

Review

The Role of TRAIL in Apoptosis and Immunosurveillance in Cancer

Julio M. Pimentel ^{1,2,3} , Jun-Ying Zhou ^{1,3} and Gen Sheng Wu ^{1,2,3,4,*}

¹ Molecular Therapeutics Program, Karmanos Cancer Institute, School of Medicine, Wayne State University, Detroit, MI 48201, USA; pimentej@karmanos.org (J.M.P.); zhouj@karmanos.org (J.-Y.Z.)

² Cancer Biology Program, School of Medicine, Wayne State University, Detroit, MI 48201, USA

³ Department of Oncology, School of Medicine, Wayne State University, Detroit, MI 48201, USA

⁴ Department of Pathology, School of Medicine, Wayne State University, Detroit, MI 48201, USA

* Correspondence: wug@karmanos.org; Tel.: +1-313-578-4750; Fax: +1-313-831-7518

Simple Summary: Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) plays an important role in apoptosis and tumor immunosurveillance. Because TRAIL selectively induces apoptosis in tumor cells, there is growing interest in using it as a cancer therapy agent, but the development of TRAIL resistance has limited its clinical development. Recent evidence suggests that the TRAIL pathway can activate the immunological checkpoint protein programmed death-ligand 1 (PD-L1), which has recently been found to play an important role in TRAIL resistance and tumor invasion. Thus, targeting PD-L1 could be a promising new therapeutic strategy to improve TRAIL-based treatments in human cancers.

Abstract: Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily that selectively induces apoptosis in tumor cells without harming normal cells, making it an attractive agent for cancer therapy. TRAIL induces apoptosis by binding to and activating its death receptors DR4 and DR5. Several TRAIL-based treatments have been developed, including recombinant forms of TRAIL and its death receptor agonist antibodies, but the efficacy of TRAIL-based therapies in clinical trials is modest. In addition to inducing cancer cell apoptosis, TRAIL is expressed in immune cells and plays a critical role in tumor surveillance. Emerging evidence indicates that the TRAIL pathway may interact with immune checkpoint proteins, including programmed death-ligand 1 (PD-L1), to modulate PD-L1-based tumor immunotherapies. Therefore, understanding the interaction between TRAIL and the immune checkpoint PD-L1 will lead to the development of new strategies to improve TRAIL- and PD-L1-based therapies. This review discusses recent findings on TRAIL-based therapy, resistance, and its involvement in tumor immunosurveillance.

Keywords: TRAIL; apoptosis; resistance; immunosurveillance; PD-L1



Citation: Pimentel, J.M.; Zhou, J.-Y.; Wu, G.S. The Role of TRAIL in Apoptosis and Immunosurveillance in Cancer. *Cancers* **2023**, *15*, 2752. <https://doi.org/10.3390/cancers15102752>

Received: 17 March 2023

Revised: 1 May 2023

Accepted: 10 May 2023

Published: 13 May 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a cytokine of the TNF superfamily that plays an important role in apoptosis and tumor surveillance. TRAIL was discovered by comparing the sequence of its c-terminal (CTE) domain to that of other TNF superfamily members, such as FasL and TNF- α [1,2]. Since then, TRAIL has been shown to be expressed by immune cells as a homotrimeric type 2 transmembrane or soluble protein. TRAIL binds to its two death receptors, DR4 and DR5, to induce apoptosis in tumor cells without harming normal cells [3,4]. Because of these features, the TRAIL pathway is regarded as a more appealing cancer therapy agent than FasL and TNF- α , both of which activate similar pathways but cause unacceptable systemic toxicity when administered. The safety of TRAIL-based therapy in cancer patients has been well established in Phase I and Phase II clinical trials [5–7]. However, the development of resistance to TRAIL-based therapies and poor pharmacokinetic profiles limit their clinical utility [8]. Therefore, novel

approaches are needed to improve the pharmacokinetic profile of TRAIL-based therapies and reduce resistance to TRAIL-based treatment.

2. TRAIL Ligand

TRAIL is a type 2 transmembrane protein with two forms: membrane-bound and solubilized [9]. Membrane-bound TRAIL is a native form of TRAIL that consists of the C-terminal extracellular domain (CTE), the transmembrane (TM), an extracellular stalk (ES) region, and N-terminal cytoplasmic domain (NTC) [9]. The CTE domain is conserved and homologous to CD95L and TNF- α . When the ES region (aa 89–106) is catalyzed by cathepsin E, TRAIL detaches from the cell membrane [10]. Consequently, the TRAIL monomer is assembled and converted into a soluble homotrimer composed of monomers arranged in a jellyroll topology with two antiparallel beta sheets connected by a zinc atom [11–13]. The zinc atom chelates the cysteine (cys)-230 sulfhydryl group in each TRAIL monomer, linking them in the trimeric core [13]. Therefore, cys-230 plays an important role in the trimeric structure of TRAIL while distinguishing it from other members of the TNF family [13,14]. Many tissues express TRAIL, with the immune and lymphatic systems having high levels [15], suggesting the role of TRAIL in regulating immune surveillance.

3. TRAIL Receptors

There are five receptors for TRAIL, including membrane-bound receptors DR4 (TRAIL-R1, TNFRSF10A, CD261, APO2), DR5 (TRAIL-R2, TNFRSF10B, CD262, KILLER, TRICK2, ZTNFR9), DcR1 (TRAIL-R3, TRID), DcR2 (TRAIL-R4, TRUND), and the soluble receptor osteoprotegerin (OPG) [16–21]. DR4 and DR5 are death receptors. TRAIL binds to and activates DR4 and DR5 to induce apoptosis. DR4 and DR5 are type 1 transmembrane proteins that comprise a cysteine-rich extracellular domain, a transmembrane domain, and an intracellular domain that contains a death domain (DD) [22,23]. The first cys of the extracellular domain contains a preligand assembly domain (PLAD), which promotes the oligomerization of the DR4 and DR5 trimers, and thus, improves TRAIL-DR4/5 binding [24]. O-glycosylation and other post-translational modifications of DR4 and DR5 have also been shown to enhance the binding of TRAIL-DR4/DR5 by stabilizing the assembly of death receptors and preventing endocytosis [25]. Although the regulation of death receptor expression is not fully understood, the tumor suppressor p53 has been shown to regulate the expression of the TRAIL death receptor [19,26,27]. While an initial study showed that the chemotherapeutic agent doxorubicin and etoposide induce DR5 [19], subsequent studies have shown that several agents can induce the expression of DR4 and DR5, which can sensitize cancer cells to chemotherapy and radiation therapy [6].

In addition to the TRAIL death receptors DR4 and DR5, there are three TRAIL decoy receptors, DcR1, DcR2, and OPG. DcR1 and DcR2 are cell surface receptors; the former is a glycosylphosphatidylinositol (GPI) anchored receptor lacking DD and the latter has a functionally inactive truncated DD [6,9,28]. In contrast, OPG is a secreted receptor with DD that inhibits apoptosis [9]. Thus, these decoy receptors can compete for TRAIL binding to DR4 and DR5, thus inhibiting TRAIL-induced apoptosis [22].

4. The TRAIL Apoptosis Pathway

The first step in the TRAIL apoptosis pathway is the formation of death-inducing signaling complexes (DISCs), which are initiated by recruiting Fas-associated death domain (FADD) adapter proteins. When TRAIL binds to DR4 and DR5, the conformation of DR4 and DR5 changes, which promotes interaction through the death domains between DR4 and DR5 with FADD (Figure 1). Once FADD binds to DR4 and DR5, it readily recruits cysteine protease precursors known as procaspases 8/10 [29,30]. Procaspases 8/10 are known to have an N-terminal pro-domain, two death effector domains (DED1/DED2), and a C-terminal protease domain with large (p20/p18) and small (p12/p10) subunits linked by a short linker region [29,30]. Previous studies on the protein sequence alignment of the two caspases revealed that caspase 10 differs from caspase 8 in cleavage sites in the short linker

region and subunit size [31]. It is unknown whether two death receptors play an equal role in inducing apoptosis. Studies suggest that DR5 binds to TRAIL more efficiently than DR4 through a stepwise binding mechanism [32,33]. Although leukemic cells prefer to initiate apoptosis through DR4 [34,35], many cancer cells, including those from colorectal cancer, are equally sensitive to DR4- and DR5-induced apoptosis [36,37]. Therefore, more research is needed to better understand the conditions under which one or both death receptors are preferred over another in inducing apoptosis in cancer cells.

