4 (DcR2) or by signaling events mediated by any of the other TRAIL-receptors which are not triggered by the respective antibody, such a TRAIL-R1 (DR4)- or TRAIL-R2 (DR5)-specific agonist would still be able to transmit the death signal despite the expression of other receptors.
- TRAIL or TRAIL-R agonist mAbs can be combined with diverse chemotherapeutic drugs or radiotherapy to induce synergistic cell death of tumor cells.
- Due to the promising pre-clinical results of TRAIL as an anti-cancer agent several companies are developing TRAIL-receptor-targeting therapeutics. These TRAIL receptor agonists (TRAs) are currently being tested in Phase I and II clinical trials.
- A design that pre-selects the patients most likely to benefit from rhTRAIL/agonist antibody therapy by identifying suitable biomarkers will be helpful in fulfilling the promise of personalized medicine for the exploitation of the TRAIL/TRAIL receptor pathway in cancer therapy and other autoimmune disorders.

This box summarizes key points contained in the article.

can affect normal cells leading to side effects. Ongoing attempts to improve chemotherapy efficacy are now entering a new era with rationally designed targets. The process of selectively targeting the cell death pathways in cancer cells while sparing the normal cells is of interest in cancer therapy. Members of the TNF superfamily including Fas ligand, TNF and TNF-related apoptosis-inducing ligand (TRAIL) have been identified as targets for cancer biotherapy. TRAIL triggers apoptosis in cancers, irrespective of the p53 status and appears to have a therapeutic index in preclinical studies.

2. TRAIL and its receptors

In humans, the gene that encodes TRAIL is located on chromosome 3q26, which is not adjacent to other TNF family members. The genomic structure of the TRAIL gene extends to about 20 kb and consists of five exonic segments of 222, 138, 42, 106 and 1245 nucleotides and four introns of approximately 8.2, 3.2, 2.3 and 2.3 kb. The TRAIL gene lacks TATA and CAAT boxes and the promoter region contains putative response elements for GATA, activator protein 1 (AP1), CCAAT/enhancer binding protein (C/EBP), specific protein-1 (SP1), octamer-binding transcription factor 1 (OCT-1), AP3, polyomavirus enhancer activator 3 homolog (PEA3), cleavage stimulation factor (CF-1), and interferon-sensitive responsive element (ISRE) [1]. Kuribayashi *et al.* recently reported a direct regulation of TRAIL gene by p53 protein [2]. In addition, early growth response protein (EGR) [3], interferon regulatory factor 1 (IRF1) [4], NF-κB [5], SP1 [6] and PU1 [7] have been implicated in the regulation of TRAIL. TRAIL shows homology with other members of the TNF superfamily. It consists of 281 amino acids and has characteristics of a type II transmembrane protein (i.e., no leader sequence and an internal transmembrane domain). The N-terminal cytoplasmic domain is not conserved across family members, however, the C-terminal extracellular domain is conserved and can be proteolytically cleaved from the cell surface. TRAIL forms a homotrimer that binds three receptor molecules. TRAIL is present in various tissues, particularly in the spleen, lung and prostate.

TRAIL binds to two different types of receptors: death receptors that trigger TRAIL-induced apoptosis and decoy receptors inhibit this pathway. TRAIL can also bind to osteoprotegerin (OPG) (a soluble inhibitor of receptor activator of NF-κB (RANK) ligand) at low affinity. To date, four human receptors specific for TRAIL have been recognized: the death receptors TRAIL-R1 (also know as DR4) and TRAIL-R2 (also known as DR5) and the putative decoy receptors TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2). TRAIL-R1 (DR4) is expressed at very low levels in most human tissues including the spleen, thymus, liver, peripheral blood leukocytes, activated T cells, small intestine and some tumor cell lines. TRAIL-R2 (DR5) is ubiquitiously distributed both in normal and tumor cell lines but is more abundant in spleen, peripheral blood leukocytes, activated lymphocytes and hepatocyes.

3. TRAIL-induced apoptosis and mechanisms of cell death resistance

3.1 Apoptotic signaling

To signal cell death, TRAIL trimerizes and binds to its receptors, TRAIL-R1 and TRAIL-R2, thereby recruiting Fas-Associated protein with Death Domain (FADD) and forms the Death Inducing Stimulating Complex (DISC) (**Figure 1**). FADD recruits Caspase 8 or 10 through its death effector domain (DED). Activated caspases 8 or 10 then cleaves the effector caspase 3 which in turn proceeds with the cleavage of the death substrates [8]. Both low levels of FADD-like interleukin converting enzyme-like inhibitory protein (FLIP) [9] and the process of caspase 8 ubiquitination [10] appear to be involved in caspase 8 activation. In type I cells, the DISC-induced caspase cascade is adequate for the activation

