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Review Article

# Therapeutic targeting of TRAIL death receptors

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The discovery of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) along with its potent and selective antitumor effects initiated a decades-long search for therapeutic strategies to target the TRAIL pathway. First-generation approaches were focused on the development of TRAIL receptor agonists (TRAs), including recombinant human TRAIL (rhTRAIL) and TRAIL receptor-targeted agonistic antibodies. While such TRAIL pathway-targeted therapies showed promise in preclinical data and clinical trials have been conducted, none have advanced to FDA approval. Subsequent second-generation approaches focused on improving upon the specific limitations of first-generation approaches by ameliorating the pharmacokinetic profiles and agonistic abilities of TRAs as well as through combinatorial approaches to circumvent resistance. In this review, we summarize the successes and shortcomings of first- and second-generation TRAIL pathway-based therapies, concluding with an overview of the discovery and clinical introduction of ONC201, a compound with a unique mechanism of action that represents a new generation of TRAIL pathway-based approaches. We discuss preclinical and clinical findings in different tumor types and provide a unique perspective on translational directions of the field.

## Introduction

The evasion of apoptosis was recognized in the late 1980s as a mechanism of cancer development and progression and was included in 2000 among the original key hallmarks of cancer. Since then, much work has been done to pursue the targeted activation of apoptotic pathways in cancer cells as a viable therapeutic strategy [1,2].

Apoptosis itself was first identified as a controlled cell death pathway in 1972, and years since have seen the emergence of two broad 'pathways' driving the process: cell 'intrinsic' and cell 'extrinsic' [3,4]. Though interplay between the two pathways has been observed, the intrinsic pathway largely relies on the mitochondria and the BCL-2 family of proteins to trigger apoptosis in response to internal stress borne by the cell, while for the extrinsic pathway, ligand binding to cell surface death receptors translates various death signals into a caspase cascade which ultimately leads to cell death [5] (Figure 1).

One such death receptor ligand is tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which binds death receptors DR4 and DR5 and several decoy receptors. While DR4 and DR5 are tumor cell-expressed TRAIL-binding receptors that contain death domains to trigger the induction of apoptosis, decoy receptors lack the death domain and are primarily expressed on normal cells. This ability of TRAIL to preferentially induce apoptosis in tumor cells, but not normal cells, provides a

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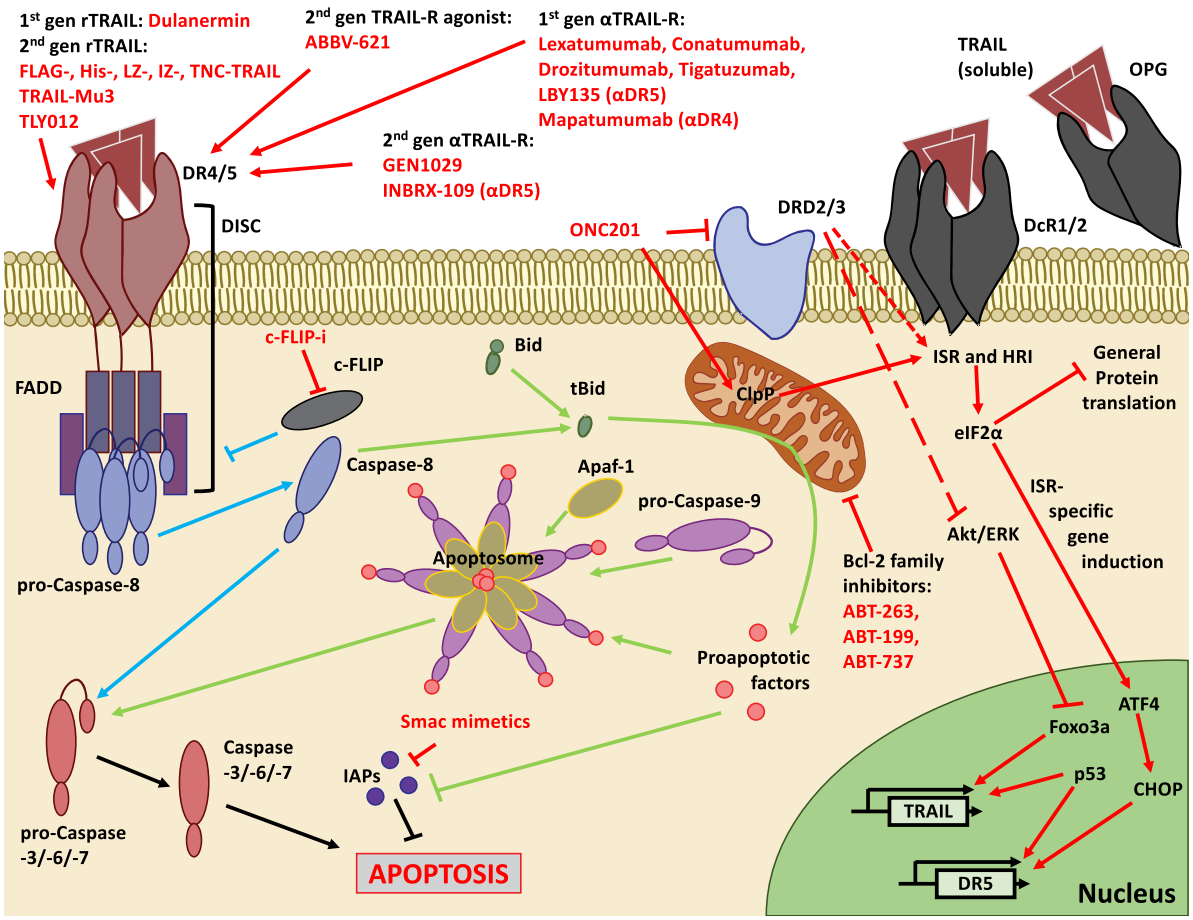


