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TRAIL pathway targeting therapeutics

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Abstract

Introduction: Despite decades of focused research efforts, cancer remains a significant cause of morbidity and mortality. Tumor necrosis factor(TNF)-related apoptosis-inducing ligand (TRAIL) is capable of inducing cell death selectively in cancer cells while sparing normal cells.

Areas covered: In this review, the authors cover TRA therapy and strategies that have been undertaken to improve their efficacy, as well as unconventional approaches to TRAIL pathway activation including TRAIL-inducing small molecules. They also discuss mechanisms of resistance to TRAIL and the use of combination strategies to overcome it.

Expert commentary: Targeting the TRAIL pathway has been of interest in oncology, and although initial clinical trials of TRAIL receptor agonists (TRAs) showed limitations, novel approaches represent the future of TRAIL-based therapy.

Keywords

Apoptosis; cancer; TRAIL; DISC; apoptosome; TRAIL-R agonist; Imipridone; atrimer

1. Introduction

Apoptosis, activated under pathological and physiological conditions, results in the programmed destruction of a cell. The hallmarks of apoptosis include cell shrinkage, nuclear DNA fragmentation, and membrane blebbing [1]. Apoptosis can be triggered following the activation of the cell-intrinsic pathway or the cell extrinsic pathway. Activation of the intrinsic, or mitochondrial, pathway is regulated by the B-cell lymphoma-2 (Bcl-2) family of proteins. The Bcl-2 family includes pro-apoptotic proteins and anti-apoptotic proteins, all of which function to control the permeability of the mitochondrial outer membrane [2].

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Declaration of Interest

WS El-Deiry is a co-Founder and shareholder in Oncoceutics, a company that is developing ONC201 in the clinic. WS El-Deiry is compliant with institutional and NIH disclosure guidelines. Oncoceutics provided no financial support for the review and has not been involved in any way in its writing. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Cellular stresses such as DNA damage activate pro-apoptotic and inhibit anti-apoptotic Bcl-2 family proteins in a p53-dependent manner. This results in mitochondrial outer membrane permeabilization (MOMP) and release of cytochrome *c*. Cytochrome *c* associates with Apaf-1 and activator caspase-9, forming the apoptosome [3]. Caspase-9, as a part of the apoptosome, can cleave and activate effector caspase-3 [4], which goes on to cleave downstream targets and induce cell death.

The extrinsic pathway is activated following binding of pro-apoptotic stimuli such as tumor necrosis factor(TNF)-related apoptosis-inducing ligand (TRAIL) to cell surface receptors. TRAIL/Apo2L was discovered as an inducer of apoptosis [5, 6] that, unlike other TNF superfamily members, selectively targets cancer cells while leaving normal cells unharmed [7]. TRAIL binds to cell surface receptors DR4 (TRAIL-R1) [8] and DR5 (TRAIL-R2) [9] as a homotrimer [10]. DR4 and DR5 have functional intracellular carboxyl terminal death domains (DDs), and ligand binding leads to receptor oligomerization and recruitment of adaptor protein Fas-associated death domain (FADD). Alternative TRAIL receptors TRAIL-R3, TRAIL-R4, and the soluble osteoprotegerin (OPG) lack a viable DD [11–13]. TRAIL-R3 and TRAIL-R4 may function to act as decoy receptors and inhibit TRAIL-induced apoptosis [14]. Through its death effector domain (DED), FADD recruits pro-caspase-8 and pro-caspase-10 [15, 16]. Proper formation of this death inducing signaling complex (DISC) is required for apoptosis induction. Once recruited to the DISC, pro-caspase-8 and −10 are cleaved and activated [17]. In type I cells, caspase-8 cleavage is sufficient to induce cleavage of caspase-3 and cell death. In type II cells, an extra step is required: caspase-8 mediated BH3-interacting-domain death agonist (BID) cleavage activates the intrinsic apoptosis pathway [18]. The cleaved form of Bcl-2 family protein BID, tBID, enables mitochondrial outer membrane permeabilization (MOMP) and cytochrome *c* release [19, 20]. Release of mitochondrial cytochrome *c* is necessary for formation of the apoptosome and ultimately caspase-3 cleavage and the induction of cell death in type II cells.