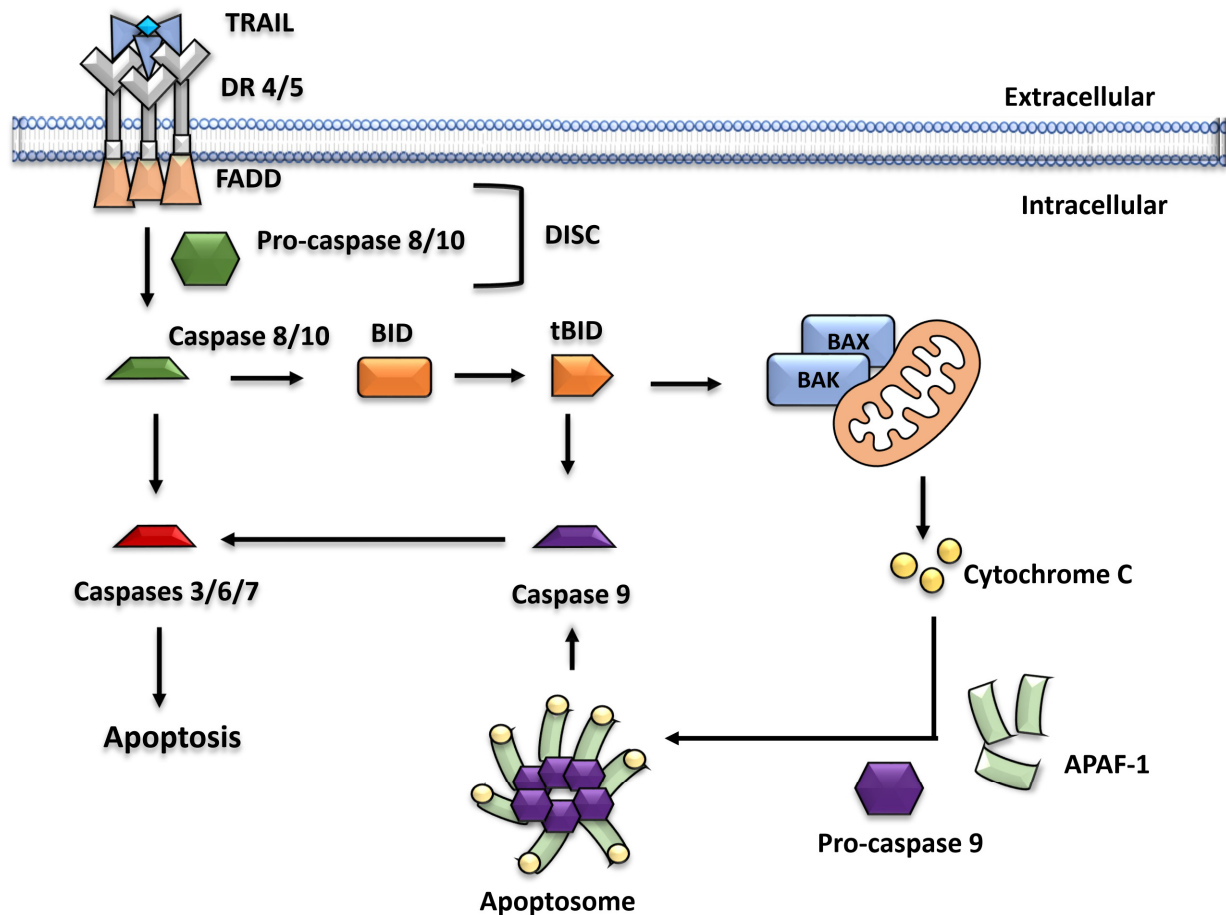


Figure 1. Schematic overview of the TRAIL apoptosis pathway. TRAIL induces apoptosis by binding to death receptors 4/5. This promotes the trimerization of the death receptor, which recruits FADD and pro-caspase 8/10 to form DISC. The latter activates caspase 8/10, which activates caspase 3 to induce apoptosis. Caspase 8/10 can also convert BID to tBID by cleavage, increasing apoptosis. To accomplish this, tBID translocates to the mitochondria, activating BAX/BAK. The formation of mitochondrial pores is then mediated by activated BAX/BAK, resulting in the release of cytochrome c. An apoptosome is formed when Apaf-1 and pro-caspase 9 bind together. Caspase 9 is activated, which then activates caspases 3, 6, and 7 to increase apoptosis.

When bound to FADD, DED1s dimerize procaspases 8/10, resulting in autocatalytic cleavage and activation of caspases 8/10 [38]. Caspases 8/10 are regulated by a cellular FLICE-inhibitory protein (c-FLIP), a cellular FADD-like interleukin (IL)-1-converting enzyme (FLICE)-inhibitory protein [39]. c-FLIP is a DED-containing protein that is structurally similar to caspases 8/10 but lacks protease activity due to the absence of a critical NH₂ amino acid residue at the active site [39]. c-FLIP inhibits caspase activation by interfering with the interaction between FADD and procaspases 8/10. Active caspases 8/10 can directly activate caspases 3, 6, and 7 to induce cell death.

Furthermore, activated caspases 8/10 can enhance apoptosis by cleaving the B-cell lymphoma-2 (BCL-2) family protein BID [40,41]. BID is a cytosolic protein that is cleaved

by activated caspase 8 on the Asp-60 residue into two fragments: c-terminal (truncated BID [tBID], p15) and p7 [40,41]. tBID activates the pro-apoptotic proteins BAX and BAK [42]. Active BAX/BAK undergoes a conformational change, resulting in dimerization and the formation of pores in the mitochondrial outer membrane (MOM) or MOM permeabilization (MOMP) [43]. MOMP induced by BAX/BAK releases cytochrome c (Cyt c) and other factors, including the DIABLO homolog (second mitochondrial-derived activator of caspases [SMAC/DIABLO]) from the mitochondria [44] to the cytosol, where it combines with ATP and the adaptor protein apoptosis-protease activating factor 1 (Apaf-1) to form an apoptosome [45,46]. As a result, caspase 9 is activated [46,47]. Furthermore, SMAC/DIABLO can increase apoptosome formation by inhibiting anti-apoptotic proteins called inhibitors of apoptosis (IAP) [44]. Active caspase 9 activates caspases 3, 6, and 7 in the same way that caspases 8/10 do [48].

Caspase 3 activation affects several downstream substrates, resulting in DNA fragmentation and cell disintegration. An example is caspase-activated DNase (CAD)/DNA fragmentation factor [DFF]. CAD is a caspase-3-activated endonuclease activated by the proteolytic cleavage of the CAD inhibitor (ICAD, DFF45) [49,50]. Activated CAD can then dimerize and bind to A/T-rich DNA regions, resulting in double-stranded DNA fragments [50,51]. The deactivation of DNA repair proteins, such as poly (ADP-ribose) polymerase-1 (PARP-1) can also increase CAD-dependent DNA fragmentation [52,53]. Furthermore, caspase 3 can deactivate several other survival proteins to enhance apoptosis, including those involved in nuclear structure maintenance, transcription, cell cycle, membrane-bound, cell adhesion, cell–cell communication, RNA synthesis/splicing, and protein translation/modification [53]. As a result, cells eventually disintegrate and form apoptotic bodies, which are consumed by phagocytic cells [54].

5. TRAIL-Mediated Non-Apoptotic Signaling

In addition to the induction of apoptosis, TRAIL has also been shown to activate several non-apoptotic signaling pathways. Among these pathways are the extracellular signal-regulated kinase (ERK) [55–57], AKT [56,58,59] and NF- κ B [60–62] pathways. These pathways are usually cell-type specific, and the activation of these pathways inhibits apoptosis [55,57,58,61,63,64]. For example, a previous study suggests that TRAIL can activate the ERK, AKT, and NF- κ B pathways through a secondary complex that forms after the formation of DISC [65]. This complex contains FADD, caspase-8, c-FLIP, receptor-interacting protein 1 (RIP1), TNF receptor-associated factor 2 (TRAF2), I κ B kinase, TNF receptor 1-associated death domain protein (TRADD), and NF- κ B essential modulator (NEMO) [60,65–67]. Furthermore, the location of death receptors affects whether apoptosis occurs. It has been demonstrated that death receptor distribution in lipid rafts induces apoptosis, while non-apoptotic signaling can occur outside these rafts [68,69]. However, the precise mechanisms and circumstances that drive TRAIL to promote the formation of secondary complexes are still being explored. Furthermore, TRAIL can generate a tumor-supportive immune microenvironment by producing cytokines/chemokines, including CXCL1, CXCL5, CCL2, IL-8, and NAMPT, to polarize monocytes to M2-like cells [70]. Furthermore, TRAIL death receptor 2 is overexpressed in KRAS-mutated tumors, and this overexpression activates the Rac1/PI3K pathway, which in turn promotes KRAS-driven tumor progression, invasion, and metastasis [71]. A recent study showed that TRAIL could induce the expression of the immune checkpoint protein programmed death-ligand 1 (PD-L1) via the ERK pathway, and that inhibiting it made TRAIL-resistant cells susceptible to TRAIL-induced apoptosis [57]. These findings show that the TRAIL pathway can activate oncogenic signaling pathways and immunological checkpoint responses and produce cytokines/chemokines that promote cancer cell survival.