**Figure 1. Cell death signaling activated by TRAIL and TRAIL pathway therapeutics.**

TRAIL pathway signaling is initiated upon TRAIL binding to DR4 or DR5, driving receptor trimerization and recruitment of the FADD to form the DISC. DR4 and DR5 compete with decoy receptors DcR1, DcR2, and the soluble OPG for TRAIL. Activation of DR4 or DR5 triggers apoptotic signaling initially through initiator caspases, primarily caspase-8. Extrinsic apoptotic signaling (blue arrows) continues through activation of executioner caspases-3, -6, and -7, resulting in apoptotic death. Caspase-8 additionally activates intrinsic apoptotic signaling (green arrows) through facilitating conversion of Bid to tBid, driving proapoptotic factor release from the mitochondrion, apoptosome assembly, and ultimately also converging on executioner caspase activation. Several therapies, as described in the text, have been developed to engage at several points along this path (red labels and red arrows). Some, including first and second generation rTRAIL, TRAs, and antibodies, directly agonize DR4 and DR5 (unless otherwise indicated, these agonists have described activity against both DR4 and DR5). Downstream, inhibitors of c-FLIP prevent its competition with pro-caspase-8 for the FADD to enhance caspase-8 activation. Bcl-2 family inhibitors act to mitigate the function of antiapoptotic members of the Bcl-2 family, especially Bcl-2 and Bcl-xL. Smac mimetics also help to sequester anti-apoptotic IAPs. On a transcriptional level, ONC201 (initially referred to as TIC10 for TRAIL-inducing compound 10) antagonizes DRD2 and DRD3 while agonizing ClpP, ultimately bringing about growth arrest through the integrated stress response (ISR). Moreover, downstream effects of ONC201 (alongside p53) drive increased expression of TRAIL and DR5. Pointed arrows indicate activation; blunted arrows indicate inhibition.

therapeutic window. Thus, following its discovery in 1996, the tumoricidal potential of TRAIL was pursued along with a search for TRAIL-based treatments [6,7] (Figure 1). First explored were TRAIL receptor-based therapies, namely recombinant human TRAIL (rhTRAIL) and death receptor agonistic antibodies. We cloned Death Receptor 5 (DR5; KILLER/DR5) as a p53 target gene which provided a rationale for the combination of TRAIL with DNA damaging therapeutics [8]. These first-generation TRAIL treatments were found to both directly lead to tumor cell death as well as result in the sensitization of tumor cells to other chemotherapies and



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radiotherapies [9]. However, such strategies have limitations, ranging from weak binding to death receptors, to poor pharmacokinetics, to acquired resistance. As such, novel second-generation treatment strategies have been sought to address these shortcomings, working to either increase or supplement the core efficacy of TRAIL receptor-based therapies, or to prevent the acquisition of resistance and re-sensitize tumors to first-generation agents. In addition, the recent discovery and emerging clinical success of TIC10/ONC201 have led to its recognition as a 'next-generation' approach.

In this review, we provide a brief overview of key therapies exploiting the TRAIL pathway, along with their limitations and subsequent advances, concluding with a perspective on novel strategies of therapeutic TRAIL pathway-mediated induction of tumor cell death.

# TRAIL signaling pathway

TRAIL itself can exist in one of two forms: either as a transmembrane protein form expressed by various cell types, or as a soluble form composed of the shed extracellular domain of membrane-bound TRAIL which self-trimerizes [9]. As previously mentioned, both forms are capable of binding target receptors DR4 and DR5 to trigger an apoptotic signal on the death-receptor expressing cell, but may also bind membrane-bound decoy receptors DcR1 and DcR2 or soluble decoy receptor OPG [10]. Consequently, an initial point of apoptotic signal modulation exists through the expression of such decoy receptors, which ultimately can reduce TRAIL-induced death signaling through competition with DR4 and DR5. Indeed, decoy receptor expression has been implicated, among other mechanisms, in the resistance of normal cells to TRAIL [11,12] as well as in cancer cell resistance to TRAIL [13].