TRAIL is expressed on the surface of activated NK cells [21] and can bind to death receptors expressed on tumor cells, triggering their apoptosis [22, 23]. TRAIL-deficient mice are more susceptible to tumor initiation and metastasis following carcinogen exposure, in a partially NK-cell-dependent manner [24]. Similarly, TRAIL-deficient mice show increased susceptibility to the growth of primary tumors and their spontaneous liver metastasis following inoculation of syngeneic breast cancer cells [25]. In addition to triggering apoptosis, death receptor engagement can activate pro-inflammatory transcription factor NF-KB [26–28] and induce TRAIL-resistant cancer cells to proliferate [29] and secrete tumor promoting cytokines [30, 31].The mechanism by which TRAIL activates NF-KB involves nuclear translocation following phosphorylation and degradation of inhibitor of KB (IKB) proteins [32].

The identification of TRAIL as an extrinsic apoptosis pathway ligand with such a strong selectivity for cancer cells over normal cells [7] sparked an interest in developing TRAIL receptor agonists (TRAs) as anti-cancer therapeutics.

2. TRAIL-R agonists

2.1: Soluble recombinant human TRAIL

Two subtypes of TRAs have been developed for use in the clinic: recombinant forms of the TRAIL protein and DR4/5 agonistic antibodies. The initial efficacy of recombinant human TRAIL against cancer cells was shown in the early 1990's [10]. Early forms of recombinant TRAIL contained poly-histidine or FLAG epitope tags [5, 6] used to aid in the protein purification process. These forms of TRAIL showed toxicity against primary hepatocytes *in vitro* [33], which prompted concern about potential *in vivo* hepatotoxicity until it was determined that untagged versions of the molecule did not have the same effect [34]. Recombinant TRAIL was clinically developed as rhApo2L.0/dulanermin. Preclinically, dulanermin binds to both DR4 and DR5 to induce cell death in cancer cells but not normal cells [10]. While safe and well tolerated in patients [35], the protein was also disappointingly inactive when tested in the setting of a randomized phase II trial [36]. The protein's extremely short half-life [37] has been blamed for these discouraging results. The second class of TRAs is made up of monoclonal death receptor binding antibodies. These antibodies boasted a half-life on the order of days, much longer than that of dulanermin, the plasma concentration of which halves in under an hour after injection.

2.2: TRAIL receptor agonistic antibodies

Death receptor agonistic antibodies that have been tested clinically include one DR4 agonistic antibody (mapatumumab), and five DR5 agonistic antibodies (drozitumumab, conatumumab, lexatumumab, tigatuzumab, LBY-135). A recent study compared the efficacy of anti-DR5 antibody tigatuzumab plus nanoparticle albumin-bound paclitaxel or nanoparticle albumin-bound paclitaxel alone in patients with triple negative breast cancer. Prolonged survival of several patients treated with the combination support further study of these agents [38]. While TRA antibodies including tigatuzumab have been generally well tolerated, they have not yet advanced into phase III.

3. Strategies for improving the efficacy of TRAIL-R agonists

3.1: Increasing the stability and trimerization ability of TRAIL

Despite the unsatisfactory performance of early TRAs in the clinic, scientists and clinicians are not ready to give up on targeting the TRAIL pathway. Cell death induction by TRAIL requires ligand trimerization prior to death receptor binding. Tagged formats of recombinant human TRAIL with trimerization domains were initially abandoned for development after the discovery that his-TRAIL and FLAG-TRAIL exhibited toxicity against primary hepatocytes [33]. TRAIL with an attached leucine zipper motif (LZ-TRAIL) [7], an attached isoleucine zipper motif (iz-TRAIL) [39], and an attached tenascin-C oligomerization domain (TNC-TRAIL) [40] effectively trimerize and are non-toxic to hepatocytes [39]. These forms of TRAIL have been shown to be more potent and have longer half-lives than rhApo2L.0/dulanermin, the untagged version of TRAIL used in clinical trials. Permutation of the TRAIL amino acid sequence has been undertaken to improve pharmacokinetic properties and increase potency of killing. The single chain TRAIL trimer (scTRAIL) protein is expressed as three linker-connected TRAIL extracellular domains [41], eliminating the need

for trimerization *in vivo*. Recombinant mutant human TRAIL (rmhTRAIL), also known as circularly permutated TRAIL (CPT) contains a flexible linker connecting the N terminus of amino acids 121–135 to the C terminus of amino acids 135–281 [42]. Both novel TRAIL forms show increased potency above the wildtype protein [41, 42]. CPT has been tested in a phase II study of patients with relapsed and refractory multiple myeloma in combination with thalidomide. The protein was shown to be well-tolerated and have an overall response rate (ORR) of 22% [43]. An alternative approach to improve the efficacy of TRAIL by increasing its stability has involved the covalent linkage of TRAIL to polyethylene glycol (PEGylated TRAIL) [44].