6. TRAIL Resistance Mechanisms

The clinical development of TRAIL-based cancer therapy faces several challenges. To begin with, many cancer cells are intrinsically resistant to TRAIL. Second, previously

sensitive cancer cells develop resistance to TRAIL (acquired resistance). Third, no patient population that can benefit from TRAIL has been selected. Finally, the mechanisms underlying the resistance of TRAIL are not fully understood [6]. Therefore, these features warrant further investigation into the mechanisms that confer TRAIL resistance to develop TRAIL-based therapies for clinical use.

Increasing evidence suggests that TRAIL resistance mechanisms are diverse and can occur anywhere along the TRAIL signaling pathway, from the cell surface to downstream caspases (Figure 2). Specifically, resistance to TRAIL can be conferred by the dysfunction, degradation, or polymorphisms of the death receptor on the cell surface, resulting in reduced binding of TRAIL to its death receptors and increased cancer cell survival. For example, a loss-of-function mutation of DR5, found in human head and neck cancer, is TRAIL resistant [72]. Furthermore, previous studies using TRAIL death receptor knockout mice with diethyl nitrosamine-induced liver tumors or lymphoma showed increased cancer metastasis [73]. Based on these findings, several therapeutics, including the use of proteasome (to prevent death receptor degradation) and histone deacetylase (HDAC) (to block death receptor acetylation) inhibitors, have been proposed [74,75].

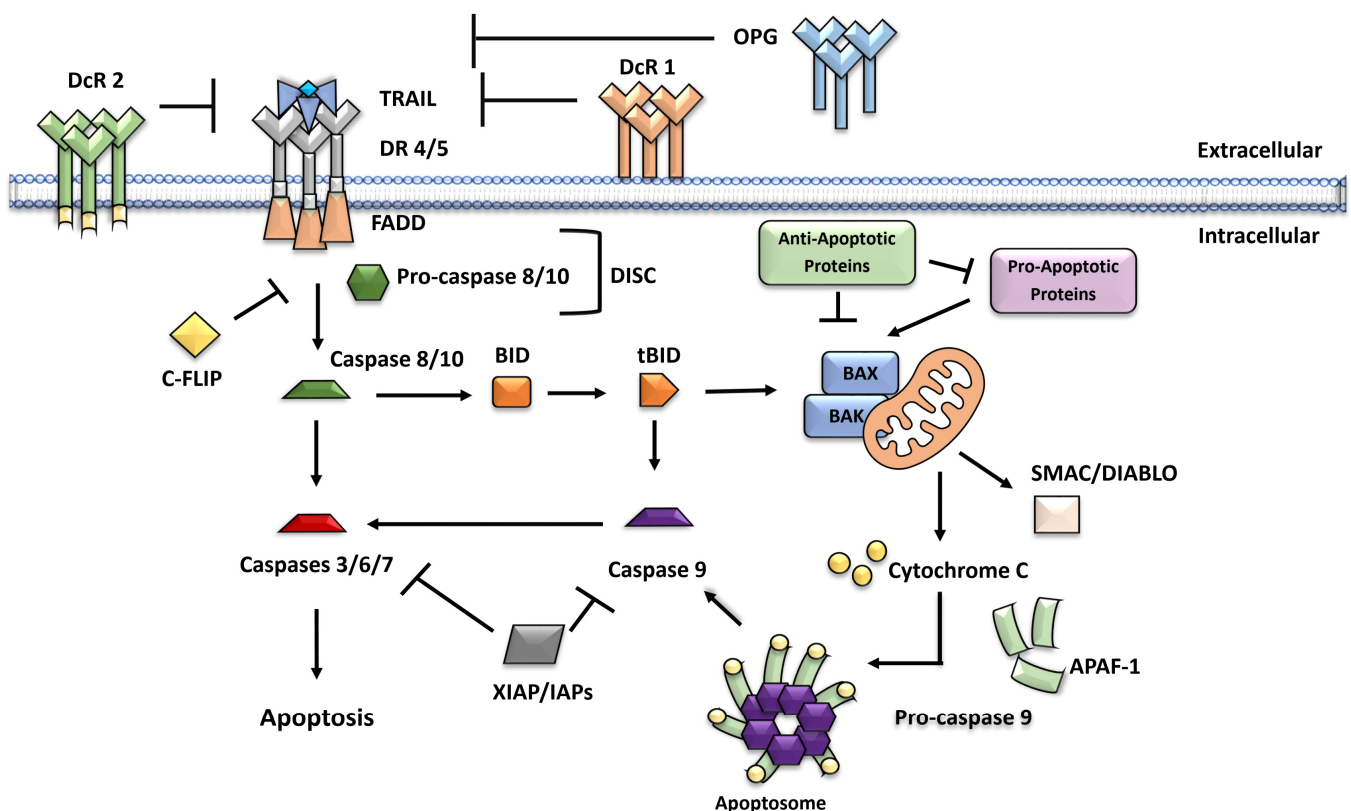


Figure 2. Mechanisms of TRAIL resistance. TRAIL resistance mechanisms can occur at any point along the TRAIL signaling pathway. Overexpression of decoy receptors, such as DcR1, DcR2, and OPG, can inhibit TRAIL-induced activation of DR4 and DR5 at the cell surface. TRAIL-induced apoptosis can also be inhibited by the inhibitory protein c-FLIP. This is accomplished by c-FLIP binding to FADD or procaspases 8/10 and inhibiting DISC formation. Antiapoptotic proteins (e.g., BCL-2, BCL-XL) can inhibit the TRAIL signaling pathway. These proteins bind to pro-apoptotic proteins (e.g., BAX), preventing MOMP, SMAC/DIABLO, and cytochrome c release. Two more downstream TRAIL signaling inhibitors are XIAP and IAP. These proteins prevent caspase 9, as well as caspases 3, 6 and 7 activation, which in turn inhibits apoptosis.

DcR (DcR1, DcR2, and OPG) expression can also confer TRAIL resistance by competing for the binding of TRAIL to DR4 and DR5 to inhibit apoptosis [76–78]. It has been shown that TRAIL-DcR2 binding activates tumor-promoting downstream pathways in cervical

cancer cells and the NF- κ B pathway in large granular lymphocyte leukemia [79,80]. DcR1 has also been found in lipid rafts and has been shown to inhibit the formation of DISC associated with DR5-TRAIL [76]. Therefore, these findings suggest that targeting TRAIL receptors has an important implication in the prevention of cancer and the induction of apoptosis.

Furthermore, when TRAIL-DR4/DR5 is activated, proteins such as c-FLIP are recruited to DISC and replace procaspases 8/10, forming an inactive complex [81]. Because of this, caspases 8/10 are rendered inactive. In this regard, the c-FLIP protein has been found to be overexpressed in human cancers, including prostate cancer (DU145) and non-small cell lung cancer (A549) cells, and has been linked to poor prognoses [82]. In addition, caspases 8/10 mutations can confer TRAIL resistance in cancer cells. A p10 mutation in procaspase 8 was found in acute myeloid leukemia, impairing the dimerization of procaspase 8 [83]. Caspase 10 mutations have also been found in colon, gastric, and NHL cancers, leading to TRAIL-induced apoptosis resistance [84]. Finally, though rare, mutations in the DISC-forming FADD protein can confer TRAIL resistance in cancer cells such as NSCLC cells [85]. Thus, several mutations in the DISC protein can confer resistance to TRAIL by preventing the activation of downstream mechanisms that lead to extrinsic and intrinsic apoptosis pathways.

In addition, anti-apoptotic proteins can promote TRAIL resistance. BCL-2 and BCL-XL proteins, for instance, inhibit TRAIL-induced apoptosis [86,87]. Several studies have shown that small-molecule BCL-2 inhibitors can be used to inhibit these proteins [88]. Members of the IAP family, including XIAP (X-linked inhibitor of apoptosis protein) and survivin, which inhibit caspase 9 and caspase 3 activity, are negative regulators of the TRAIL apoptosis pathway. Targeting IAP expression has been shown to sensitize cancer cells to TRAIL-induced cell death [89,90], suggesting that in cancer cells whose IAP is over-expressed, inhibition of IAP is a strategy to overcome resistance to TRAIL. Although several resistance mechanisms to TRAIL have been identified [91], a complete understanding of these resistance mechanisms is still needed to develop better cancer therapies.