Cell death via the extrinsic apoptotic pathway is initiated by ligands such as TRAIL binding to their target death receptors. Upon ligand binding, the death receptors trimerize, leading to their activation. This is followed by the recruitment of the adaptor protein Fas-associated protein with death domain (FADD) as well as initiator caspases-8 and -10. Together, these molecules assemble into the death-inducing signaling complex (DISC) [14]. While lower-order death receptor clustering (i.e. trimerization) is essential to efficient DISC formation, the higher-order clustering of multiple complexes has emerged as being another important factor in apoptotic signal transduction [15]. Downstream of DISC formation, resultant activation of initiator caspase-8 leads to the activation of executioner caspases-3, -6, and -7, which leads to cell death.

In so-called Type I cells, this pathway alone is sufficient to activate the executioner caspases and induce cell death. Conversely, in Type II cells, additional amplification via the intrinsic mitochondrial apoptotic pathway is required. In these cells, Bid cleavage by caspase-8 is followed by its myristoylation and translocation to the mitochondria, where it, along with Bax and Bak, allows for the release of proapoptotic factor cytochrome C [16,17]. Cytochrome C complexes with apoptotic peptidase activating factor 1 (Apaf-1) and initiator caspase-9 to form an executioner caspase cascade-activating apoptosome. TRAIL signaling at DR5 receptors has additionally been shown to translocate proapoptotic Bim and Bax to lysosomes, resulting in a release of cathepsin B that further enhances mitochondrial outer membrane permeabilization and executioner caspase activation through the recruitment of phosphofurin acidic cluster sorting protein-2 (PACS-2) to DR5-positive endosomes [18]. C-Myc was identified as a major determinant of TRAIL sensitivity [19].

# TRAIL receptor-based therapies: first generation

An early study in the exploration of TRAIL pathway-based therapeutics assessed the expression of TRAIL receptors by immunohistochemistry in primary glioblastoma (GBM) specimens. The study showed that higher receptor expression is correlated with increased patient survival, supporting the important role played by TRAIL in commanding patient prognosis [20,21]. Thus, receptor-targeted therapies garnered much attention based on their potential as effective anticancer agents. Many first-generation TRAIL receptor-targeted agents focused on recombinant forms of TRAIL as well as agonistic antibodies against TRAIL death receptors.

## Recombinant forms of TRAIL

One strategy to target the TRAIL pathway is to develop bioactive recombinant soluble forms of TRAIL. One such example is dulanermin (AMG-951), which is a soluble recombinant human Apo2Ligand/TRAIL (rhApo2L/TRAIL) protein that induces apoptosis in target cells via its binding and activation of DR4 (i.e. TRAIL-R1) and DR5 (i.e. TRAIL-R2), though it is also known to bind to decoy receptors [22]. Developed by Amgen/Genentech, dulanermin is the only rhTRAIL to have reached clinical trials. In a phase 1 dose-escalation

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study in patients with advanced cancer, dulanermin was shown to have a tolerable safety profile; however, its observed anticancer activity was limited [22]. Of note, the mean terminal phase half-life ($t_{1/2}$) of rhTRAIL ranges from 0.56 to 1.02 h, likely restricting its ability to reach tumor cells [22,23]. Moreover, because rhTRAIL binds to decoy receptors, its delivery to DR4/5 is diluted. This weakened agonism is worsened by the fact that although rhTRAIL induces lower-order receptor trimerization, it has a limited capacity to induce higher-order receptor clustering, resulting in a weak apoptotic signal [24,25]. Meanwhile, other first-generation TRAIL pathway-based therapies were developed.

### Death receptor agonist antibodies

The development of agonistic antibodies against DR4/5 presented another strategy to target TRAIL receptors. Unlike rhTRAIL, which binds to both death receptors and decoy receptors, diluting its apoptotic effect, agonistic antibodies specifically target DR4 or DR5 to activate apoptosis in target cells. Moreover, receptor-targeted monoclonal antibodies (TRAIL-R mAbs) have a significantly longer half-life than rhTRAIL. Therefore, the development of these therapeutic antibodies was pursued as an anti-cancer strategy.

Preclinical studies showed promising results, with significant antitumor activity without toxicity seen both *in vivo* and *in vitro* [26]. Consequently, several first-generation TRAIL-R mAbs have been tested in clinical trials including DR5-targeted agents such as lexatumumab, conatumumab, drozitumab, tigatuzumab, and LBY135. DR5-targeted mAbs showed results in early clinical trials that only modestly improved upon clinical results observed with rhTRAIL. For example, in a phase 1 trial of lexatumumab in patients with advanced cancers, the mAb was well-tolerated and was associated with stable disease in several patients [27]. Safety was also demonstrated in the first-in-human study of conatumumab in adult patients with advanced solid tumors [28]. In this study, a single patient with colorectal cancer (CRC) had stable disease for 24 weeks and showed a 24% reduction in tumor size by RECIST (Response Evaluation Criteria in Solid Tumors) criteria. Drozitumab is another DR5-targeted mAb that was evaluated in a phase 1 study in patients with advanced malignancies [29]. In this study, three minor responses were observed in patients with CRC, granulosa cell ovarian cancer, and chondrosarcoma, although no objective responses were observed. Moreover, in a phase 1 trial of DR5-targeted tigatuzumab, stable disease and a tolerable safety profile were observed [30]. A DR4-targeted agent, mapatumumab, which is a fully human mAb also entered clinical trials. In a phase 1 study in patients with advanced solid malignancies, mapatumumab was observed to be well-tolerated, however, no objective responses were observed [31]. Another approach using DR4 atrimers was explored although this was not pursued in the clinic [32].