3.2: Directly targeting TRAIL to cancer cells

In addition to making alterations to the TRAIL protein itself, researchers have devised creative delivery strategies to effectively target TRAIL to cancer cells. Anticancer therapeutics can be coupled with components such as lipids or polymers to form nanoparticles. Nanoparticle delivery to tumor tissue relies on a phenomenon called the enhanced permeability and retention (EPR) effect. Solid tumors possess abnormal blood and lymphatic vessels with increased permeability compared to those found in healthy tissues, and as a result, macromolecules will leak out of vessels and collect within the tumor [45]. A variety of TRAIL-containing nanoparticles are under development. Some groups have attached and immobilized TRAIL onto the surface of the nanoparticle and other have encapsulated TRAIL within. TRAIL-containing nanoparticles have been reviewed in greater detail by de Miguel et. al [46]. Fusion proteins combining the antigen specific single-chain variable-region (scFv) of an antibody with the TRAIL protein represent an alternative strategy for targeting TRAIL to cancer cells. scFv regions targeting a variety of antigens expressed on cancer cells and immune cells have been developed. TRAIL-scFv fusion proteins have been reviewed in greater detail by de Bruyn et. al [47].

4. Alternative approaches to TRAIL pathway activation

4.1: TRAIL-inducing (imipridone) compounds

A novel approach for targeting the TRAIL pathway involves small molecules and peptide TRAIL pathway activators. ONC201, an anti-cancer compound belonging to the novel imipridones class, was originally identified in a luciferase reporter screen as a p53-independent transcriptional inducer of the TRAIL gene [48]. ONC201 was selected as the lead candidate from the screen following its success in preclinical studies, where it exhibited a favorable safety profile and showed anti-tumor effects *in vivo* with a single dose [49]. The mechanism by which ONC201 induces transcription of the TRAIL gene involves inactivation of pro-survival kinases Akt and ERK, leading to decreased phosphorylation of transcription factor FOXO3a. Dephosphorylated FOXO3a is activated and translocates to the nucleus to activate its target gene TRAIL [49]. More recently, it was determined that ONC201 also induces cell death through activation of a PERK-independent ISR [50, 51]. The compound also exerts TRAIL dependent [52] anti-cancer stem cell activity [52, 53]. ONC201 has shown efficacy in against a variety of tumor types preclinically including breast cancer [54], glioblastoma [55], pancreatic cancer, and prostate cancer. The compound has also been shown the recruit NK cells to tumors [56]. The compound has completed its

first-in-human clinical trial in advanced solid tumors [57] and is currently being tested in multiple phase II trials. Analogs of ONC201 able to upregulate TRAIL with increased potency have been synthesized [58] and will hopefully enter the clinic soon.

4.2: TRAIL-R activating atrimers, TRAIL-R activating small molecules

Other unconventional approaches to activating the TRAIL pathway have included small DR5 binding peptides [59], DR5 activating small molecules that induce receptor clustering and aggregation [60], and trivalent DR4 atrimer complexes [61].

5. TRAIL resistance and the use of combination therapy to overcome it

5.1: TRAIL resistance is common in tumor cells

While the use of TRAs with increased potency and ability to target to cancer cells is a valid approach, it does not address the problem of TRAIL resistance. Resistance mechanisms have been identified at multiple points in the TRAIL pathway, from cell surface ligand binding to intracellular caspase cleavage. The pathway became of interest as a therapeutic target due to its ability to induce cell death in cancer cell while sparing normal cells [7]. Different mechanisms of resistance to TRAIL have been identified in normal cells, including post-translational modification of caspase-8 [62], high levels of c-FLIP [63, 64] and cell surface decoy receptors [64].