7. Targeting TRAIL and TRAIL Death Receptors for Cancer Therapies

Because TRAIL selectively induces apoptosis in cancer cells, clinical trials were conducted to test the efficacy of TRAIL and agonist antibodies targeting death receptors in cancer patients (Table 1) [7,92]. Unlike most chemotherapeutic drugs, TRAIL ligand-based therapy causes apoptosis in tumor cells in a p53-independent manner [22]. Therefore, several TRAIL-based cancer monotherapies and combinations have been tested in human clinical trials [7,92]. The earlier forms of TRAIL used were recombinant TRAIL (rTRAIL), which was purified with a poly-Histidine (His) or FLAG epitope (FLAG-TRAIL) tag in the NTC domain [93]. Despite promising in vitro/vivo results, His- and FLAG-tagged rTRAIL aggregated and caused hepatotoxicity in vitro [3,93–95]. An example of rTRAIL used in clinical trials is Dulanermin (Apo2L.O or AMG-915) [93]. Dulanermin can form stable bioactive trimers that bind to DR4 and DR5 and induce apoptosis. Dulanermin has previously been shown to selectively induce apoptosis in cancer cells and work in tandem with a number of chemotherapeutic agents [3,96]. Dulanermin has been evaluated in non-small cell lung cancer (NSCLC) (phase III: NCT03083743) and B-cell non-lymphoma Hodgkin's (phase II: NCT01258608) [7,97]. The addition of Dulanermin (i.v., 75 g/kg) to standard therapy (cisplatin [i.v., 30 mg/m²] or Vinorelbine [i.v., 25 mg/m²]) improved progression-free survival (PFS) (6.4 months) in patients with advanced untreated NSCLC compared to placebo with standard therapy (3.5 months) [97]. Furthermore, the combination demonstrated acceptable toxicity while increasing the overall response rate (ORR) in the Dulanermin arm [97]. Despite promising results, Dulanermin has limitations, including a poor pharmacokinetic (PK) profile, a short half-life, and the ability to bind to DcRs [98,99].

Table 1. Clinical trials of rTRAIL and death-receptor-targeting antibodies.

Type	Name	Cancer	Phase	Clinical Trial ID
rTRAIL	Dulanermin	Non-small cell lung cancer	III	NCT03083743
			II	NCT00508625
		Non-Hodgkin's lymphoma	II	NCT00400764
			I	NCT00671372
	SCB-313	Peritoneal malignancies	I	NCT03443674
		Peritoneal carcinomatosis	I	NCT04047771
	ABBV-621	Advanced solid tumors and hematological malignancies	I	NCT03082209
		Multiple myeloma	II	NCT04570631
DR4 targeting	Mapatumumab	Multiple myeloma	II	NCT00315757
		Non-Hodgkin's lymphoma	II	NCT00094848
		Hepatocellular carcinoma	II	NCT01258608
		Non-small cell lung cancer	II	NCT00583830
		Cervical cancer	II	NCT01088347
		Non-small cell lung cancer	II	NCT00991796
DR5 targeting	Tigatuzumab	Pancreatic cancer	II	NCT00521404
		Triple-negative breast cancer	II	NCT01307891
		Ovarian cancer	II	NCT00945191
		Colorectal cancer	I	NCT01124630
		Colorectal cancer	I	NCT01124630
	INBRX-109	Solid tumors, malignant pleura mesothelioma, gastric, colorectal, sarcoma (Ewing and chondrosarcoma), pancreatic	I	NCT03715933
		Chondrosarcoma	II	NCT04950075
	IGM-8444	Solid tumors, colorectal, lymphoma (non-Hodgkin and small lymphocytic), sarcoma (chondrosarcoma), leukemia (chronic lymphocytic and acute)	I	NCT04553692
	GEN1029	Colorectal, non-small cell lung, triple-negative breast, renal cell, gastric, pancreatic, and urothelial cancers	II	NCT03576131
	BI 905711	Gastrointestinal, pancreatic, and cholangiocarcinoma	I	NCT04137289
		Gastrointestinal cancers	I	NCT05087992

The limitations of rTRAIL have led to research on novel ways of improving its therapeutic efficacy. The conjugation of rTRAIL with cytotoxic agents is one strategy for enhancing rTRAIL's therapeutic efficacy. rTRAIL combined with chemotherapy has been shown to improve clinical outcomes in Phase III clinical studies for patients with advanced NSCLC [7,97]. Furthermore, TRAIL-based therapy can be combined with immunotherapy (NCT02991196), biological treatment (NCT00400764, NCT00508625), and targeted therapy (NCT01258608, NCT00315757) to improve overall survival (OS) in a variety of cancers [7,92].

Despite new drug combinations and formulations, rTRAIL's poor pharmacokinetic profile continues to limit its clinical development, including its short biological half-life [100,101]. Therefore, efforts have been made to overcome these limitations to improve rTRAIL's poor bioactivity, stability, and tumor specificity. For example, the addition of a tenascin C oligomerization domain, the formation of single-chain TRAIL trimers, and the covalent attachment of TRAIL to molecules (e.g., human serum albumin [HSA] and polyethylene glycol) have been used to improve the stability of rTRAIL [93]. An example of

trimeric forms of the TRAIL protein is SCB-313, which was tested in Phase I clinical trials for peritoneal malignancies (NCT03443674 and NCT04051112) [102]. Furthermore, ABBV-621 (Eftozanermin) is a hexavalent TRAIL-Fc fusion protein that has shown antitumor activity with acceptable toxicity in Phase I clinical trials in solid tumors (NCT03082209) [103]. Currently, ABBV-621 is being studied in Phase II clinical trials for multiple myeloma (NCT04570631). One challenge with TRAIL-based therapy is its distribution to tumors. To address this, the combination of TRAIL with nanoparticles is a unique approach to improving the delivery of TRAIL to tumors [104]. Several TRAIL-containing nanoparticles have been developed, with compositions ranging from human serum albumin [104–106] to poly (lacto-co-glycolic) acid (PLGA) microspheres [107,108] to liposomes [109,110]. Thus, more research is needed to fully comprehend the clinical potential of nanoparticles containing TRAIL. Despite these modified forms of rTRAIL showing promising in vitro/in vivo results, such as a better pharmacokinetic profile, longer half-life, and higher clearance rate, the delivery of rTRAIL to tumors has remained challenging [93].

In contrast, TRAIL agonist antibodies against its death receptors have several advantages over TRAIL ligands, including directly targeting DR4 or DR5 and being more stable [111]. Therefore, the focus of studies has shifted to the development of agonistic antibodies against death receptors for treating cancer. Antibodies against death receptors are classified into two groups: (1) DR4-targeting antibodies (e.g., mapatumumab) [112]; and (2) DR5-targeting antibodies (e.g., conatumumab, lexatumumab, tigatuzumab, drozitumab, and LBY-135) [113–117]. Several DR4-targeting antibodies have been developed. One of them is mapatumumab (Map), which has been studied in Phase II clinical trials for multiple myeloma (NCT00315757), NHL (NCT00094848), hepatocellular carcinoma (NCT01258608), cervical cancer (NCT01088347), and lung cancer (NCT00583830) [118]. Based on these studies, Map is well tolerated at 20 mg/kg/day, with a favorable safety profile [7]. However, Map has not been shown to improve response rates in cancer patients when combined with paclitaxel, gemcitabine, carboplatin, or bortezomib [7,119]. Similarly, several DR5-targeting antibodies have been developed and tested in patients with different cancers. The DR5 antibody tigatuzumab has been studied in Phase II clinical trials for NSCLC (NCT00991796), pancreatic cancer (NCT00521404), ovarian cancer (NCT00945191), colorectal cancer (NCT01124630), and triple-negative breast cancer (TNBC) (NCT01307891) [118]. Most DR5- and DR4-targeting antibodies are safe and well tolerated in patients at 20 mg/kg/day [119]. In addition, several new DR5-targeting antibodies are now entering clinical studies. The tetravalent DR5 antibody INBRX-109 is one of them, where it will be examined in a variety of solid tumors and sarcomas (NCT03715933 and NCT04950075) [120]. INBRX-109 will be studied alone and in combination with other treatment agents, including 5-fluorouracil/irinotecan- with INBRX-109 to treat pancreatic ductal cancer (NCT03715933). Another candidate is IGM-8444, which is a multimeric anti-DR5 agonist antibody that will be tested in patients with newly diagnosed, relapsed, or refractory cancers (NCT04553692) alone or in combination with other chemotherapies. Preclinical studies with IGM-8444 showed tumor cytotoxicity in vitro and in vivo with a favorable safety profile and synergized with chemotherapeutic agents, including paclitaxel and the BCL-2 inhibitor ABT-199 [121]. DR5 antibodies, such as GEN1029 and BI 905711, have also been studied in clinical trials. GEN1029 is a hexamerizing IgG that forms hexamers when it binds to its target due to a mutation of E430G in its Fc domains [122]. GEN1029 underwent Phase I and Phase II clinical studies in various malignancies (NCT03576131). However, the study's sponsor halted the clinical trial with GEN1029 due to a narrow therapeutic window after the dose-escalation phase of the study. Lastly, BI 905711, a bispecific tetravalent antibody DR-5 and CDH17, is recruiting for Phase I clinical trials for gastrointestinal, pancreatic, and cholangiocarcinomas (NCT04137289, NCT05087992) [123]. These clinical trials were prompted by a recent preclinical study showing that BI 905711 could selectively induce apoptosis in CDH17-positive colorectal cancer cells in vitro and in vivo with a favorable safety profile [123].