Thus, although successful in preclinical studies, TRAIL-R mAbs have performed modestly in clinical trials. Despite improving upon the pharmacokinetic ability and specificity of rhTRAIL, the agonistic ability of TRAIL-R mAbs remains limited. One factor contributing to this is that mAbs have an inherently bivalent structure, only allowing for their cross-linking to two death receptors. As lower-order receptor trimerization is required to ensure efficient DISC-formation, additional cross-linking is needed for sufficient apoptotic signaling [33]. Another limiting factor is that while first-generation TRAIL-R mAbs are DR4- or DR5-specific, there are differences in the ability of each receptor to transmit apoptotic signals that vary between tissue types [34], pointing to the need for antibodies that target both receptors.

## Improving upon TRAIL receptor-based therapies

First-generation therapies were thus divided into two categories: recombinant forms of TRAIL and receptor-specific agonistic antibodies. Although these early TRAIL receptor agonists (TRAs) were shown to be promising in preclinical data [35,36] and well-tolerated in patients [22,37], they ultimately proved to have limited clinical anticancer activity. This can be attributed to several factors, including poor pharmacokinetics, limited agonistic ability, and inherent or acquired resistance [2,38]. Second-generation TRAs were developed to target these limitations by improving upon the pharmacokinetic profile and agonistic ability of these agents and circumventing resistance through combinatorial approaches.

### TRAIL derivatives

#### N-terminal tags

An early second-generation approach was to modulate the rhTRAIL protein through the addition of N-terminal tags to improve its pharmacokinetic profile and agonistic ability. The small molecular weight of rhTRAIL is what leads to its rapid clearance via renal filtration. Adding a molecular tag to rhTRAIL increases

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its size and thus improves its half-life [34,39]. Several 'tagged' forms of rhTRAIL were developed, including the addition of Flag (FLAG-TRAIL), poly-histidine (His-TRAIL), leucine zipper (LZ-TRAIL), isoleucine zipper (IZ-TRAIL), or tenascin-C (TNC) oligomerization domain (TNC-TRAIL) [40]. Both FLAG-TRAIL and His-TRAIL were created to facilitate the purification process [38]. On the other hand, LZ-TRAIL, IZ-TRAIL, and TNC-TRAIL were all developed to stabilize rhTRAIL homotrimerization through hydrophobic interactions within the LZ, IZ, and TNC sequences [6,41,42]. This increased stability enhances the agonistic activity and apoptotic potential of rhTRAIL. Despite promising preclinical data, FLAG-TRAIL and His-TRAIL were found to induce significant apoptosis in primary human hepatocytes [43]. While LZ-TRAIL, IZ-TRAIL, and TNC-TRAIL were not found to be significantly hepatotoxic [41], concern over on-target or off-target toxicity hindered the translation of tagged rhTRAIL into the clinic.

## TLY012

Another approach to improving rhTRAIL is to covalently link it to molecules that enhance its pharmacokinetic profile. One such fusion product is TLY012, an N-terminal PEGylated rhTRAIL. Like the tags, the covalent attachment of polyethylene glycol (PEG) to a therapeutic protein increases its size, thereby reducing its clearance by renal filtration and increasing its half-life [44–46]. Indeed, TLY012 was shown to have a greatly increased half-life compared with rhTRAIL in a molecular weight-dependent manner, with 20K- and 30K-PEG-TRAIL having circulating half-lives of 12 and 18 h, respectively, compared with the 1 h half-life of rhTRAIL in ICR mice [47]. This increased half-life was associated with a greater anti-tumor effect both *in vitro* and *in vivo* in CRC models [47]. Later, TLY012 was shown also to have activity against fibrotic cells [48,49]. While TLY012 is entering clinical trials for the treatment of fibrotic diseases and was granted Orphan Drug Designation (ODD) by the FDA in 2019 for the treatment of systemic sclerosis, further exploration of its anticancer potential as a single agent and in combination has been ongoing (further discussed below).