5.2: Resistance at the level of the death receptors

In cancer cells, low surface expression of TRAIL receptors DR4 and DR5 correlates with decreased sensitivity to TRAIL. Specific genetic and epigenetic changes responsible for decreased DR4 and DR5 expression have been identified and include promoter hypermethylation [65, 66], loss-of-function mutations [67, 68], gene deletion [69]. Impaired death receptor transport to [70] and constitutive receptor endocytosis from the cell surface [71] also modulate cell surface expression and thus TRAIL sensitivity. Therapies that induce DNA damage and activate p53, such as chemotherapy and radiation, can upregulate DR4 [72] and DR5 [73] and have been tested in combination with TRAIL. Strategies to overcome TRAIL resistance associated with low cell surface death receptor levels have also included combinations with histone deacetylase (HDAC) inhibitors [74, 75], proteasome inhibitors [76], and ER stress inducer tunicamycin [77]. Interestingly, post-translational modifications of death receptors can also modulate TRAIL sensitivity. O-glycosylation of DR4 and DR5 enhances ligand-mediated receptor clustering, DISC formation, and caspase activation. Genes encoding o-glycosylating enzymes are overexpressed in TRAIL-sensitive cells, and their inhibition causes resistance [78].

5.3: Resistance at the level of the caspase-8 and c-FLIP

Resistance can also occur at the level of caspase-8. Deletion and hypermethlyation of the caspase-8 gene has been observed in childhood neuroblastomas [79] and small cell lung carcinomas [66]. An endogenous mediator of TRAIL resistance is cellular flice inhibitory protein (c-FLIP). Like caspase-8, FLIP contains a death-effector domain (DED) that allows it to bind to FADD. Unlike caspase-8, c-FLIP lacks the protease activity that is required for proper cleavage of effector caspases and thus apoptosis induction [80]. The short splice

variant of c-FLIP, c-FLIP$_S$, prevents caspase-8 cleavage at the DISC [81]. High c-FLIP expression is associated with resistance to TRAIL in cancer cells, as reviewed by Newsom-Davis [82]. Interestingly, c-myc has been shown to modulate TRAIL sensitivity through negative regulation of c-FLIP transcription [83]. Reduction of c-FLIP expression by a variety of compounds, including HDAC inhibitor LBH589 [84] and multiple PPARγ ligands [85], sensitizes to TRAIL-mediated apoptosis.

5.4: Resistance mediated by B-cell lymphoma-2 (Bcl-2) family proteins

Pro-apoptotic Bcl-2 family protein Bid is highly relevant to TRAIL-induced apoptosis, as discussed previously. In type II cells, caspase-8 mediated Bid cleavage and activation is required for induction of the intrinsic apoptosis pathway and cell death [18]. Type II cancer cells can develop resistance to TRAIL following overexpression of anti-apoptotic Bcl-2 family proteins Mcl-1 [86] and Bcl-2 [87] or loss of pro-apoptotic Bcl-2 family protein Bax [88]. BH3-mimetics designed to antagonize anti-apoptotic proteins are being tested clinically and can sensitize cancer cells to TRAIL [89, 90].

5.5: Resistance mediated by Inhibitor of apoptosis (IAP) family proteins

A class of caspase inhibitors known as the inhibitor of apoptosis (IAP) family proteins can also regulate TRAIL-mediated cell death. IAP proteins such as XIAP contain baculovirus IAP repeat domains, essential for direct binding to and inhibition of caspases 3,7, and 9 [91]. Smac/DIABLO, an endogenous XIAP inhibitor, is released following MOMP, binds to XIAP, and inhibits its function [92, 93]. Small molecule Smac mimetics with the capability to antagonize IAPs are being tested in the clinic. These compounds sensitize cancer cells to TRAIL-mediated apoptosis in preclinical models [94, 95]. Other IAP proteins, including cellular-IAP1 (c-IAP1) and cellular-IAP2 (c-IAP2) are able to ubiquitinate protein targets, including caspase-3 and caspase-7, and target them for degradation [96] through an E3-ligase Really Interesting New Gene (RING) domain [97]. Interestingly, multikinase inhibitor sorafenib has been shown to sensitize resistant cells to TRAIL through downregulation of Mcl-1, c-IAP2, and c-FLIP [98, 99] through inhibition of the JAK/STAT3 signaling pathway [100].