Although it is unknown why DR5 antibodies outnumber DR4 antibodies, DR5 antibodies have been studied in a variety of cancers and combinations in the past [119]. Despite promising results, the inability of TRAIL death receptor antibodies to induce death receptor trimerization limits their clinical development [119,124]. Despite a favorable safety profile in patients, death receptor agonist antibodies have shown limited efficacy. Current efforts have been made to improve antibody-based therapies by modifying death receptor antibodies to enhance death receptor clustering [111]. In addition, combining with other chemotherapies is another viable strategy for improving death-receptor-based therapies. Because TRAIL and its death receptor agonist antibodies have shown limited efficacy in clinical trials, alternative strategies have been tested, including the search for small molecules that can induce TRAIL and DR5 as therapeutics. One of the promising small molecules identified using this approach is ONC201 [125]. ONC201 was initially discovered as a compound that can induce TRAIL expression and is potent against several types of tumors [126–128]. Subsequent studies have shown that ONC201 has several targets, including the induction of DR5 and the activation of an integrated stress response and caseinolytic protease P (CLPP) [129,130]. ONC201 has been tested in clinical trials, and its efficacy has been observed in some cancer patients, particularly those with glioblastoma that harbor H3K27M mutations [129]. ONC201 is now in Phase III clinical trials for treating adult recurrent H3 K27M-mutant high-grade glioma.

8. TRAIL and Tumor Immunosurveillance

The immune system is involved in tumor prevention and elimination of tumors [131]. For example, viral infections must be eradicated to prevent virus-induced tumors. Additionally, inflammatory conditions must be resolved to prevent tumor development. In addition, tumors must be identified and removed. The TRAIL pathway has been shown to play a critical role in viral infection and tumor immune surveillance [132,133]. Increasing evidence suggests that TRAIL expression is abundant in innate and adaptive immune system cells [134]. TRAIL-expressing cells from the innate immune system include macrophages (MPs) and dendritic cells (DCs), and those from the adaptive immune system include B and T cells (CD4+ and CD8+ T cells) [135–137]. TRAIL is also expressed by natural killer cells (NKC) [138]. Although TRAIL expression varies by cell types, TRAIL is stored in the intracellular pool of many immune cells from which it is secreted in response to stimuli [139,140]. Lipopolysaccharide (LPS) and pro-inflammatory cytokines, such as interferons (IFN- α , β), TNF- α , and IL2, act as stimuli by activating transcription factors that increase TRAIL transcription, resulting in increased soluble and membrane-bound TRAIL expression in immune cells [136,141–144].

Tumors are complex tissues composed of malignant and surrounding cells, including immune cells that interact with tumor cells [145]. TRAIL-expressing cytotoxic cells (e.g., MPs, NKCs, and T cells) and antigen-presenting cells (APCs) (e.g., DCs, MPs, and B cells) serve as the first line of defense against cancer in both the innate and adaptive immune systems [146]. Previous studies suggest that MPs recognize tumor-associated antigens (TAA) through Fc γ or lectin-like receptors, as well as LDL receptor-related protein 1 (LRP1) [147,148]. Then, in response to LPS and IFN stimulation, MPs are activated, resulting in increased TRAIL expression [136,141]. Therefore, through functional interactions with immune cells, TRAIL can directly inhibit tumor cell growth by inducing cancer cell apoptosis and promoting the recruitment of immune cells (monocytes/macrophages) through chemokine secretion to kill cancer cells [146]. IFN has also been shown to increase TRAIL expression in DC [142] and NKC [132]. For DCs, IFN-stimulated TRAIL expression induces apoptosis in TRAIL-sensitive cells, including tumor cells [142]. In the case of NKCs, IFN-stimulated TRAIL induction is critical for the antitumor metastasis activity of NK cells [132].

9. TRAIL and the Immune Checkpoint PD-L1

Recent evidence suggests that the TRAIL pathway functionally interacts with the immune checkpoint PD-L1. PD-L1 is a checkpoint molecule that binds to inhibitory receptor programmed death protein 1 (PD-1) on the surface of immune cells, such as T and B cells [149]. The PD-1/PD-L1 signaling axis maintains immune homeostasis by suppressing T-cell function to prevent autoimmunity [149,150]. Many tumor cells exploit this mechanism to evade immune surveillance by overexpressing PD-L1 [151]. Although TRAIL has previously been implicated in tumor immune surveillance [133,152,153], TRAIL has been shown to induce PD-L1 expression and promote the epithelial–mesenchymal transition in the squamous cell carcinoma of the esophagus [154]. TRAIL does this by activating the ERK/STAT3 signaling pathways, which promote EMT via PD-L1 [154]. In gastric cancer cells, TRAIL can also increase the expression of PD-L1 by inhibiting miR-429 [155]. PD-L1 binds to the epidermal growth factor receptor (EGFR) and activates the cell survival signaling pathway mTOR/AKT, which reduces the sensitivity of TRAIL [155]. Furthermore, a recent study found that DR5 agonistic antibodies induce PD-L1 expression [156]. The underlying mechanism is that the activation of caspase 8 by DR5 agonistic antibodies increases Rho-associated kinase1 (ROCK1) activity to induce PD-L1 expression, which promotes immune evasion [156]. A recent study showed that PD-L1 expression confers resistance to TRAIL in tumor cells in a non-canonical manner [57]. TRAIL can induce PD-L1 expression in triple-negative breast cancer (TNBC) cells via an ERK-dependent mechanism [57]. Furthermore, PD-L1 expression has been shown to confer resistance to TRAIL in tumor cells in a non-canonical manner [57]. Inhibiting PD-L1 expression in TRAIL-resistant TNBC cells increased TRAIL sensitivity in the absence of immune cells [57]. Combining an anti-PD-L1 antibody with TRAIL effectively induced cancer cell death [157]. Thus, an increase in PD-L1 expression by the TRAIL pathway promotes EMT, cell survival, immune evasion, and TRAIL resistance (Figure 3). Based on these findings, we speculate that targeting PD-L1 may improve TRAIL/TRAIL death-receptor-based therapies in tumor cells. We also speculate that targeting the TRAIL pathway may improve PD-L1-based cancer immunotherapy.

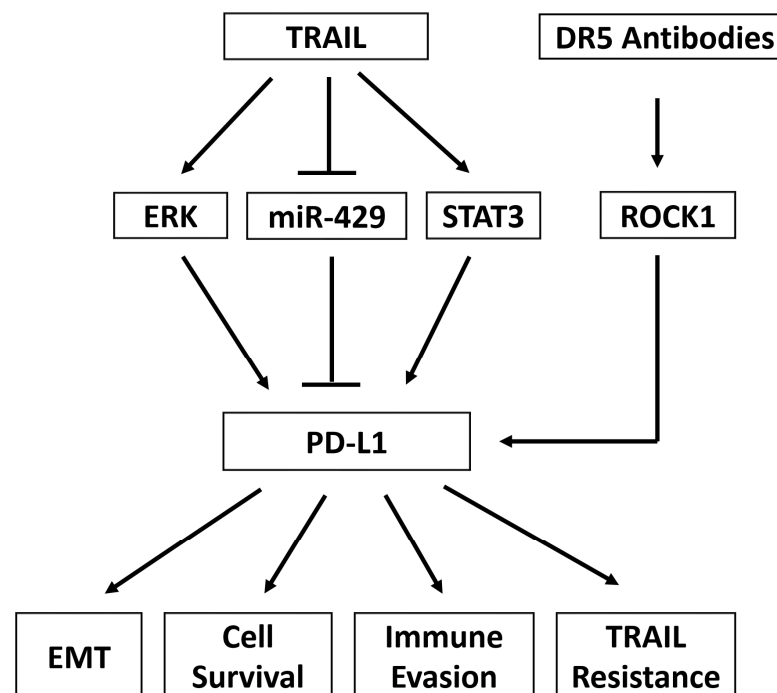


Figure 3. TRAIL and DR5 agonist antibodies increase PD-L1 expression. TRAIL can induce PD-L1 expression by activating the ERK and STAT3 pathways or inhibiting miR-429. DR5 agonist antibodies can induce PD-L1 expression by activating ROCK1. Induction of PD-L1 then results in EMT, cell survival, immune evasion, and resistance to TRAIL.