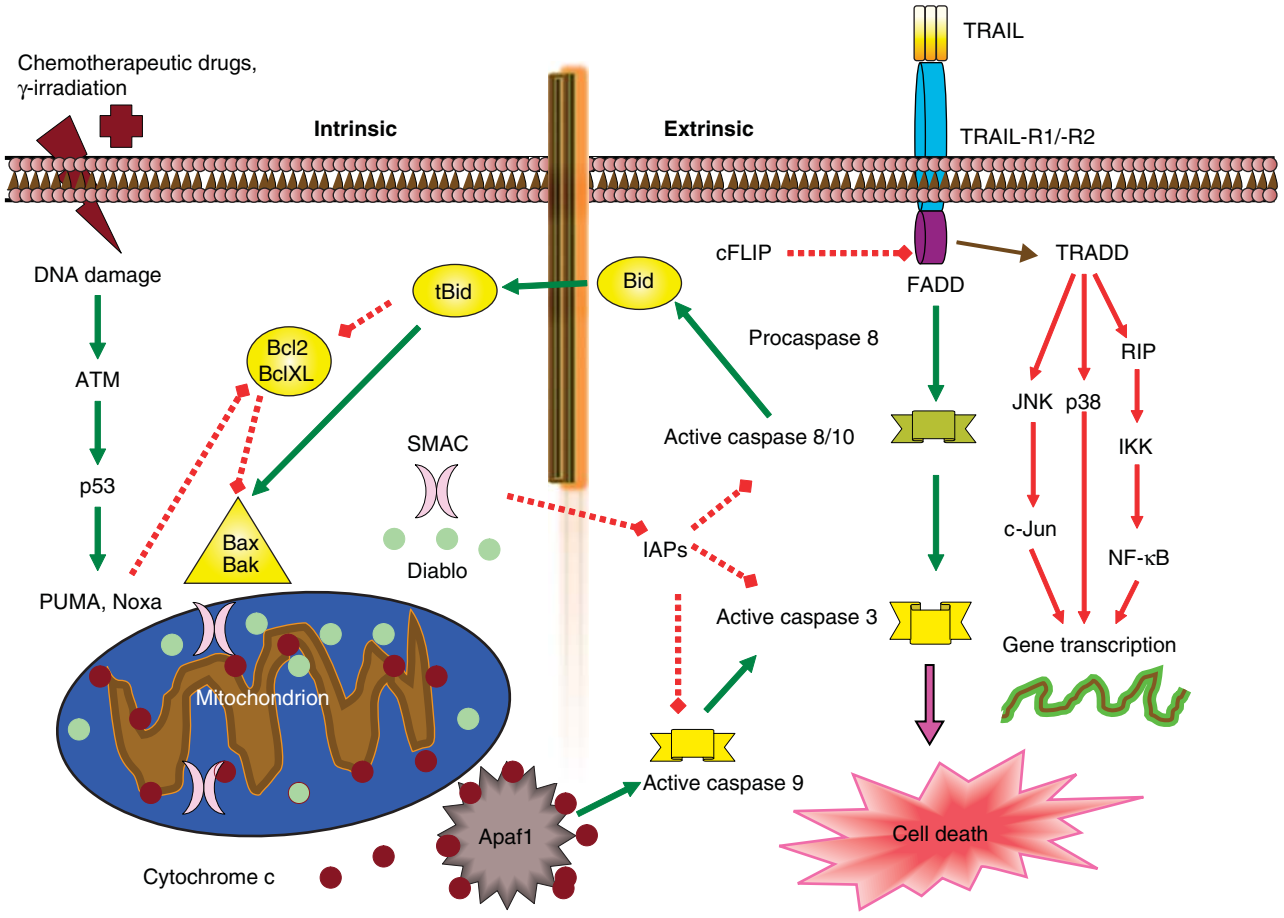

Figure 1. Signaling through the Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) pathway. Binding of TRAIL or the TRAIL receptor agonist antibody to TRAIL death receptors results in recruitment of the FAS-associated protein with death domain (FADD) and caspase 8 to form a functional death-inducing complex (DISC). DISC formation leads to the cleavage and activation of caspase 8 which can activate caspase 3 and the BH3-only protein Bid. In type 1 cells, caspase 8 activation is sufficient to activate other downstream caspases leading to cell death. However, in type II cells, the amplification of the mitochondrial loop by Bid is essential to induce cell death. Chemotherapeutic drugs and radiation cause DNA damage which is sensed by the ataxia telangiectasia mutated homolog (ATM) and ataxia telangiectasia and Rad3 related (ATR) kinases and their downstream effector checkpoint kinase 2 (Chk2). This leads to the activation of p53 dependent activation of genes like PUMA, Bax and Noxa leading to mitochondrial permeabilization. In TRAIL resistant cells, dissociation of FADD and caspase 8 from the receptor signals survival through NF-κB, p38, PI3K, AKT and JNK pathway.
Apaf1: Apoptotic peptidase activating factor 1; Bak: BCL2-antagonist/killer; Bax: Bcl2-associated X protein; Bcl2: B cell leukaemia/lymphoma-2; BclXL: B cell lymphoma like X; cFLIP: CellularFADD-like interleukin converting enzyme-like inhibitory protein; Diablo: Direct inhibitor of apoptosis protein binding protein with low pI; IAPs: Inhibitors of apoptosis proteins; IKK: Inhibitor of NF-κB kinase; PUMA: p53 upregulated modulator of apoptosis; RIP: Receptor interacting protein; SMAC: Second mitochondria-derived activator of caspases; tBid: Truncated Bid, TRADD: Tumor necrosis factor receptor type 1 associated death domain protein.

of effector caspases and hence the induction of apoptosis. On the other hand, type II cells depend on the activation of the mitochondrial amplification loop, also known as the intrinsic or B cell leukaemia/lymphoma-2 (Bcl2)-controlled, apoptosis pathway [11]. The death receptor-mediated extrinsic pathway and the mitochondrial intrinsic apoptosis pathway are connected via the BH3-only protein, BH3 interacting domain death agonist (Bid). Upon DISC formation, Bid is cleaved to truncated Bid (tBid) by caspase 8 or caspase 10. tBid translocates to the mitochondria, leads to the activation of Bcl2-associated X protein (Bax) and BCL2-antagonist/killer (Bak) and, consequently, to the release of cytochrome *c* and other pro-apoptotic proteins from the mitochondria. Cytochrome *c*, together with dATP, apoptotic protease activating factor 1 (Apaf-1) and caspase 9, forms the apoptosome, which provides a platform for activation of the initiator caspase 9 that in turn activates downstream effector caspases. Additionally, the second mitochondria-derived activator of caspases/direct inhibitor of apoptosis protein binding protein with low pI (Smac/DIABLO), which is released from the

mitochondria during apoptosis, counterchecks the function of X-linked inhibitor of apoptosis protein (XIAP), thereby allowing for the full activation of caspases 3, 7 and 9 [12]. Furthermore, chemotherapeutic drugs and radiation cause DNA damage which is sensed by the ataxia telangiectasia mutated homolog (ATM) and ataxia telangiectasia and Rad3 related (ATR) kinases and their downstream effector checkpoint kinase 2 (Chk2). This leads to the activation of p53-dependent activation of genes like p53 upregulated modulator of apoptosis (PUMA), Bax and Noxa leading to mitochondrial permeabilization [13].

3.2 Other signal transduction pathways mediated through TRAIL receptors

Depending on the cell type, the relative strength and duration of the ligand signal, and either the presence, absence or activation state of the intracellular proteins that signal downstream of TRAIL receptors, treatment with TRAIL may stimulate either apoptosis or in rare instances cell proliferation [14-16]. Indeed TRAIL may simultaneously induce multiple intracellular signal transduction pathways that involve proteins such as nuclear NF-κB, MAPKs, including extracellular signal-regulated kinases (ERKs), JNKs, PI3K and Akt/protein kinase B (PKB) [17]. There is at present no clear consensus as to the make-up of the protein complexes that are necessary for TRAIL-mediated activation of these so called 'alternative' signaling pathways but various combinations of FADD, FADD-like apoptosis regulator (CFLAR, also known as c-FLIP), caspases 8 and 10, TNF receptor-associated factor 2 (TRAF2), NF-κB essential modulator (NEMO) and receptor-interacting protein (RIPK1) are involved, possibly in a cell-type-dependent manner (**Figure 1**). If one or more of these 'alternative' cell proliferative signaling pathways are engaged in a manner that dominates over the pro-apoptotic signal this may have dramatic effects on the physiological or therapeutic activities of TRAIL or agonistic anti-TRAILR mAbs. TRAIL has also been reported to induce autophagy [18].