5.6: Resistance mediated by PI3K-Akt signaling

Phosphatidylinositol 3-phosphate kinase (PI3K) is a well-studied regulator of cellular proliferation, growth, and survival. Paradoxically, treatment with TRAIL can activate this prosurvival pathway [101]. Binding of growth factors to cell surface PI3K receptors leads to the generation of second messenger phosphotidylinositol-3,4,5-triphosphate (PIP3). PIP3 binds to kinase Akt, which regulates many downstream targets such as mammalian target of rapamycin (mTOR) complex 1 and 2 through phosphorylation. Activating mutations of PIK3CA, the gene that encodes the p110alpha catalytic subunit of PI3K, may confer resistance to TRAIL [102]. Similary, loss of expression of PI3K negative regulator PTEN or overexpression of its downstream target Akt lead to decreased TRAIL sensitivity [103]. Combination of TRAIL with PI3K [104], Akt [105], and mTORC1 inhibitors [106] enhances apoptosis.

6. Conclusion

Interest in the TRAIL pathway developed following the observation that TRAIL could induce cell death in transformed cells but showed no toxicity towards normal cells [7]. TRAIL receptor agonists have been developed for use in the clinic and include recombinant human soluble TRAIL and death receptor agonistic antibodies. Unfortunately, these approaches have yet to show efficacy in a clinical trial. Multiple strategies have been used to improve the efficacy of these agonists. Forms of TRAIL with increased stability have been developed, and multiple systems for improved TRAIL delivery have been identified. A novel approach for TRAIL pathway activation involves small molecule and peptide TRAIL pathway activators. ONC201, originally called TRAIL inducing compound 10 (TIC10) is one such small molecule that is currently being tested in clinical trials. TRAIL resistance in cancer cells is a significant problem, and combination therapies with existing drugs have been explored as a method for combating it. In conclusion, although early TRA therapies showed limited clinical efficacy, novel and innovative approaches have potential and merit further clinical testing.

7. Expert Commentary

The TRAIL pathway holds enormous potential among the therapeutic strategies currently employed to treat cancer. The field has evolved significantly since the 1980's when significant toxicities with TNF were being observed in clinical trials. There have been hurdles in the development of TRAIL as a therapeutic and the field has shifted towards TRAIL receptor agonists or other TRAIL pathway activating small molecules. Activation of the TRAIL pathway which is part of the innate immune system for cancer and metastasis suppression offers hope and a bright future given that tumors with various oncogenic drivers can be targeted. Unlike TNF, the TRAIL ligand as well as TRAIL receptor agonists have generally proven to not be limited by toxicity in the clinic. There was some concern early on that hepatic expression of DR5 might result in more toxicity from therapeutic targeting of TRAIL-R2 versus TRAIL-R1 in the clinic. However, this did not materialize in the clinical trials. Of course, neither ant-DR5 or anti-DR4 alone is equivalent to TRAIL and it is complicated to combine 2 unapproved therapeutics in early phase trials. Thus, the answers are unfortunately not there at present to address the issue of whether targeting TRAIL-R1 (DR4) or TRAIL-R2 (DR5) or both in the path forward in the clinic.

The TRAIL pathway is active in cancer suppression despite p53 tumor suppressor gene mutations or common oncogene mutations such as KRAS, BRAF, EGFR, among others. While there are numerous TRAIL pathway resistance mechanisms in cancer including TRAIL decoys, TRAIL receptor glycosylation and translocation to the cell membrane, NFkB, Bcl-XL, Mcl-1, the IAP family, Akt, FLIP, mutation or loss of caspase 8 expression, there are also promising combinatorial therapeutics that can address resistance. There are effects of TRAIL on the tumor microenvironment that need to be further examined in treated human tumors in the clinic with regard to a potential immune suppressive milieu. The development of predictive biomarkers for specific TRAIL pathway therapeutics or combinations would help advance the field and translation to the clinic. Unfortunately, no reliable predictive biomarkers have emerged in the clinical trials to help with our

understanding of response or resistance of specific patients to TRIAL receptor targeted therapeutics, despite available preclinical data. This includes M3, glycosylated receptors, TRAIL-R expression, or a variety of intracellular biomarkers. There is opportunity for a more integrated genomic and proteomic approach to address this unmet need in the future.