10. Conclusions—Concluding Remarks

The discovery of TRAIL as a cancer-selective agent has led to extensive research into the TRAIL signaling pathway and its potential application in cancer therapy. Following promising preclinical results, TRAIL-based treatments were developed, which included recombinant TRAIL and its death receptor agonistic antibodies. While several TRAIL-based therapies have advanced to clinical trials and have been shown to be well tolerated in patients, the low efficacy of TRAIL and death receptor antibodies as cancer therapeutics limits their further development. A number of TRAIL resistance mechanisms has been identified, and treatments that target these mechanisms have been developed, including TRAIL and its death receptor antibody variants with structural modifications, nanoparticles, and novel combination strategies. Additionally, new evidence suggests that TRAIL functionally interacts with the immune checkpoint molecule PD-L1 and that targeting PD-L1 enhances the antitumor activity mediated by TRAIL. These findings are encouraging because they capitalize on TRAIL's role in tumor immunosurveillance and pave the way for clinical trials by combining TRAIL-based therapies with immune checkpoint blockers. Finally, a better understanding of the pathways that regulate the TRAIL pathway in the context of the immune checkpoint PD1/PD-L1 axis may lead to improving TRAIL-based therapy. Thus, it is conceivable that understanding these issues will aid in better developing TRAIL-based cancer therapy.

Author Contributions: Conceptualization, J.M.P. and G.S.W.; writing—original draft, J.M.P. and G.S.W.; writing—review and editing, J.M.P., J.-Y.Z. and G.S.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was, in part, supported by the National Institute of Health [Grant R01CA174949] through the NCI (National Cancer Institute) (GSW), T32 Fellowship [T32-CA009531] (JMP), and Dean's Diversity Fellowship of Wayne State University (JMP).

Data Availability Statement: All representative data are contained within the article.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Abbreviations

Apaf-1: apoptosis-protease activating factor 1; APC: antigen-presenting cell; BAK: BCL-2 antagonist killer; BAX: BCL-2-associated x protein; BID: BH3-interacting domain; c FLIP: cellular FLICE-inhibitory protein; CAD: caspase-activated Dnase; CLPP: caseinolytic protease P; CTE: C-terminal extracellular; cys: cysteine; Cyt C: cytochrome C; DC: dendritic cell; DcR: decoy receptor; DD: death domain; DED: death effector domain; DFF: DNA fragmentation factor; DISC: death-inducing signaling complex; EGFR: epidermal growth factor receptor; EMT: epithelial–mesenchymal transition; ERK: extracellular signal-regulated kinase; ES: extracellular stalk; FADD: Fas-associated death domain; FLICE: FADD-like interleukin-1-converting enzyme; GPI: glycosylphosphatidylinositol; HDAC: histone deacetylase; His: histidine; HSA: human serum albumin; IAP: inhibitor of apoptosis protein; IL2: interleukin-2; INF: interferon; LRP1: LDL receptor-related protein 1; LPS: lipopolysaccharide; Map: mapatumumab; MIM: mitochondrial inner membrane; MOM: mitochondrial outer membrane; MOMP: mitochondrial outer membrane permeabilization; MP: macrophage; NEMO: NF- κ B essential modulator; NKC: natural killer cell; NSCLC: non-small cell lung cancer; NTC: N-terminal cytoplasmic; OPG: osteoprotegerin; ORR: overall response rate; PARP-1: poly (ADP-ribose) polymerase-1; PD-1: programmed death protein 1; PD-L1: programmed death-ligand 1; PK: pharmacokinetic; PLAD: preligand assembly domain; PLGA: poly (lacto-co-glycolic) acid; RIP1: receptor-interacting protein 1; ROCK1: rho-associated kinase 1; rTRAIL: recombinant TRAIL; SMAC: second mitochondrial-derived activator of caspases; TAA: tumor-associated antigen; tBID: truncated BID; TM: transmembrane; TNBC: triple-negative breast cancer; TNF: tumor necrosis factor; TRAF2: TNF receptor-associated factor 2; TRAIL: TNF-related apoptosis-inducing ligand; XIAP: X-linked inhibitor of apoptosis protein.

References

- Wiley, S.R.; Schooley, K.; Smolak, P.J.; Din, W.S.; Huang, C.P.; Nicholl, J.K.; Sutherland, G.R.; Smith, T.D.; Rauch, C.; Smith, C.A.; et al. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* **1995**, *3*, 673–682. [\[CrossRef\]](#) [\[PubMed\]](#)
- Pitti, R.M.; Marsters, S.A.; Ruppert, S.; Donahue, C.J.; Moore, A.; Ashkenazi, A. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J. Biol. Chem.* **1996**, *271*, 12687–12690. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ashkenazi, A.; Pai, R.C.; Fong, S.; Leung, S.; Lawrence, D.A.; Marsters, S.A.; Blackie, C.; Chang, L.; McMurtrey, A.E.; Hebert, A.; et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. *J. Clin. Invest.* **1999**, *104*, 155–162. [\[CrossRef\]](#) [\[PubMed\]](#)
- Walczak, H.; Miller, R.E.; Ariail, K.; Gliniak, B.; Griffith, T.S.; Kubin, M.; Chin, W.; Jones, J.; Woodward, A.; Le, T.; et al. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat. Med.* **1999**, *5*, 157–163. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ralff, M.D.; El-Deiry, W.S. TRAIL pathway targeting therapeutics. *Expert. Rev. Precis. Med. Drug Dev.* **2018**, *3*, 197–204. [\[CrossRef\]](#)
- Yuan, X.; Gajan, A.; Chu, Q.; Xiong, H.; Wu, K.; Wu, G.S. Developing TRAIL/TRAIL death receptor-based cancer therapies. *Cancer Metastasis Rev.* **2018**, *37*, 733–748. [\[CrossRef\]](#) [\[PubMed\]](#)
- Snajdauf, M.; Havlova, K.; Vachtenheim, J., Jr.; Ozaniak, A.; Lischke, R.; Bartunkova, J.; Smrz, D.; Strizova, Z. The TRAIL in the Treatment of Human Cancer: An Update on Clinical Trials. *Front. Mol. Biosci.* **2021**, *8*, 628332. [\[CrossRef\]](#)
- Kelley, S.K.; Harris, L.A.; Xie, D.; Deforge, L.; Totpal, K.; Bussiere, J.; Fox, J.A. Preclinical studies to predict the disposition of Apo2L/tumor necrosis factor-related apoptosis-inducing ligand in humans: Characterization of in vivo efficacy, pharmacokinetics, and safety. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 31–38.
- Wajant, H. Molecular Mode of Action of TRAIL Receptor Agonists-Common Principles and Their Translational Exploitation. *Cancers* **2019**, *11*, 954. [\[CrossRef\]](#)
- Kawakubo, T.; Okamoto, K.; Iwata, J.; Shin, M.; Okamoto, Y.; Yasukochi, A.; Nakayama, K.I.; Kadowaki, T.; Tsukuba, T.; Yamamoto, K. Cathepsin E prevents tumor growth and metastasis by catalyzing the proteolytic release of soluble TRAIL from tumor cell surface. *Cancer Res.* **2007**, *67*, 10869–10878. [\[CrossRef\]](#)
- Hymowitz, S.G.; Christinger, H.W.; Fuh, G.; Ultsch, M.; O'Connell, M.; Kelley, R.F.; Ashkenazi, A.; de Vos, A.M. Triggering cell death: The crystal structure of Apo2L/TRAIL in a complex with death receptor 5. *Mol. Cell* **1999**, *4*, 563–571. [\[CrossRef\]](#) [\[PubMed\]](#)
- van der Sloot, A.M.; Mullally, M.M.; Fernandez-Ballester, G.; Serrano, L.; Quax, W.J. Stabilization of TRAIL, an all-beta-sheet multimeric protein, using computational redesign. *Protein Eng. Des. Sel.* **2004**, *17*, 673–680. [\[CrossRef\]](#) [\[PubMed\]](#)
- Hymowitz, S.G.; O'Connell, M.P.; Ultsch, M.H.; Hurst, A.; Totpal, K.; Ashkenazi, A.; de Vos, A.M.; Kelley, R.F. A unique zinc-binding site revealed by a high-resolution X-ray structure of homotrimeric Apo2L/TRAIL. *Biochemistry* **2000**, *39*, 633–640. [\[CrossRef\]](#)
- Bodmer, J.L.; Meier, P.; Tschopp, J.; Schneider, P. Cysteine 230 is essential for the structure and activity of the cytotoxic ligand TRAIL. *J. Biol. Chem.* **2000**, *275*, 20632–20637. [\[CrossRef\]](#) [\[PubMed\]](#)
- Guicciardi, M.E.; Gores, G.J. Life and death by death receptors. *FASEB J.* **2009**, *23*, 1625–1637. [\[CrossRef\]](#)
- Pan, G.; Ni, J.; Wei, Y.F.; Yu, G.; Gentz, R.; Dixit, V.M. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* **1997**, *277*, 815–818. [\[CrossRef\]](#)
- Pan, G.; O'Rourke, K.; Chinnaiyan, A.M.; Gentz, R.; Ebner, R.; Ni, J.; Dixit, V.M. The receptor for the cytotoxic ligand TRAIL. *Science* **1997**, *276*, 111–113. [\[CrossRef\]](#)
- Walczak, H.; Degli-Esposti, M.A.; Johnson, R.S.; Smolak, P.J.; Waugh, J.Y.; Boiani, N.; Timour, M.S.; Gerhart, M.J.; Schooley, K.A.; Smith, C.A.; et al. TRAIL-R2: A novel apoptosis-mediating receptor for TRAIL. *EMBO J.* **1997**, *16*, 5386–5397. [\[CrossRef\]](#)
- Wu, G.S.; Burns, T.F.; McDonald, E.R.; Jiang, W.; Meng, R.; Krantz, I.D.; Kao, G.; Gan, D.D.; Zhou, J.Y.; Muschel, R.; et al. KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. *Nat. Genet.* **1997**, *17*, 141–143. [\[CrossRef\]](#)
- Wu, G.S.; Burns, T.F.; Zhan, Y.; Alnemri, E.S.; El-Deiry, W.S. Molecular cloning and functional analysis of the mouse homologue of the KILLER/DR5 tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptor. *Cancer Res.* **1999**, *59*, 2770–2775.
- Sheridan, J.P.; Marsters, S.A.; Pitti, R.M.; Gurney, A.; Skubatch, M.; Baldwin, D.; Ramakrishnan, L.; Gray, C.L.; Baker, K.; Wood, W.I.; et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* **1997**, *277*, 818–821. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wang, S.; El-Deiry, W.S. TRAIL and apoptosis induction by TNF-family death receptors. *Oncogene* **2003**, *22*, 8628–8633. [\[CrossRef\]](#) [\[PubMed\]](#)
- Walczak, H. Death receptor-ligand systems in cancer, cell death, and inflammation. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a008698. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chan, F.K. Three is better than one: Pre-ligand receptor assembly in the regulation of TNF receptor signaling. *Cytokine* **2007**, *37*, 101–107. [\[CrossRef\]](#)
- Wagner, K.W.; Punnoose, E.A.; Januario, T.; Lawrence, D.A.; Pitti, R.M.; Lancaster, K.; Lee, D.; von Goetz, M.; Yee, S.F.; Totpal, K.; et al. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. *Nat. Med.* **2007**, *13*, 1070–1077. [\[CrossRef\]](#)
- Wu, G.S.; Burns, T.F.; McDonald, E.R.; Meng, R.D.; Kao, G.; Muschel, R.; Yen, T.; El-Deiry, W.S. Induction of the TRAIL receptor KILLER/DR5 in p53-dependent apoptosis but not growth. *Oncogene* **1999**, *18*, 6411–6418. [\[CrossRef\]](#)