## TRAIL-Mu3

A similar approach to enhancing the pharmacokinetic profile of rhTRAIL involves making modifications to the amino acid sequence to alter the chemical properties of the complete peptide. Mutagenesis of amino acid sequence VRERGPQR (114–121) into RRRRRRRR, for instance, has been shown to enhance the membrane-penetrating abilities of wild-type TRAIL, leading to the development of a novel membrane-penetrating TRAIL-Mu3 [50]. TRAIL-Mu3 has been shown to have enhanced efficacy both in *in vitro* and *in vivo* preclinical models against pancreatic cancers over both rhTRAIL as well as gemcitabine [50]. TRAIL-Mu3 has a greater tendency to activate caspase cascade in pancreatic cancer cell lines compared with rhTRAIL [51]. Though TRAIL-Mu3 is currently confined to the preclinical setting, its deployment into the clinic may allow for a novel therapeutic strategy against gemcitabine-resistant pancreatic cancers.

## Eftozanermin alfa

As previously noted, a major limitation of both first- and second-generation rhTRAIL, receptor-specific mAbs, and TRAIL derivatives is their inability to induce efficient lower- and higher-order receptor clustering, leading to reduced apoptotic signaling. In the case of mAbs, it has been shown that due to the bivalent structure of antibodies, additional cross-linking of the Fc region of antibodies to Fcγ receptors (FcγR) is necessary for lower-order clustering and trimerization to occur [33,52]. However, IgG is known to compete with these antibodies for this interaction. Mouse models have very low levels of IgG as compared with cancer patients, providing a likely explanation for the discrepancy between preclinical studies and clinical trials of receptor-specific mAbs [33]. With this in mind, a new family of TRAIL-R agonists was produced.

APG350 is a first-in-class prototype of Eftozanermin alfa (ABBV-621). It was designed to increase receptor clustering while remaining independent of FcγR cross-linking. The molecule is composed of two single chain TRAIL trimers dimerized via fusion to the Fc region of IgG1 and is therefore able to bind to six TRAIL receptors (both DR4 and DR5) with limited FcγR binding capability [33]. APG350 was shown to have an enhanced lower-order clustering efficiency as compared with TRAIL-R mAbs, and because it can simultaneously bind two death receptor trimers, it has a greater ability to induce higher-order receptor clustering as compared with rhTRAIL and its derivatives [53,54]. Moreover, the later developed Eftozanermin alfa has an estimated human half-life of ∼2 days [54]. Thus, Eftozanermin alfa clearly improves upon the agonistic ability and pharmacokinetic profile of first-generation TRAIL therapeutics and has consequently progressed into clinical trials (Table 1). A first-in-human study showed Eftozanermin alfa was well-tolerated in patients with advanced solid

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**Table 1 Ongoing clinical trials involving TRAIL-based therapeutics**

| Cancer type | Combined with | Phase | Status | Clinicaltrials.gov ID |
|---|---|---|---|---|
| *Eftozanermin alfa* | | | | |
| Multiple myeloma | – | 1 | Recruiting | NCT04570631 |
| Advanced solid tumors, hematological malignancies | – | 1 | Completed | NCT03082209 |
| *SCB-313* | | | | |
| Peritoneal malignancies | – | 1 | Completed | NCT03443674 |
| Malignant pleural effusions | – | 1 | Completed | NCT03869697 |
| Peritoneal carcinomatosis | – | 1 | Recruiting | NCT04047771 |
| Malignant ascites | – | 1 | Completed | NCT04051112 |
| Malignant pleural effusions | – | 1 | Completed | NCT04123886 |
| *GEN1029* | | | | |
| Solid tumors | – | 1/2 | Terminated | NCT03576131 |
| *INBRX-109* | | | | |
| Metastatic solid tumors, including sarcomas | – | 1 | Recruiting | NCT03715933 |
| Conventional chondrosarcoma | – | 2 | Recruiting | NCT04950075 |
| *IGM-8444* | | | | |
| Solid tumors | Numerous agents | 1 | Recruiting | NCT04553692 |
| *ONC201* | | | | |
| Neuroendocrine | – | 2 | Active, not recruiting | NCT03034200 |
| Acute myeloid leukemia | – | 1 | Recruiting | NCT03932643 |
| Leukemia | – | 1/2 | Recruiting | NCT02392572 |
| Multiple myeloma | – | 1/2 | Active, not recruiting | NCT02863991 |
| Metastatic colorectal cancer | – | 1/2 | Terminated | NCT03791398 |
| Ovarian cancer | Paclitaxel | 2 | Recruiting | NCT04055649 |
| Glioma | – | 2 | Active, not recruiting | NCT03295396 |
| H3 K27M Glioma | – | 1 | Recruiting | NCT03416530 |
| Recurrent GBM, H3 K27M Glioma, midline glioma | – | 2 | Active, not recruiting | NCT02525692 |
| Midline glioma | Radiation, Paxalisib, or Panobinostat | 2 | Recruiting | NCT05009992 |