It remains to be seen in the clinic what resistance mechanisms ultimately will prove to be insurmountable by TRAIL pathway-directed monotherapy and which mechanisms may be overcome by combination therapy including immunotherapy. The major limitation to combination therapeutics revolves around which TRAIL receptor or TRAIL pathway targeting therapeutic will ultimately serve as the anchor for a combination regimen. It remains for future development to establish specific therapy combinations targeting specific tumor types. Certainly, the combination of a TRAIL-R2 agonist antibody plus docetaxel for triple negative breast cancer holds promise. The combination of TRAIL or TRAIL receptor agonistic targeting plus sorafenib has much support mostly from preclinical data. While anti-DR4 plus sorafenib was disappointing in hepatocellular cancer, the reason for failure has remained unclear, and it is possible that with better patient selection such a combination could be more effective. A number of other combinations are emerging from preclinical studies, e.g. with ONC201 such as combination with anti-VEGF targeting, anti-PD-1 targeting, sorafenib, or everolimus, among others. Clinical trials with specific combinations in specific indications will ultimately direct the most promising agent combinations towards further development. The TRAIL pathway remains a powerful host mechanism for cancer suppression and one that has not yet been adequately exploited for patient benefit in oncology.

8. Five-year view

There are currently no FDA-approved agents specifically targeting activation of the TRAIL pathway. The TRAIL pathway is a powerful innate immune tumor suppressive mechanism that has yet to be harnessed in cancer therapy. The availability of TRAIL receptor agonist antibodies or TRAIL pathway stimulating/activating small molecules such as ONC201 in multiple clinical trials points towards the future. It is expected that while the agents have single agent anti-tumor effects, the combinations for specific tumor types is likely the path forward. Moreover, in the era of precision medicine and careful patient selection, there is expectation that the patients most likely to respond would be the ones who are selected for specific therapy combinations. In the case of ONC201, preliminary indications for H3K27M mutated gliomas such as DIPG, with altered dopamine receptor DRD2 expression appear promising. How the dopamine receptor expression plays out in predicting efficacy of ONC201 or analogues for other tumor types is expected to be unraveled from ongoing or yet to be initiated clinical trials.

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Reference annotations

* Of interest

** Of considerable interest

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Key issues

- Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is capable of inducing cell death selectively in cancer cells while sparing normal cells. Targeting the TRAIL pathway has been of interest in oncology since the 1990's.
- Limited clinical efficacy has been observed in trials of recombinant human TRAIL (rhTRAIL) or death receptor agonistic antibodies.
- Novel approaches for targeting the TRAIL pathway involve directly upregulating TRAIL and its receptors or using combination therapies to target resistance mechanisms.