27. Guan, B.; Yue, P.; Lotan, R.; Sun, S.Y. Evidence that the human death receptor 4 is regulated by activator protein 1. *Oncogene* **2002**, *21*, 3121–3129. [\[CrossRef\]](#)
28. Locksley, R.M.; Killeen, N.; Lenardo, M.J. The TNF and TNF receptor superfamilies: Integrating mammalian biology. *Cell* **2001**, *104*, 487–501. [\[CrossRef\]](#)
29. Chang, D.W.; Xing, Z.; Capacio, V.L.; Peter, M.E.; Yang, X. Interdimer processing mechanism of procaspase-8 activation. *EMBO J.* **2003**, *22*, 4132–4142. [\[CrossRef\]](#)
30. Tummers, B.; Green, D.R. Caspase-8: Regulating life and death. *Immunol. Rev.* **2017**, *277*, 76–89. [\[CrossRef\]](#)
31. Wachmann, K.; Pop, C.; van Raam, B.J.; Drag, M.; Mace, P.D.; Snipas, S.J.; Zmasek, C.; Schwarzenbacher, R.; Salvesen, G.S.; Riedl, S.J. Activation and specificity of human caspase-10. *Biochemistry* **2010**, *49*, 8307–8315. [\[CrossRef\]](#)
32. Ramamurthy, V.; Yamniuk, A.P.; Lawrence, E.J.; Yong, W.; Schneeweis, L.A.; Cheng, L.; Murdock, M.; Corbett, M.J.; Doyle, M.L.; Sheriff, S. The structure of the death receptor 4-TNF-related apoptosis-inducing ligand (DR4-TRAIL) complex. *Acta Crystallogr. F Struct. Biol. Commun.* **2015**, *71*, 1273–1281. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Kelley, R.F.; Totpal, K.; Lindstrom, S.H.; Mathieu, M.; Billeci, K.; Deforge, L.; Pai, R.; Hymowitz, S.G.; Ashkenazi, A. Receptor-selective mutants of apoptosis-inducing ligand 2/tumor necrosis factor-related apoptosis-inducing ligand reveal a greater contribution of death receptor (DR) 5 than DR4 to apoptosis signaling. *J. Biol. Chem.* **2005**, *280*, 2205–2212. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Tur, V.; van der Sloot, A.M.; Reis, C.R.; Szegezdi, E.; Cool, R.H.; Samali, A.; Serrano, L.; Quax, W.J. DR4-selective tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) variants obtained by structure-based design. *J. Biol. Chem.* **2008**, *283*, 20560–20568. [\[CrossRef\]](#)
35. MacFarlane, M.; Kohlhaas, S.L.; Sutcliffe, M.J.; Dyer, M.J.; Cohen, G.M. TRAIL receptor-selective mutants signal to apoptosis via TRAIL-R1 in primary lymphoid malignancies. *Cancer Res.* **2005**, *65*, 11265–11270. [\[CrossRef\]](#)
36. Luster, T.A.; Carrell, J.A.; McCormick, K.; Sun, D.; Humphreys, R. Mapatumumab and lexatumumab induce apoptosis in TRAIL-R1 and TRAIL-R2 antibody-resistant NSCLC cell lines when treated in combination with bortezomib. *Mol. Cancer Ther.* **2009**, *8*, 292–302. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Abdulghani, J.; Allen, J.E.; Dicker, D.T.; Liu, Y.Y.; Goldenberg, D.; Smith, C.D.; Humphreys, R.; El-Deiry, W.S. Sorafenib sensitizes solid tumors to Apo2L/TRAIL and Apo2L/TRAIL receptor agonist antibodies by the Jak2-Stat3-Mcl1 axis. *PLoS ONE* **2013**, *8*, e75414. [\[CrossRef\]](#)
38. Boatright, K.M.; Renatus, M.; Scott, F.L.; Sperandio, S.; Shin, H.; Pedersen, I.M.; Ricci, J.E.; Edris, W.A.; Sutherlin, D.P.; Green, D.R.; et al. A unified model for apical caspase activation. *Mol. Cell* **2003**, *11*, 529–541. [\[CrossRef\]](#)
39. Boatright, K.M.; Deis, C.; Denault, J.B.; Sutherlin, D.P.; Salvesen, G.S. Activation of caspases-8 and -10 by FLIP(L). *Biochem. J.* **2004**, *382*, 651–657. [\[CrossRef\]](#)
40. Li, H.; Zhu, H.; Xu, C.J.; Yuan, J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* **1998**, *94*, 491–501. [\[CrossRef\]](#)
41. Luo, X.; Budihardjo, I.; Zou, H.; Slaughter, C.; Wang, X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* **1998**, *94*, 481–490. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Kim, H.; Tu, H.C.; Ren, D.; Takeuchi, O.; Jeffers, J.R.; Zambetti, G.P.; Hsieh, J.J.; Cheng, E.H. Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis. *Mol. Cell.* **2009**, *36*, 487–499.
43. Westphal, D.; Kluck, R.M.; Dewson, G. Building blocks of the apoptotic pore: How Bax and Bak are activated and oligomerize during apoptosis. *Cell Death Differ.* **2014**, *21*, 196–205. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Du, C.; Fang, M.; Li, Y.; Li, L.; Wang, X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* **2000**, *102*, 33–42. [\[CrossRef\]](#)
45. Li, P.; Nijhawan, D.; Budihardjo, I.; Srinivasula, S.M.; Ahmad, M.; Alnemri, E.S.; Wang, X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* **1997**, *91*, 479–489. [\[CrossRef\]](#)
46. Acehan, D.; Jiang, X.; Morgan, D.G.; Heuser, J.E.; Wang, X.; Akey, C.W. Three-dimensional structure of the apoptosome: Implications for assembly, procaspase-9 binding, and activation. *Mol. Cell* **2002**, *9*, 423–432. [\[CrossRef\]](#)
47. Bao, Q.; Shi, Y. Apoptosome: A platform for the activation of initiator caspases. *Cell Death Differ.* **2007**, *14*, 56–65. [\[CrossRef\]](#)
48. Slee, E.A.; Harte, M.T.; Kluck, R.M.; Wolf, B.B.; Casiano, C.A.; Newmeyer, D.D.; Wang, H.G.; Reed, J.C.; Nicholson, D.W.; Alnemri, E.S.; et al. Ordering the cytochrome c-initiated caspase cascade: Hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J. Cell Biol.* **1999**, *144*, 281–292. [\[CrossRef\]](#)
49. Sakahira, H.; Enari, M.; Nagata, S. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. *Nature* **1998**, *391*, 96–99. [\[CrossRef\]](#)
50. Nagata, S.; Nagase, H.; Kawane, K.; Mukae, N.; Fukuyama, H. Degradation of chromosomal DNA during apoptosis. *Cell Death Differ.* **2003**, *10*, 108–116. [\[CrossRef\]](#)
51. Widlak, P.; Li, P.; Wang, X.; Garrard, W.T. Cleavage preferences of the apoptotic endonuclease DFF40 (caspase-activated DNase or nuclease) on naked DNA and chromatin substrates. *J. Biol. Chem.* **2000**, *275*, 8226–8232. [\[CrossRef\]](#)
52. Lazebnik, Y.A.; Kaufmann, S.H.; Desnoyers, S.; Poirier, G.G.; Earnshaw, W.C. Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature* **1994**, *371*, 346–347. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Timmer, J.C.; Salvesen, G.S. Caspase substrates. *Cell Death Differ.* **2007**, *14*, 66–72. [\[CrossRef\]](#)
54. Boada-Romero, E.; Martinez, J.; Heckmann, B.L.; Green, D.R. The clearance of dead cells by efferocytosis. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 398–414. [\[CrossRef\]](#) [\[PubMed\]](#)