3.3 Physiological role of TRAIL and its receptors

TRAIL is a cytokine that is expressed by effector lymphocytes. TRAIL is known to contribute to host immunosurveillance against primary tumor development and metastasis. TRAIL knockout mice are viable and display no apparent hematological or reproductive disorders [19]. The expression level of TRAIL is extremely low in freshly-isolated lymphocytes. Only a small set of NK cells express detectable TRAIL and it seems most likely that the expression of TRAIL on the liver NK cells is regulated by the secretion of IFN-γ from the NK cells in an autocrine manner [20]. It has been previously shown that stimulation of dendritic cells (DCs) with IFN-β results in the expression of TRAIL on the DCs, therefore enhancing the cytotoxicity of DCs to tumor cells [21]. Therefore, TRAIL may play a role in regulating the innate immune response involving the IFNs, NK cells and DCs [22]. Furthermore, TRAIL can boost the host responses to the tumor and somehow change the tumor microenvironment for enhanced antigen presentation and tissue infiltration. Some studies showed that IFN-γ induction by T cells is crucial for the tumor infiltration and migration and that IFN-γ has to act on the tumor stromal cells [23]. The IFN-γ-induced expression of TRAIL might lead to greater leukocyte infiltration into the tumor. TRAIL-sensitive tumor cells might become resistant by challenging interaction with TRAIL-expressing tumor infiltrating immune cells or stromal cells [24]. Hypoxic environments, often present in solid tumors, are known to induce the up-regulation of hypoxia-inducible factor-1α, which in turn leads to the induction of the hypoxia-response genes, including VEGF, and subsequently increases tumor angiogenesis and metastasis [25]. A hypoxic environment has also been shown to confer resistance to TRAIL-induced apoptosis in TRAIL-sensitive tumors [26]. It has been suggested that TRAIL anti-metastatic activity is restricted to liver NK cells. Both neutralizing anti-mTRAIL mAb and TRAIL knockout mice supported a direct role for NK cells expressing TRAIL in the suppression of tumor metastasis, while no metastasis occurred against the TRAIL-resistant cells examined [19,20]. TRAIL strongly induces the expression of IL-8 followed by the distant metastasis of pancreatic tumors *in vivo* [27]. In addition to the role of TRAIL in tumor immunosurveillance, TRAIL has also been implicated in regulating autoimmunity. Several studies have reported increased serum levels of soluble TRAIL in patients with systemic lupus erythematosus (SLE) [28]. It has also been found that the levels of TRAIL may also serve as a potential marker in determining the sensitivity to IFN treatment for human multiple sclerosis patients [29].

The physiological role of TRAIL receptors however remains unclear. TRAIL-R knockout studies showed that TRAIL-R is not involved in the embryonic development because TRAIL-R knockout mice are viable and continue to develop normally. Recently, TRAIL-R knockout mouse studies indicated that TRAIL-R is a negative regulator of the innate immune response and that loss of TRAIL-R leads to increased levels of IFN-α, -γ and IL-12 and activation of NF-κB [30].

Loss of heterozygosity of a region on chromosome 8 where TRAIL-R2 is mapped is frequently found in head, neck and lung carcinomas [31]. TRAIL-R2 is incriminated in suppressing tumor progression and determining chemosensitivity, as silencing of TRAIL-R2 promotes tumor growth and renders tumor cells resistant to treatment with 5-fluorouracil [32]. TRAIL-R2 deficiency also increases susceptibility to chronic inflammation and tumorigenesis. Loss of a single allele of TRAIL-R2 in the B-cell lymphoma-prone Eu-myc genetic background significantly reduces median lymphoma-free survival [33]. Moreover, TRAIL-R2 knockout mice are resistant to ionizing radiation-induced apoptosis in the thymus and spleen, demonstrating the tissue specificity of TRAIL-R2 response upon irradiation [34]. Recently, TRAIL-R2 has been found to play a role in regulating anoikis in human colon

cancer cells [35,36]. Grosse-Wilde *et al.* 2008 showed that murine TRAIL-R2 functions as a metastasis suppressor and that the loss of TRAIL-R2 enhances metastasis of the primary tumors to the lymph nodes [37].

3.4 TRAIL resistance

About half of tumor cell lines may be TRAIL-resistant and this resistance may vary in primary human tumor cells [38]. Ovarian cancers show more variable responses with some primary tumor cells being effectively killed and others displaying resistance and this variation also being reflected in established cell lines [39]. Some tumor cells appear to be inherently resistant to TRAIL and resistance can also be acquired in cells that were originally found to be sensitive to TRAIL. For example, in a cell line model of breast and ovarian cancers, resistance to the anti-TRAIL-R2 antibody TRA-8 was induced by repeated exposure to non-apoptosis-inducing doses of the antibody [40]. Interestingly in this example, the resistance mechanism was selective for TRAIL-R2-directed agonists and the resistant cells retained sensitivity to TRAIL and to an antibody that activates the other death receptor, TRAIL-R1 (**Figure 2**). Recent studies demonstrate that the microRNAs miR-221 and miR-222 interfere with TRAIL signaling through their effects on the cell cycle regulator protein p27kip1 raising the possibility that microRNA patterns maybe be used to predict TRAIL resistance or sensitivity [41]. Furthermore, MET oncogene is involved in miR-221&222 activation through the c-Jun transcription factor. miR-221&222, by targeting phosphatase and tensin homologue (PTEN) and tissue inhibitor of metalloproteinase 3 (TIMP3) tumor suppressors, induce TRAIL resistance and enhance cellular migration through the activation of the AKT pathway and metallopeptidases [42].

One group of resistance mechanisms involve tumor characteristics that inhibit apoptosis in a general manner such as reduced caspase expression (e.g., epigenetic silencing of caspase-8 expression in aggressive neuroblastoma [43] and some glioblastomas [44]), increased expression of caspase inhibitors such as XIAP [45], cIAP2 [46] or over-expression of Bcl-2 (**Figure 3**) [47] and other inhibitors of the mitochondrial apoptosis pathway such as myeloid cell leukemia sequence 1 (Mcl-1) [46].

The second group of resistance mechanisms includes mechanisms that are more specific for TRAIL (or death receptor) signaling such as defects in the TRAIL receptors themselves or increased expression of inhibitors that are very selective for death receptors such as FLIP or the decoy receptors TRAIL-R3 and TRAIL-R4. FLIP is a homolog of caspase-8 with mutations in the catalytic domain that prevent its activation as a protease. Its recruitment to the DISC can block TRAIL-induced apoptosis [48]. FLIP expression is controlled by a few other important oncogenic pathways; for example, Myc regulation of TRAIL sensitivity can be achieved through Myc's ability to repress FLIP expression [50]. cFLIP may also promote cell survival by activating the NF-κB and ERK signaling pathways [51]. The decoy receptors block TRAIL signaling both by competing for binding with TRAIL and by forming stable complexes with the signaling receptors to form inactive receptor complexes thus preventing cell death [52]. Epigenetic silencing of TRAIL receptor expression has been reported [53] as well as somatic mutations in TRAIL receptors [54]. Somatic mutations can have a 'dominant-negative' phenotype whereby not only is the mutant receptor unable to send signals that induce apoptosis it also inhibits the ability of wild-type receptors in the same cell to signal cell death [55]. More importantly it was demonstrated that the underlying mechanism by which this dominant-negative effect was achieved predicted that in cells with mutant TRAIL-R2 but functional TRAIL-R1 the mutant receptor would block signaling by TRAIL-R2-specific agonists and TRAIL but would not inhibit signaling by TRAIL-R1-specific agonists. This provides a rationale for selection of the agonistic anti-TRAIL-R1 antibody like mapatumumab (from Human Genome Sciences) rather than other therapeutic agents for killing of those cells [55]. Although tumor cells may express both TRAIL-R1 and TRAIL-R2, apoptosis signaling can occur preferentially through one or the other receptor even when the other receptor is present and not mutated, depending upon the cell type. For example in chronic lymphocytic leukemia and mantle cell lymphoma, it was found that primary tumor cells that express both TRAIL-R1 and TRAIL-R2 could only be killed by agonists that activate TRAIL-R1 [56].