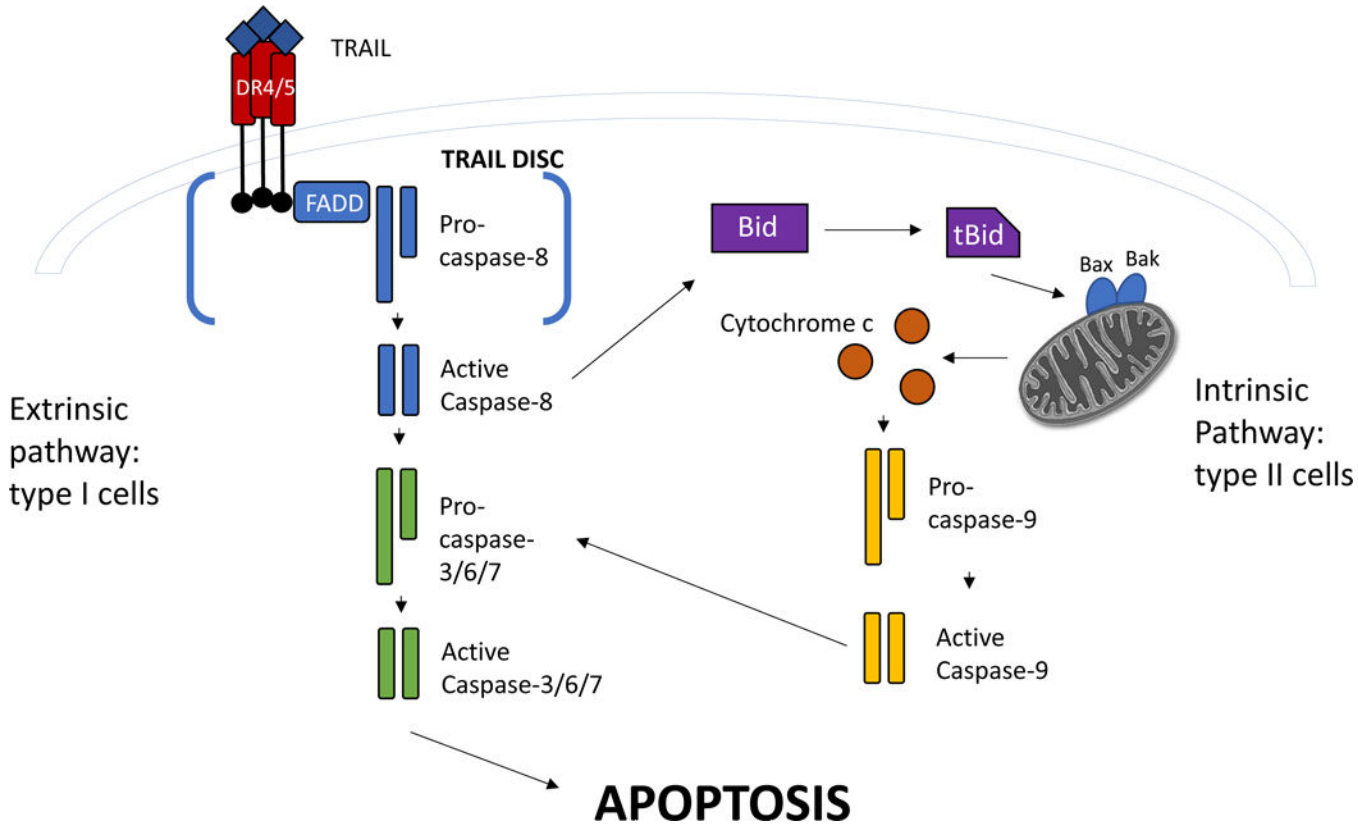

Figure 1: Signaling pathways linking the TRAIL ligand to apoptosis.

TRAIL binds to its cell surface receptors DR4 and DR5 as a trimer. Receptor clustering and recruitment of FADD to intracellular the death domains starts formation of the DISC. Association of pro-caspase-8 finalizes DISC formation. Caspase-8 is cleaved to its active form. In type I cells, caspase-8 cleavage will directly cleave effector caspases 3, 6, and 7, triggering apoptosis. In type II cells, a secondary signal through the mitochondria is required. Caspase-8 will cleave Bcl-2 family protein Bid to its truncated form, tBid. This enables permeabilization of the mitochondrial outer membrane and release of cytochrome c into the cytosol. Pro-caspase-9 will be activated and go on to cleave effector caspases 3,6, and 7, leading to the induction of apoptosis.

Table 1:

Summary of the therapeutic approaches used to target the TRAIL pathway.

Therapeutic Approach	Stage in Development
TRAIL-receptor agonists	
his-TRAIL/FLAG TRAIL	**preclinical**
rhApo2L.0/dulanermin	**discontinued**
DR4 agonistic monoclonal antibodies	
mapatumumab	**Phase II**
DR5 agonistic monoclonal antibodies	
drozitumab	**discontinued**
conatumumab	**Phase II**
lexatumumab	**discontinued**
tigatumumab	**Phase II**
LBY-135	**discontinued**
Strategies for improving the efficacy of TRAIL-receptor agonists	
LZ-TRAIL	**preclinical**
iz-TRAIL	**preclinical**
TNC-TRAIL	**preclinical**
scTRAIL	**preclinical**
CPT	**discontinued**
PEGylated TRAIL	**preclinical**
TRAIL containing nanoparticles	**preclinical**
TRAIL/scFv fusion proteins	**preclinical**
TRAIL expressing mesenchymal stem cells	**Phase I/II**
TRAIL pathway activation	
TRAIL-inducing (imipridone) compounds	**Phase I/II**
TRAIL-R activating atrimers	**preclinical**
TRAIL-R activating small molecules/peptides	**preclinical**