tumors and hematological malignancies alone and in combination with venetoclax or chemotherapeutics [55,56] (NCT03082209). The results of dose-escalation and dose-optimization cohorts of patients with advanced solid tumors receiving Eftozanermin alfa monotherapy showed partial responses according to RECIST in three patients (two with CRC and one with pancreatic cancer) among the 105 patients participating in the clinical trial (PMID 35467243). Pharmacodynamics studies demonstrated saturation of Eftozanermin alfa binding sites on the TRIAL receptors and increased levels of M30 and M65 markers of apoptosis in the peripheral blood. Analysis of paired tumor specimens collected during the clinical trial also showed increase tumor infiltration of immune cells including CD4+ T cells in post-treatment biopsies compared with baseline tumor specimens as well as increased PARP cleavage and down-regulation of MEK/Erk/AKT pathway. Another phase 1B study is currently recruiting patients with relapsed or refractory multiple myeloma (NCT04570631).

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## Modified TRAIL-R antibodies

Besides TRAIL derivatives, modified TRAIL-R mAbs are another class of second-generation therapeutics. Unlike their bivalent first-generation counterparts which are unable to induce efficient receptor trimerization, these antibodies are multivalent, which allows them to bind more than just two receptors, thereby enhancing receptor clustering.

GEN1029 (HexaBody-DR5/DR5) is novel a mixture of two DR5-specific IgG1 mAbs, each targeting two distinct DR5 epitopes and containing E430G mutations that enhance antibody hexamerization via Fc-Fc interactions [57–59]. This unique structure results in the ability to induce lower- and higher-order receptor clustering in an FcγR-independent manner. GEN1029 has shown potent antitumor activity in preclinical models in a variety of solid and hematological malignancies [59,60]. A two-part safety trial consisting of a first-in-human dose-escalating phase 1 and an expansion phase 2A for a variety of solid tumors was recently terminated by its sponsor, Genmab (Copenhagen, Denmark), for unspecified reasons (NCT03576131) (Table 1).

INBRX-109 (Inhibrx, La Jolla, CA, U.S.A.) is another multivalent DR5 agonistic antibody. Previous attempts to produce multivalent antibodies led to the realization that excessive receptor clustering can cause hepatotoxicity (as shown by the terminated phase 1 trial for TAS266, a DR5-targeting tetravalent nanobody [61]). INBRX-109 was engineered to avoid hepatotoxicity by limiting its valency to four. Preclinical studies indeed showed that INBRX-109 maintained superior induction of apoptosis in cancer cells while sparing human hepatocytes. INBRX-109 entered an ongoing phase 1 clinical study to evaluate its single agent efficacy in subjects with locally advanced or metastatic tumors (NCT04950075). The drug was shown to be well-tolerated, which led to the FDA granting it orphan drug designation (ODD) in 2021 as well as a phase 2 study of INBRX-109 in conventional chondrosarcoma (NCT03715933) (Table 1).

Finally, IGM-8444 is an IgG DR5 antibody. With 10 binding units, it is capable of binding multiple receptors at once, facilitating receptor clustering [62]. IGM-8444 was found to induce apoptosis in a broad panel of solid and hematological malignancies [62]. A phase I study is currently recruiting patients to test IGM-8444 alone and in combination in subjects with cancer (NCT04553692).

## Combination therapies

Second-generation approaches have clearly improved upon first-generation therapeutics, with larger, multivalent molecules enhancing the pharmacokinetic profiles and agonistic abilities of rhTRAIL and TRAIL-R mAbs. However, it is established that resistance to monotherapy with these TRAs is inherent or acquired [9,38,63,64]. Combinations of TRAs with chemotherapeutics and TRAIL-sensitizers are being explored to help overcome this resistance.

TRAs have been tested in combination with chemotherapeutics including gemcitabine, cisplatin, carboplatin, irinotecan, 5-FU, oxaliplatin, paclitaxel, capecitabine and doxorubicin. In the case of rhTRAIL, in a phase 1B study of patients with CRC, dulanermin was evaluated in combination with FOLFOX6 regimen plus bevacizumab. Study results showed that the combination was well-tolerated. A partial response (PR) was observed in 57% of patients, stable disease was observed in 30% of patients, and progressive disease (PD) was observed in the remaining 13% [65]. Dulanermin was also evaluated in combination with paclitaxel, carboplatin and bevacizumab in a randomized phase 2 study in patients with advanced non-small-cell lung cancer (NSCLC). The addition of dulanermin to these therapeutic regimes was tolerated but did not significantly improve patient outcomes [66]. In a phase 3 study in patients with advanced NSCLC, the combination of dulanermin plus vinorelbine and cisplatin was evaluated. Here, researchers noted that the addition of dulanermin to the vinorelbine and cisplatin regime significantly improved overall response rate (ORR) and progression-free survival (PFS) [67]. Finally, in a phase 1b open-label study, a patient with BRAF-mutant metastatic colon cancer received dulanermin and FOLFIRI. The patient maintained stable disease through 25 doses administered every two weeks, a period which extended beyond the median overall survival (OS) for patients with this disease [68]. An ongoing phase 1 trial with Eftozanermin alfa is investigating the combination with FOLFIRI and bevacizumab for treatment of patients with KRAS-mutant CRC (NCT03082209).