A novel method of resistance involving expression of the peptidyl *O*-glycosyltransferase *N*-acetylgalactosaminyltransferase 14 (GALNT14) was recently identified [57]. Loss of this enzyme correlated with reduced sensitivity to TRAIL because *O*-glycosylation of TRAIL-R1 or TRAIL-R2 promotes the ligand-stimulated clustering of receptors leading to a more efficient recruitment and activation of caspase-8. Because GALNT14 expression in multiple cell lines appeared to be a better predictor of TRAIL sensitivity or resistance than other regulators of the pathway such as c-FLIP, Bcl-2 and XIAP, it was suggested that screening for expression of GALNT14 could be used as a strategy to select patients who are more or less likely to benefit from treatment – 10% of lobular breast tumors and up to 30% of lung tumors expressed high levels of GALNT14 and would therefore be expected to be particularly likely to be sensitive to TRAIL [57].

Inhibition of PI3K/AKT pathway sensitizes tumor cells to TRAIL treatment or can even reverse TRAIL resistance [58]. TRAIL stimulates the phosphorylation of the serine/threonine kinase PKB/Akt depending on PI3K activation [59]. It was shown that inhibition of PKB/Akt sensitized HUVECs to TRAIL-mediated caspase-dependent apoptosis. In the same cell system, TRAIL stimulated the ERK- but not the p38- or the JNK-pathway and induced a remarkable increase in endothelial cell proliferation in an ERK-dependent manner [59].

Sine oculis homeobox homolog 1 (Six1), a homeobox transcription factor that is not expressed in most normal adult

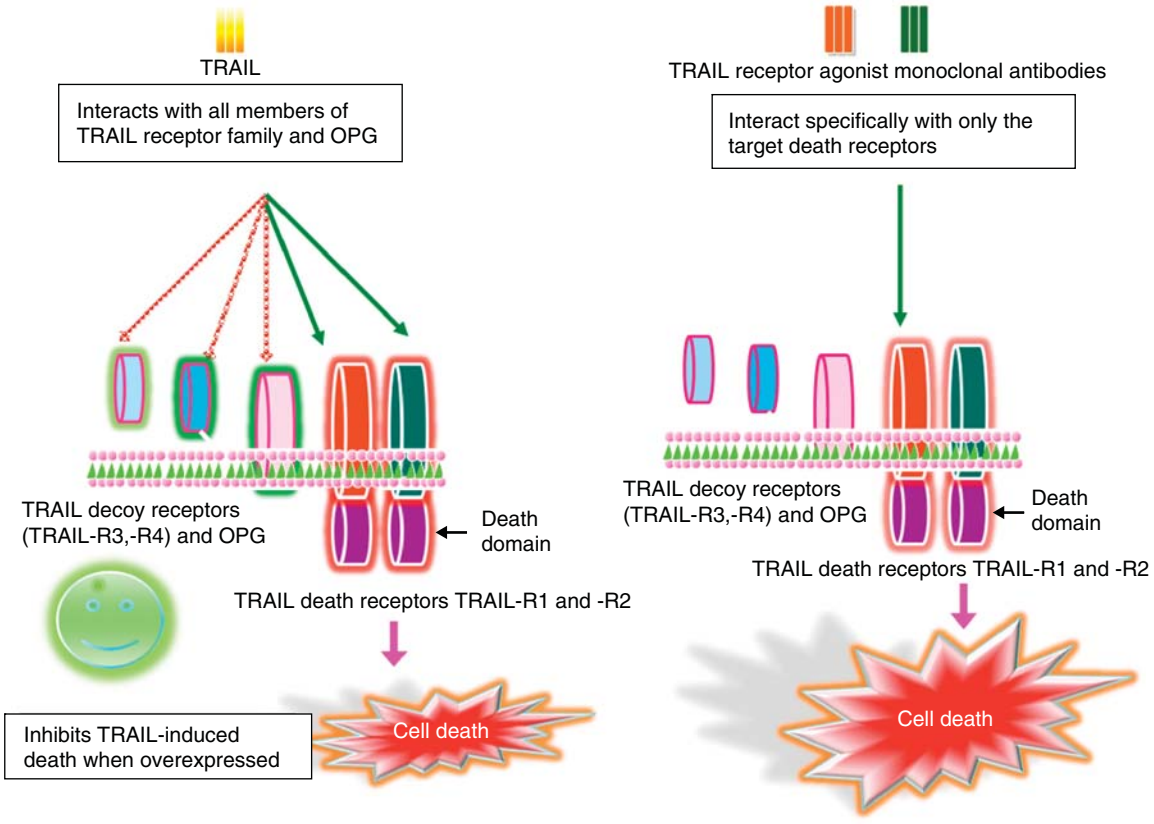

Figure 2. Selective targeting of TRAIL receptor agonist monoclonal antibodies. TRAIL interacts with all members of TRAIL receptor family including TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4 and osteoprotegerin (OPG). TRAIL binds to the death receptors TRAIL-R1 and TRAIL-R2 initiating cell death as well as the decoy receptors TRAIL-R3, TRAIL-R4 and OPG. The decoy receptors either lack the intracellular domain or contain a mutated intracellular domain and consequently do not initiate cell death. TRAIL receptor agonist antibodies selectively target the death receptors TRAIL-R1 and TRAIL-R2 causing cell death.

tissues but is often re-expressed in tumors causes marked resistance to TRAIL-induced apoptosis while having little effect on killing by another death receptor agonist, Fas ligand [60]. More importantly, Six1 expression is correlated with metastasis in breast cancer, where it is overexpressed in 50% of primary breast cancers, but an even greater 90% of metastatic lesions [61]. Furthermore, mir-185 sensitizes Six-1 overexpressing resistant cancer cells to apoptosis in general and TRAIL in particular [62]. Thus Six1 expression may represent a mechanism of TRAIL resistance that is very common in certain advanced metastatic cancers.

4. Modulation of TRAIL receptor signaling by cancer therapeutic agents

There are very few agents that are truly cancer-cell-specific in terms of efficacy or cell death induction. In recent years agents like Gleevec (imatinib), Tarceva (erlotinib) have been developed to target cancer specific mutant oncogenic proteins. TRAIL is an example of a targeted therapy that kills cancer cell lines but not most normal cells [63]. Soluble recombinant TRAIL as well as agonistic antibodies targeting either TRAIL-R1 or TRAIL-R2 can be applied to activate the extrinsic TRAIL-mediated pathway in tumor cells.

4.1 Soluble recombinant TRAIL as a cancer therapeutic agent

In order to use TRAIL-receptor agonists as anti-cancer drugs in clinical settings, they require high anti-tumor activity with low toxicity for normal cells.

A variety of recombinant forms of human TRAIL have been designed, each of them encoding the extracellular domain of human TRAIL. In addition, some preparations were generated that have been amino-terminally fused to distinct tags, including a poly-histidine tag [64], the FLAG epitope (which can be further cross-linked by anti-FLAG antibodies) [65] and the leucine zipper [38] or isoleucine zipper trimerization domain [66].

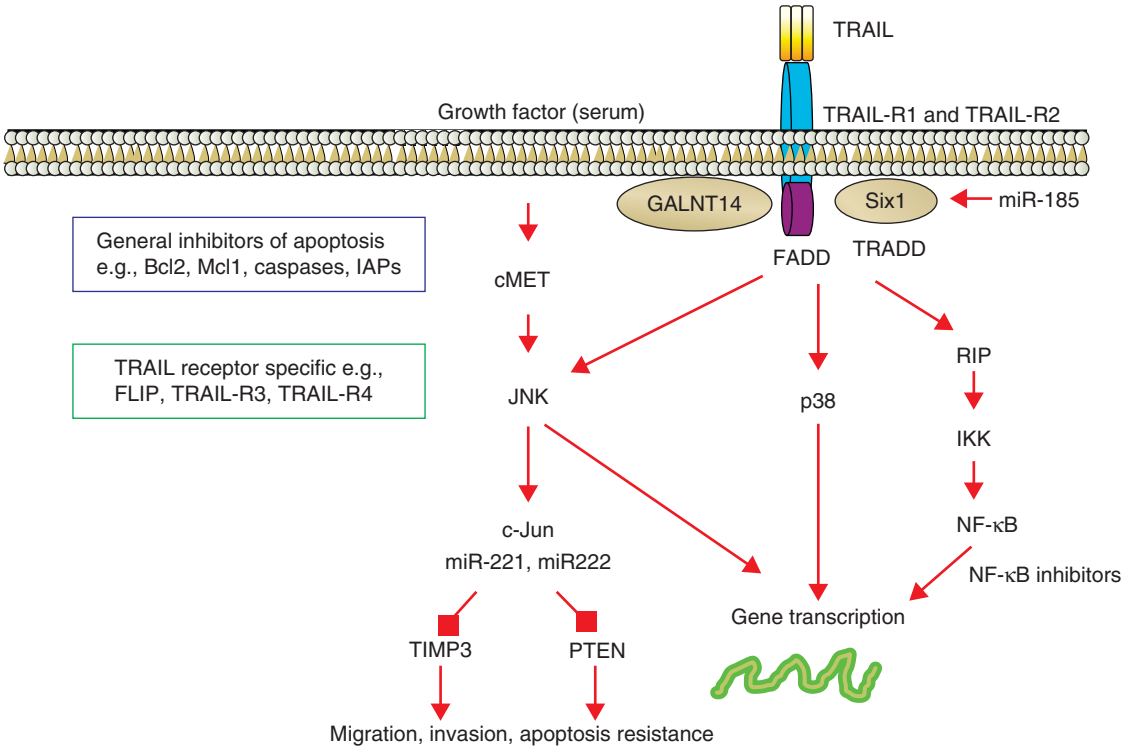

Figure 3. Molecules leading to TRAIL resistance. One group of resistance mechanisms include general inhibitors of apoptosis including B cell leukaemia/lymphoma-2 (Bcl-2) and myeloid cell leukemia sequence 1 (Mcl-1). The second group of resistance mechanism includes TRAIL-specific signaling like cellular FAS-associated protein with death domain-like interleukin converting enzyme-like inhibitory protein (FLIP) or TRAIL-R3 and TRAIL-R4. Dissociation of FAS-associated protein with death domain (FADD) and caspase 8 can lead to survival through various pathways including NF-κB, p38 and JNK. MET induces miR-221 and 222. Loss of *N*-acetylgalactosaminyltransferase 14 (GALNT14) is correlated with a reduced sensitivity to TRAIL because *O*-glycosylation of TRAIL-R1/-R2 promotes the ligand-stimulated clustering of receptors leading to a more efficient recruitment and activation of caspase 8. In addition there is emerging information on regulation of the TRAIL pathway by microRNAs (miRs). Activation through activator protein 1 (AP-1) (c-Jun) which induces the miRs leading to rise in phosphatase and tensin homologue (PTEN) and tissue inhibitor of metalloproteinase 3 (TIMP3) downregulation and subsequent apoptosis resistance, cellular migration and invasion. Sine oculis homeobox homolog 1 (Six1) is often re-expressed in tumors causing marked resistance to TRAIL-induced apoptosis while having little effect on killing by another death receptor agonist, Fas ligand. mir-185 sensitizes Six-1 overexpressing resistant cancer cells to apoptosis in general and TRAIL in particular.

IKK: Inhibitor of NF-κB kinase; RIP: Receptor interacting protein; TRADD: Tumor necrosis factor receptor type 1 associated death domain protein.

In vitro toxicities were observed for some recombinant forms of TRAIL which are most likely to represent either cell culture artefacts or to be related to the specific recombinant proteins used in these particular studies. Since non-tagged TRAIL exhibits lower anti-tumour activity compared with tagged versions, it thereby also features the lowest potential for toxicity to normal cells [66]. Leucine zipper- and isoleucine zipper-TRAIL represent an intermediate state as they on the one hand possess similar anti-tumor activity to FLAG-TRAIL and His-TRAIL *in vitro*, but are on the other hand less toxic than the latter, highly aggregated preparations.

A major drawback and therefore also a limitation of TRAIL-based anti-cancer therapies is the need for huge amounts of recombinant, soluble TRAIL which has a relatively short half-life *in-vivo*. A possible alternative to overcome this demand includes the generation of a replication-deficient adenovirus encoding the human TRAIL (Ad5-TRAIL). Administration of this adenovirus at the site of tumor implantation dramatically inhibited the outgrowth of human prostate tumor xenografts in SCID mice [67]. While treatment with soluble, recombinant TRAIL requires consecutive applications due to its short half-life of about 30 min [38], a single Ad5-TRAIL localized therapy leads to regionally high, sustained concentration levels of TRAIL. Although only minimal toxicity of adenoviral injections into the prostate has been observed so far [68], more studies are required to demonstrate the safety and efficacy of adenoviral systems in anti-cancer therapy.

4.2 Anti-TRAIL-R1 and TRAIL-R2 mAbs as cancer therapeutic agents

While soluble recombinant TRAIL may bind to all TRAIL-receptors, including TRAIL-R3 and -R4, antibodies targeting TRAIL-R1 or -R2 only bind to their respective apoptosis-inducing receptor. Thus, if tumor cells were overcoming TRAIL sensitivity by expression of TRAIL-R3 (DcR1) and -R4 (DcR2) or by signaling events mediated by any of the other TRAIL-receptors which are not triggered by the respective antibody, such a TRAIL-R1 (DR4) or TRAIL-R2 (DR5)-specific agonist would still be able to transmit the death signal despite the expression of other receptors [69].

In contrast to the short plasma half-life of recombinant ligands (approximately 30 min in non-human primates), antibodies are characterized by an improved localization within the tumor site due to their significantly increased half-life (approximately 1 – 2 weeks). Although antibody-mediated tumor cell killing might be prolonged in patients, the risk of toxic side effects is increased at the same time. Human studies directly comparing the anti-tumor efficiency and toxicity of TRAIL-R1- (DR4) and -R2 (DR5)-specific antibodies to soluble recombinant TRAIL have not been carried out. Due to significant tumor penetration, recombinant Apo2L/TRAIL possesses high anti-tumor activity *in vivo* [70].

4.3 Combination of TRAIL receptor targeting agents and other cancer therapeutics

TRAIL or TRAIL-R agonist mAbs can be combined with diverse chemotherapeutic drugs or radiotherapy to induce synergistic cell death of tumor cells. There are a number of drugs that have been found to enhance TRAIL-induced apoptosis by upregulating TRAIL-R1 or TRAIL-R2. A summary and the mechanism of drugs upregulating the death receptors is shown in **Table 1**.