In the case of TRAIL-R antibodies, in a phase 2 study in chemotherapy-naïve patients with unresectable or metastatic pancreatic cancer, the combination of tigatuzumab with gemcitabine was well-tolerated. Here, an ORR of 13.1% was reported while 45.9% of patients had stable disease and 23% of patients had progressive disease [71]. LBY135 has been evaluated in combination with capecitabine in a phase 1 clinical trial of patients with advanced solid tumors. Here, there were some signs of clinical activity and the mAb was shown to be well-

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tolerated, once again [72]. Numerous mechanisms have been proposed to explain chemotherapy-induced TRA-sensitization, including receptor up-regulation and suppression of anti-apoptotic proteins [38]. Nonetheless, this sensitization remains limited due to the previously mentioned agonistic and pharmacokinetic issues associated with rhTRAIL and TRAIL-R mAbs.

We discovered a potent synergy between rhTRAIL and sorafenib through effects on NF-kB and Mcl-1 [69]. This strategy was pursued in the clinic through a randomized phase II study using mapatumumab in combination with sorafenib in hepatocellular cancer, but with limited efficacy [70].

TRAs have also been tested in combination with apoptotic pathway agents that act as TRAIL-sensitizers. The rationale is that cancer cells often become resistant to TRAIL therapy through cross-talk between the extrinsic and intrinsic pathways and by up-regulating anti-apoptotic proteins [73]. For instance, c-FLIP is known to inhibit DISC formation by competing with caspase-8/-10 for binding to FADD. FLIP is often overexpressed in cancer [19], leading to the development of FLIP inhibitors and siRNAs that silence the protein [74]. Such approaches were shown to sensitize cancer cells to TRAIL therapy [75]. In addition, XIAP interacts with and inhibits caspases, and its high expression is associated with TRAIL resistance. Smac mimetics have been developed to mimic the binding site of the XIAP antagonist Smac and have shown promising preclinical activity [76]. siRNA silencing of XIAP has been shown to effectively sensitize cells to TRAIL [77]. Finally, overexpression of BCL-2 family anti-apoptotic proteins, including BCL-2, Mcl-1, and Bcl-xl, are associated with TRAIL resistance [78]. BH3-mimetics such as ABT-263, ABT-199 and ABT-737 bind to and inhibit one or more of these proteins and have also been shown to sensitize cells to TRAIL therapy [54,79,80].

## Moving away from TRAIL-R agonists: an alternative approach

Regardless of the improvements made by combination therapies, resistance continues to be a limiting factor for some receptor agonist-based therapies, as cancer cells often up-regulate anti-apoptotic proteins and down-regulate TRAIL and its receptors [9]. Thus, agents that increase the expression of TRAIL and its receptors have some therapeutic potential. One such agent is TIC10/ONC201 [81].

Originally discovered as TRAIL-inducing compound 10 (TIC10), ONC201 is a first-in-class molecule and a founding member of the imipridone small molecule family. ONC201 acts directly to agonize ClpP, which leads to the activation of the integrated stress response (ISR) and downstream ATF4 and CHOP-mediated up-regulation of DR5 [82]. Earlier work has shown that ONC201 also inhibits Akt and ERK, which allows for the nuclear translocation of Foxo3a and subsequent up-regulation of TRAIL [83]. Thus, ONC201 up-regulates TRAIL in all cells and DR5 selectively in tumor cells. This up-regulation has been shown to translate to increased expression of TRAIL and DR5 at the cell surface which leads to downstream induction of apoptosis [84,85]. In addition, ONC201 inhibits cancer stem cells and has documented immunostimulatory effects [86,87]. Thus, ONC201 induces tumor-selective apoptosis and promotes immune function through several mechanisms, many of which are still being studied.

ONC201 has shown preclinical activity as a single agent against a wide variety of solid and hematological [88] tumors, including lung [89], breast [90], pancreatic [91], ovarian [92], colorectal [81], prostate [81], hepatocellular [93], leukemia [94] and lymphoma [95]. Of note, ONC201 has shown efficacy against neuroendocrine tumors and brain tumors [96,97]. Such activity can be attributed to ONC201's ability to cross the blood-brain barrier as well as its ability to antagonize DRD2/3. Indeed, ONC201's activity against neuroendocrine tumors including pheochromocytomas and paragangliomas is correlated with their high expression of dopamine receptor type II [97]. Treatment with ONC201 reduced tumor growth in multiple glioma xenograft models, and a single dose doubled the overall survival of mice with an intracranial xenograft of human GBM [81,98]. ONC201 has also shown preclinical anti-metastatic activity in various tissue types, including breast, CRC, and endometrial cancers [99]. This is explained by the fact that ONC201 inhibits cell adhesion, migration, and invasion in a TRAIL-dependent manner [99,100].