4.4 Barrier to clinical therapy

It is clear that the potential toxicity of therapy versus the efficacy of tumor treatment can be limiting in patients. Induction of apoptosis in normal human cells, such as hepatocytes or keratinocytes, by some rTRAIL and anti-TRAIL-R mAbs *in vitro* has been reported [71-74] and two studies have recently shown that the TRAIL–TRAIL-R2 pathway may contribute to hepatotoxicity and bile duct toxicity in mice treated with anti-mouse TRAIL-R2 mAb [75]. Thus, administration of rTRAIL or agonistic anti-TRAIL-R mAbs have the potential to induce cytotoxicity in some normal cells in patients. Hepatotoxicity with increased serum alanine aminotransferase, aspartate aminotransferase and bilirubin was reported in a few patients when treated with higher doses (20 mg per kg) of lexatumumab (TRAIL-R2 agonist antibody from Human Genome Sciences) [76]. This further supports the recent mouse studies of Takeda and colleagues showing that the anti-TRAIL-R2 mAb can induce cholangitis and cholestatic liver injury in mice [75]. Even if this unfavorable effect were observed only in a dose-limiting manner and using one anti-human anti-TRAIL-R mAb, the mechanisms underlying hepatotoxicity would need to further study, as this will provide novel information for safer treatment with anti-TRAILR2 (DR5) mAbs in humans in the future. Intriguingly, it was recently reported that TRAIL strongly induced apoptosis in explants from steatotic and hepatitis C virus-infected livers [77]. The authors of this study have therefore cautioned care in the use of TRAIL in patients with inflammatory liver diseases. In an older study, Ozoren and colleagues showed that inhibitors of caspase 9 may protect human hepatocytes from TRAIL [78].

4.5 Clinical application of TRAIL-R mAbs

Due to the promising pre-clinical results of TRAIL as an anti-cancer agent several companies are developing TRAIL-receptor-targeting therapeutics. These TRAIL receptor agonists (TRAs) are currently being tested in Phase I and II clinical trials. To date, one recombinant ligand (from Genentech), one anti-TRAIL-R1 (from Human Genome Sciences (HGS, Rockville MD, USA)), five anti-TRAIL-R2 antibodies alone or in combination with distinct chemotherapeutics and Ad5-TRAIL are being evaluated [79].

HGS has completed three Phase II clinical trials of HGS-ETR1 (mapatumumab) as monotherapy, and has initiated additional Phase II and Phase Ib combination studies of HGS-ETR1 to evaluate its potential for the treatment of specific cancers.

HGS was the first company to test a TRA in clinical trials and is currently investigating the activity and toxicity of two fully humanized monoclonal antibodies activating TRAIL-R1 (HGS-ETR1; Mapatumumab) and TRAIL-R2 (HGS-ETR2; Lexatumumab), respectively [80,81]. In pre-clinical models, both antibodies rapidly increased the activation of cell death cascades and potently induced apoptosis across a wide range of human tumor cell lines and primary cells from both solid and hematological malignancies [82,83].

So far, the activity of HGS-ETR1 could be validated in three Phase II clinical studies with patients suffering from non-Hodgkin's lymphoma (NHL), colorectal cancer (CRC) and NSCLC [84,85]. Around 30% of the patients enrolled in the NSCLC or CRC studies exhibited stable disease after treatment with HGS-ETR1 as monotherapy. Clinical response or stable disease was observed in 14 out of 17 patients with NHL diagnosed with follicular lymphomas. At the same time, no dose limiting toxicity, even at the highest doses applied (10 mg/kg), could be noticed in these clinical trials.

Moreover, Phase Ib clinical studies were initiated investigating the anti-tumor effect of HGS-ETR1 in combination with different panels of chemotherapeutic agents. From 32 patients with advanced tumors treated with HGS-ETR1 in combination with gemcitabine and cisplatin, 26 of the 37 patients with measurable disease showed a decrease in tumor lesions, and 12 patients achieved a partial response [86]. The observed adverse effects may be attributable to the toxicity profile associated with the concomitantly applied

Table 1. Death receptor regulation by various therapeutic agents in different cell types.

Receptor upregulated	Therapeutic agent used	Mechanism of action	Tumor type
TRAIL-R1	Radiotherapy	Induction of cell cycle arrest and apoptosis	Leukemia [101]
TRAIL-R1	HDAC inhibitors	Enables transcription by blocking gene acetylation	Leukemia [102]
TRAIL-R2	HDAC inhibitors	Histone deacetylase inihbition	Malignant tumor cells [103]
TRAIL-R2	Proteasome inhibitors	Inhibits protein degradation	Prostate [104], colon [105], lung [106]
TRAIL-R2	Radiotherapy	Induction of DNA damage	Prostate [106], leukemia [107], breast, lung, colon [108]
TRAIL-R2	NSAIDs	Inhibition of COX-1 and 2	Colon [109], lung [110], prostate and colon [111]
TRAIL-R1 and TRAIL-R2	Proteasome inhibitors	Inhibits protein degradation	Leukemia [112]
TRAIL-R1 and TRAIL-R2	HDAC inhibitors	Enables transcription by blocking gene deacetylation	Multiple myeloma [113], breast [114]
TRAIL-R1 and TRAIL-R2	NSAIDs	Inhibits COX 1 and 2	Colon [115]
TRAIL-R1 and TRAIL-R2	Doxorubicin	DNA intercalation and interrupts DNA replication	Sarcoma [116], renal [117]
TRAIL-R1 and TRAIL-R2	Kinase inhibitor lapatinib	Mechanism unknown	Colon cancer [118]

chemotherapeutics. Accordingly, the combination of HGS-ETR1 with paclitaxel and carboplatin showed that five patients (19%) achieved a confirmed radiologic partial response (including one pathological complete response), and 12 patients (44%) had stable disease as their best response [87]. Dose limiting toxicities (DLT) observed were neutropenic fever (attributed to chemotherapy) and hypersensitivity (attributed to HGS-ETR1) [87].

The efficacy and safety of HGS-ETR1 in combination with the proteasome inhibitor bortezomib in patients with advanced multiple myeloma is currently under investigation in Phase II clinical studies (National Cancer Institute (NCI) Clinical Trials Identifier Number: NCT00315757).

Abdulghani *et al.* 2007 have found that sorafenib in combination with agonist antibodies (lexatumumab and mapatumumab) enhances cell death in hepatocellular carcinoma (HCC) *in vitro* [88]. Previously hepatotoxicity with increased serum alanine aminotransferase, aspartate aminotransferase and bilirubin was reported in a few patients when treated with higher doses (20 mg per kg) of lexatumumab. Therefore, mapatumumab in combination with sorafenib is now being evaluated in a two-stage, multi-center open label, dose escalation study in subjects with advanced hepatocellular carcinoma (NCI Clinical Trials Identifier Number: NCT00712855).

HGS-ETR2, the TRAIL-R2-specific antibody developed by HGS, is currently being evaluated in Phase Ib clinical trials in combination with chemotherapeutic agents [89]. In particular, the combination with folinic acid, fluorouracil and irinotecan (FOLFIRI) and doxorubicin was well tolerated and associated with the induction of tumor shrinkage and partial responses in some gastric malignancies. However, several adverse effects including anemia, fatigue and dehydration were related to HGS-ETR2 treatment. Nevertheless, HGS-ETR2 could be safely administered, making further evaluations in combination with chemotherapy warranted. Interestingly, pre-clinical evaluation demonstrated a complete regression of different tumor cell line xenografts *in vivo* upon combinatorial treatment with HGS-ETR2 and the Smac mimetic SM-164 (Ascenta Therapeutics, Malvern, PA, USA) [90].

Daiichi Sankyo (Tokyo, Japan) is developing a TRAIL-receptor agonist for the treatment of advanced solid tumors and lymphomas. TRA-8/CS-1008, a humanized anti-TRAIL-R2 antibody, exhibited high anti-tumor activity against astrocytoma and leukemia cells *in vitro* and engrafted breast cancer cells *in vivo* [91,92]. A Phase I clinical trial has been completed in patients with advanced solid malignancies and lymphoma (without leukemic component) [93]. Phase II trials using CS-1008 with gemcitabine in untreated and unresectable pancreatic cancer and another combination trial with paclitaxel and carboplatin in ovarian cancer are underway (NCI Clinical Trials Identifier Number: NCT00521404).

The chimeric TRAIL-R2-targeting antibody LBY135 from Novartis induced apoptosis in 50% of a panel of 40 human colon cancer cell lines with an IC_{50} of 10 nM or less. Since the *in-vivo* anti-tumor activity of LBY135 has been verified in human colorectal xenograft models in mice [94], Novartis is recruiting patients for an open-label, multi-center, two-arm Phase I/II trial of LBY135 alone and in combination with capecitabine in advanced solid tumors (Nevada Cancer Institute, Las Vegas, NV, USA) [95].

The fully humanized monoclonal anti-TRAIL-R2 antibody Apomab generated by Genentech (South San Francisco, CA, USA) is currently being investigated in Phase I and II clinical trials in advanced cases of solid tumors. Pharmacokinetic studies have indicated that Apomab is generally well-tolerated even at doses up to 20 mg/kg, yet two disparate dose-limiting toxicities, that is one asymptomatic transaminitis and one pulmonary embolism developed among eleven patients treated with 10 mg/kg [96]. However, a 28% shrinkage of the target lesions and symptomatic improvement could be shown in at least 1 out of 6 patients receiving at least four cycles of Apomab [79,97]. Accordingly, a Phase II study with Apomab as monotherapy for sarcoma or in combination with Avastin against NSCLC was initiated in 2007 and a further treatment of NHL in combination with rituximab, the CD20-targeting antibody is underway [98].

AMG655 (TRAIL-R2), another fully human antibody targeting TRAIL-R2 which was developed by Amgen (Thousand Oaks, CA, USA) is currently being tested in Phase Ib clinical studies. The anti-tumor activity of AMG655 was confirmed by partial response in NSCLC and a metabolic partial response in colorectal cancer. Although no dose limiting toxicity or severe side effects have been reported so far even when administered at doses of up to 20 mg/kg every two weeks, adverse effects including fever, fatigue and hypomagnesaemia were observed in nine patients enrolled in the study [99]. A combination of AMG655 with chemotherapy might further enhance the anti-tumor responses, making the analysis of such treatment protocols warranted. Amgen is recruiting patients for a study of AMG 655 in combination with AMG 479 [fully human monoclonal antibody that targets type 1 IGF receptor (IGF-1R)] in advanced, refractory solid tumors. This is a multi-center, 2-part Phase Ib/II study of AMG 655 in combination with AMG 479 to be conducted in the United States and Spain. Part 1 is a dose escalation segment to identify a dose of AMG 655 in combination with AMG 479 that is safe and tolerable. Part 2 will evaluate the safety and estimate the efficacy of AMG 655 at the dose selected in Part 1 in combination with AMG 479 for the treatment of patients with advanced NSCLC (non-squamous histology; squamous histology), CRC, pancreatic cancer, ovarian cancer, and sarcoma (Clinical Trials Identifier number: NCT00819169).

Furthermore, Genentech and Amgen are jointly developing a recombinant untagged version of human TRAIL (Apo2L or AMG951), which is currently the only TRAIL receptor ligand being evaluated in clinical trials. Phase Ib/II pharmacokinetic and safety studies in patients suffering from low-grade NHL have already shown Apo2L/TRAIL to be active and safe both, alone or in combination with rituximab, without any incidence of dose limiting toxicity or severe side effects (**Table 2**). In combination with rituximab, Five NHL subjects have undergone tumor response assessment: there have been two patients with complete response, one with partial response and two with stable disease [100].

5. Expert opinion

Cancer therapeutics targeting the TRAIL/TRAIL receptor signaling pathway hold great promise for molecularly targeted pro-apoptotic anti-cancer therapy. Since the initial identification of TRAIL and TRAIL-Rs a little over a decade ago there has been continued progress in understanding how TRAIL selectively kills different tumor cells, to identify and molecularly decipher the pro- and anti-apoptotic pathways that are activated following TRAIL-R ligation and to develop therapeutic agents that can engage the cell death pathway.

The development of rTRAIL and agonistic anti-TRAIL-R mAbs has rapidly advanced and encouraging clinical trial results have been reported in the last few years, but questions still remain regarding the role(s) of the decoy TRAIL-Rs and the cellular and molecular contexts in which activation of NF-κB, PI3K–Akt and other signal transduction pathways impinge on the physiological and therapeutic activities of TRAIL. Moreover, there is experimental evidence that ligation of TRAIL-Rs in cells that cannot initiate an apoptotic signal results in activation of pathways that may stimulate cell proliferation, survival and migration, and the physiological effects of such situations need to be fully appreciated. Answers to these questions will undoubtedly provide a clearer scientific rationale for combining agents that activate the TRAIL pathway with small molecules or biologicals that are most likely to be therapeutically advantageous. There are some agents that induce the expression of TRAIL-R1 and TRAIL-R2 as shown in **Table 1**. Therefore its possible that these drugs can be used in combination with TRAIL receptor agonists to enhance cell death. Moreover there are a number of Phase II clinical trials with other combinations sponsored by Genentech, Human Genome Sciences, Amgen and Daiichi Sankyo, Inc. on the horizon. These combinatorial therapies under consideration may develop into effective regimens for cancer therapy in specific tumor types.

It is still not clear which molecular mechanisms contribute to the determination of TRAIL sensitivity between normal versus cancer cells. Defining the context in which TRAIL may eliminate or spare normal tissues and why to a large extent most normal cells are generally resistant to TRAIL is of great importance when considering manipulation of the TRAIL pathway in novel cancer therapy. It is crucial to find effective ways to overcome the resistance to TRAIL or conventional chemotherapy at multiple levels, including death receptors and the intrinsic pro-apoptotic or pro-survival pathways. There is no clear indication yet whether targeting TRAIL-R1 or TRAIL-R2 or both receptors might be the best approach. With the ongoing clinical trials a better picture can be obtained which would enable us to compare the anti-tumor efficacy of wild type rhTRAIL to TRAIL-R1/TRAIL-R2 agonist mAbs. Whether it is possible to develop a recombinant TRAIL that would bind to TRAIL-R1 and TRAIL-R2 but not the decoy receptors for clinical use

Table 2. TRAIL-based clinical trials.