The preclinical outcomes of ONC201 led to its approval by the FDA for testing in phase 1 trials for patients with various cancers. ONC201 has been evaluated in 16 clinical trials as a single agent (Table 1). The first-in-human study found ONC201 was well-tolerated in patients with advanced solid tumors. Although objective responses were not achieved by RECIST criteria, a subset of the patients experienced tumor regression, including one patient with chemotherapy-resistant endometrial cancer [101]. More recently, a phase 2 study of ONC201 monotherapy in patients with recurrent GBM once again found that ONC201 was well-

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tolerated. Of note, this study was the second report of a patient with a H3K27M mutation achieving a complete regression of enhancing multifocal lesions that was maintained for >1.5 years. It is noteworthy that the more potent ONC201 analog ONC212 is active against H3K27M-mutated DIPG cells in preclinical studies, but this analog has not been reported to antagonize dopamine receptors [102].

Although ONC201 has shown some success as a single agent, its activity is limited by resistance-inducing factors such as PI3K/AKT/mTOR upregulation [103] and insufficient TRAIL induction [104,105]. This, along with its multifaceted mechanism of action makes it a good candidate for combination therapy. There is extensive preclinical work showing synergism between ONC201 and radiation [106], chemotherapeutics, targeted therapies [107], and immune-checkpoint agents [96,99]. Most relevant to this review is the combination between ONC201 and TRAs. ONC201 was shown to be ineffective as a single agent in triple-negative breast cancer (TNBC) due to insufficient TRAIL induction [104,105]. TRAIL itself has activity only in a subset of TNBC patients. Combination therapy between ONC201 and rhTRAIL produced a potent apoptotic effect in breast cancer cells which was replicated in other cancer types [104]. A similar effect was seen in non-TNBC cells with the combination between lexatumumab and ONC201 [108]. Finally, the combination of ONC201 with TLY012 was shown to produce a synergistic apoptotic effect both *in vitro* and *in vivo* in pancreatic cancer models [109]. Such combinations have potential but remain to be tested in clinical trials. Meanwhile, clinical trials have been initiated using combinations between ONC201 and radiation (NCT05009992) or chemotherapeutics (NCT04055649) (Table 1). Preclinical data demonstrate synergy between ONC201 or other imipridones and EZH2 or HDAC inhibitors and this has been correlated with H3K27 acetylation [102,110].

## Conclusion

Unlike other members of the TNF-family, TRAIL possesses the ability to selectively target cancer cells. This exciting realization led to several decades of intense research focused on the development of TRAIL receptor-based therapies. However, clinical studies have demonstrated that such therapies have failed to meet their expectations, with many possessing an inability to induce lower- or higher-order receptor clustering, poor pharmacokinetic profiles including short half-lives, and rapidly built resistance. While novel TRAs are being developed to specifically target these shortcomings and have shown promise in the clinic, it has become clear that a more imaginative approach must be taken to advance the field. The discovery of ONC201/TIC10 provides such an approach. Unlike TRAs, ONC201 acts independently of receptor binding and instead induces TRAIL and DR5 at the transcriptional level. This unique mechanism of action represents a new generation of TRAIL-pathway based approaches. ONC201 has shown promising preclinical and clinical results. Nevertheless, it must be recognized that the nature of cancer is complex, with tumor heterogeneity, evolution, and resistance to anti-cancer therapy acting as major limiting factors to single-drug therapies. Thus, further exploration of ONC201 and other TRAIL-based approaches is needed to address such obstacles.

## Perspectives

- The TRAIL pathway is a powerful innate immune system whose therapeutic potential is yet to be realized.
- It is likely that harnessing the TRAIL pathway in cancer therapy alone or in combination with other cancer therapeutic modalities holds promise.
- Mechanistic studies with first- and second-generation TRAIL receptor agonists will help inform translational directions and combinatorial cancer therapeutics.

### Competing Interests

W.S.E.-D. is a co-founder of Oncoceutics, Inc., a subsidiary of Chimerix. Dr. El-Deiry has disclosed his relationship with Oncoceutics/Chimerix and potential conflict of interest to his academic institution/employer and is fully compliant with NIH and institutional policy that is managing this potential conflict of interest.

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## Abbreviations

CRC, colorectal cancer; DISC, death-inducing signaling complex; FADD, Fas-associated protein with death domain; GBM, glioblastoma; ISR, integrated stress response; IZ, isoleucine zipper; LZ, leucine zipper; NSCLC, non-small-cell lung cancer; ODD, Orphan Drug Designation; ORR, overall response rate; PEG, polyethylene glycol; RECIST, Response Evaluation Criteria in Solid Tumors; TIC10, TRAIL-inducing compound 10; TNBC, triple-negative breast cancer; TNC, tenascin-C; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TRAs, TRAIL receptor agonists; rhTRAIL, recombinant human TRAIL.

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*Biochemical Society Transactions* (2023) **51** 57–70
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