Study	Cancer	Intervention	Identifier/sponsors/phase	Status
A Phase Ib, open-label, dose-escalation study of the safety and pharmacokinetics of multiple doses of dulanermin administered intravenously in combination with Camptosar®/Erbitux® chemotherapy or the folfiri regimen with or without bevacizumab in subjects with previously treated metastatic colorectal cancer	Colorectal cancer	APO2L/TRAIL; cetuximab; irinotecan	NCT00671372 Genentech; Phase I	Active, not recruiting
A Phase Ib/II, open-label, multicenter study of the safety, pharmacokinetics, and efficacy of dulanermin administered intravenously in combination with rituximab to subjects with follicular and other low-grade, $CD20^+$, B-cell non-hodgkin's lymphomas that have progressed following previous rituximab therapy	Non-hodgkin's lymphoma	APO2L/TRAIL; rituximab	NCT00400764 Genentech; Amgen; Phase I/II	Active, not recruiting
A Phase Ib study of the safety and pharmacokinetics of dulanermin administered in combination with the FOLFOX regimen and bevacizumab in patients with previously untreated, locally advanced, recurrent, or metastatic colorectal cancer	Metastatic colorectal cancer	APO2L/TRAIL; bevacizumab; FOLFOX	NCT00873756 Genentech; Phase I	Recruiting
A study of AMG 951 [rhApo2L/TRAIL] in subjects with previously untreated non-small cell lung cancer (NSCLC) treated with chemotherapy +/- bevacizumab	NSCLC	Bevacizumab; AMG 951 (rhApo2L/TRAIL); carboplatin; paclitaxel	NCT00508625 Amgen; Genentech; Phase II	Active, not recruiting
Study of TRM-1(TRAIL-R1 monoclonal antibody) in subjects with relapsed or refractory non-small cell lung cancer (NSCLC)	NSCLC	TRAIL-R1 mAb (TRM-1;HGS-ETR1)	NCT00092924 Human Genome Sciences; Phase II	Completed
Study of TRM-1 (TRAIL-R1 monoclonal antibody) in subjects with relapsed or refractory non-hodgkin's lymphoma (NHL)	Lymphoma, non-hodgkin	TRAIL-R1 mAb (TRM-1; HGS-ETR1)	NCT00094848 Human Genome Sciences; Phase II	Completed
A study of mapatumumab in combination with sorafenib in subjects with advanced hepatocellular carcinoma	Hepatocellular carcinoma	Mapatumumab; sorafenib	NCT00712855 Human Genome Sciences; Phase I/II	Recruiting
A study of mapatumumab in combination with paclitaxel and carboplatin in subjects with non-small cell lung cancer	NSCLC	Mapatumumab; paclitaxel; carboplatin	NCT00583830 Human Genome Sciences; Phase II	Active, not recruiting

Table 2. TRAIL-based clinical trials (continued).

Study	Cancer	Intervention	Identifier/sponsors/phase	Status
Study of mapatumumab in combination with bortezomib (Velcade) and bortezomib alone in subjects with relapsed or refractory multiple myeloma	Multiple myeloma	Mapatumumab; bortezomib	NCT00315757 Human Genome Sciences; Phase II	Active, not recruiting
Lexatumumab with or without recombinant interferon gamma in treating young patients with solid tumors or lymphoma that have relapsed or not responded to treatment	Kidney cancer; lymphoma; neuroblastoma; sarcoma; unspecified childhood solid tumor, protocol specific	Lexatumumab; recombinant IFN-γ; genetic: protein expression analysis; flow cytometry; immunoenzyme technique; immunohistochemistry staining method; laboratory biomarker analysis	NCT00428272 National Cancer Institute (NCI); Phase I	Active, not recruiting
A Phase Ib/II open label, dose escalation study of AMG 655 in combination with AMG 479 in subjects with advanced, refractory solid tumors	Colorectal cancer; locally advanced; metastatic cancer; NSCLC; ovarian cancer; pancreatic cancer; sarcoma; solid tumors	AMG 479 (human monoclonal antibody against IGF-1R); AMG 655 (TRAIL-R2)	NCT00819169 Amgen; Phase I/II	Recruiting
Study of CS-1008 in patients with advanced solid malignancies and lymphomas (without leukemic component)	Malignancies; lymphoma	CS-1008 (humanized anti-TRAIL-R2 antibody)	NCT00320827 Daiichi Sankyo, Inc.; Phase I	Completed
Open-label study of CS1008 for subjects with untreated and unresectable pancreatic cancer	Pancreatic cancer	CS-1008 (humanized anti-TRAIL-R2 antibody) plus gemcitabine	NCT00521404 Daiichi Sankyo, Inc.; Phase II	Active, not recruiting
Combination chemotherapy with CS-1008 to treat ovarian cancer	Ovarian cancer	CS-1008; paclitaxel; carboplatin	NCT00945191 Daiichi Sankyo, Inc.; Phase II	Recruiting
Phase Ib lymphoma study of AMG 655 in combination with bortezomib or vorinostat	Hodgkin's lymphoma; low grade lymphoma; lymphoma; mantle cell lymphoma; non-hodgkin's lymphoma; diffuse large cell lymphoma	AMG 655; vorinostat; bortezomib	NCT00791011 Amgen; Phase I	Recruiting

Table 2. TRAIL-based clinical trials (continued).

Study	Cancer	Intervention	Identifier/sponsors/phase	Status
Phase Ib/II study of AMG 655 with mFOLFOX6 and bevacizumab for first-line metastatic colorectal cancer	Metastatic colorectal cancer; colon cancer; colorectal cancer; rectal cancer	Placebo; AMG 655	NCT00625651 Amgen; Phase I/II	Active, not recruiting
Phase II safety & efficacy of FOLFIRI in combination with AMG 479 or AMG 655 vs FOLFIRI in KRAS-mutant metastatic colorectal carcinoma	Metastatic colorectal cancer	FOLFIRI; AMG 655; AMG 479; placebo	NCT00813605 Amgen; Phase II	Recruiting
Phase Ib/II study of AMG 655 with doxorubicin for the first-line treatment of unresectable soft tissue sarcoma	Locally advanced or metastatic, unresectable soft tissue sarcoma; sarcoma; soft tissue sarcoma	AMG 655; placebo	NCT00626704 Amgen, Phase I/II	Active, not recruiting
A study of AMG 655 or AMG 479 in combination with gemcitabine for treatment of metastatic pancreatic cancer	Adenocarcinoma of the pancreas; metastatic pancreatic cancer; pancreatic cancer	Placebo; AMG 479; AMG 655	NCT00630552 Amgen, Phase I/II	Recruiting

remains to be seen. Also recent data suggest that there may be multiple biomarkers (e.g., GALNT14 and Six1) that might influence TRAIL sensitivity and which might be useful to select patients who are most likely to benefit from a specific treatment regimen. There are currently no studies that take into consideration the biomarker profile of the patient as to who may or may not respond to a particular regimen. Furthermore, several studies have reported increased serum level of soluble TRAIL in patients with systemic lupus erythematosus. The levels of TRAIL may also serve as a potential marker in determining the sensitivity to IFN treatment for human multiple sclerosis patients. A design that pre-selects the patients most likely to benefit from rhTRAIL/agonist antibody therapy by identifying suitable biomarkers will be helpful in fulfilling the promise of personalized medicine for the exploitation of the TRAIL/TRAIL receptor pathway in cancer therapy and other autoimmune disorders.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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