55. Vilimanovich, U.; Bumbasirevic, V. TRAIL induces proliferation of human glioma cells by c-FLIPL-mediated activation of ERK1/2. *Cell Mol. Life Sci.* **2008**, *65*, 814–826. [[CrossRef](#)]
56. Secchiero, P.; Gonelli, A.; Carnevale, E.; Milani, D.; Pandolfi, A.; Zella, D.; Zauli, G. TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. *Circulation* **2003**, *107*, 2250–2256. [[CrossRef](#)] [[PubMed](#)]
57. Pimentel, J.M.; Zhou, J.Y.; Wu, G.S. Regulation of programmed death ligand 1 (PD-L1) expression by TNF-related apoptosis-inducing ligand (TRAIL) in triple-negative breast cancer cells. *Mol. Carcinog.* **2023**, *62*, 135–144. [[CrossRef](#)] [[PubMed](#)]
58. Xu, J.; Zhou, J.Y.; Wei, W.Z.; Wu, G.S. Activation of the Akt survival pathway contributes to TRAIL resistance in cancer cells. *PLoS ONE* **2010**, *5*, e10226. [[CrossRef](#)]
59. Morel, J.; Audou, R.; Hahne, M.; Combe, B. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces rheumatoid arthritis synovial fibroblast proliferation through mitogen-activated protein kinases and phosphatidylinositol 3-kinase/Akt. *J. Biol. Chem.* **2005**, *280*, 15709–15718. [[CrossRef](#)]
60. Grunert, M.; Gottschalk, K.; Kapahnke, J.; Gundisch, S.; Kieser, A.; Jeremias, I. The adaptor protein FADD and the initiator caspase-8 mediate activation of NF-kappaB by TRAIL. *Cell Death Dis.* **2012**, *3*, e414. [[CrossRef](#)]
61. Ehrhardt, H.; Fulda, S.; Schmid, I.; Hiscott, J.; Debatin, K.M.; Jeremias, I. TRAIL induced survival and proliferation in cancer cells resistant towards TRAIL-induced apoptosis mediated by NF-kappaB. *Oncogene* **2003**, *22*, 3842–3852. [[CrossRef](#)] [[PubMed](#)]
62. Harper, N.; Farrow, S.N.; Kaptein, A.; Cohen, G.M.; MacFarlane, M. Modulation of tumor necrosis factor apoptosis-inducing ligand-induced NF-kappa B activation by inhibition of apical caspases. *J. Biol. Chem.* **2001**, *276*, 34743–34752. [[CrossRef](#)] [[PubMed](#)]
63. Zhang, X.D.; Borrow, J.M.; Zhang, X.Y.; Nguyen, T.; Hersey, P. Activation of ERK1/2 protects melanoma cells from TRAIL-induced apoptosis by inhibiting Smac/DIABLO release from mitochondria. *Oncogene* **2003**, *22*, 2869–2881. [[CrossRef](#)] [[PubMed](#)]
64. Azijli, K.; Yuvaraj, S.; Peppelenbosch, M.P.; Wurdinger, T.; Dekker, H.; Joore, J.; van Dijk, E.; Quax, W.J.; Peters, G.J.; de Jong, S.; et al. Kinome profiling of non-canonical TRAIL signaling reveals RIP1-Src-STAT3 dependent invasion in resistant non-small cell lung cancer cells. *J. Cell Sci.* **2012**, *125*, 4651–4661. [[CrossRef](#)]
65. Varfolomeev, E.; Maecker, H.; Sharp, D.; Lawrence, D.; Renz, M.; Vucic, D.; Ashkenazi, A. Molecular determinants of kinase pathway activation by Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand. *J. Biol. Chem.* **2005**, *280*, 40599–40608. [[CrossRef](#)]
66. Cao, X.; Pobeziinskaya, Y.L.; Morgan, M.J.; Liu, Z.G. The role of TRADD in TRAIL-induced apoptosis and signaling. *FASEB J.* **2011**, *25*, 1353–1358. [[CrossRef](#)]
67. Lin, Y.; Devin, A.; Cook, A.; Keane, M.M.; Kelliher, M.; Lipkowitz, S.; Liu, Z.G. The death domain kinase RIP is essential for TRAIL (Apo2L)-induced activation of IkappaB kinase and c-Jun N-terminal kinase. *Mol. Cell Biol.* **2000**, *20*, 6638–6645. [[CrossRef](#)]
68. Song, J.H.; Tse, M.C.; Bellail, A.; Phuphanich, S.; Khuri, F.; Kneteman, N.M.; Hao, C. Lipid rafts and nonrafts mediate tumor necrosis factor related apoptosis-inducing ligand induced apoptotic and nonapoptotic signals in non small cell lung carcinoma cells. *Cancer Res.* **2007**, *67*, 6946–6955. [[CrossRef](#)]
69. Ouyang, W.; Yang, C.; Liu, Y.; Xiong, J.; Zhang, J.; Zhong, Y.; Zhang, G.; Zhou, F.; Zhou, Y.; Xie, C. Redistribution of DR4 and DR5 in lipid rafts accounts for the sensitivity to TRAIL in NSCLC cells. *Int. J. Oncol.* **2011**, *39*, 1577–1586. [[CrossRef](#)]
70. Hartwig, T.; Montinaro, A.; von Karstedt, S.; Sevko, A.; Surinova, S.; Chakravarthy, A.; Taraborrelli, L.; Draber, P.; Lafont, E.; Arce Vargas, F.; et al. The TRAIL-Induced Cancer Secretome Promotes a Tumor-Supportive Immune Microenvironment via CCR2. *Mol. Cell* **2017**, *65*, 730–742.e5. [[CrossRef](#)]
71. von Karstedt, S.; Conti, A.; Nobis, M.; Montinaro, A.; Hartwig, T.; Lemke, J.; Legler, K.; Annenwarter, F.; Campbell, A.D.; Taraborrelli, L.; et al. Cancer cell-autonomous TRAIL-R signaling promotes KRAS-driven cancer progression, invasion, and metastasis. *Cancer Cell* **2015**, *27*, 561–573. [[CrossRef](#)] [[PubMed](#)]
72. Pai, S.; Wu, G.S.; Ozoren, N.; Wu, L.; Jen, J.; Sidransky, D.; El-Deiry, W.S. Rare loss-of-function mutation of a death receptor gene in head and neck cancer. *Cancer Res.* **1998**, *58*, 3513–3518. [[PubMed](#)]
73. Finnberg, N.; Klein-Szanto, A.J.; El-Deiry, W.S. TRAIL-R deficiency in mice promotes susceptibility to chronic inflammation and tumorigenesis. *J. Clin. Invest.* **2008**, *118*, 111–123. [[CrossRef](#)]
74. Earel, J.K., Jr.; VanOosten, R.L.; Griffith, T.S. Histone deacetylase inhibitors modulate the sensitivity of tumor necrosis factor-related apoptosis-inducing ligand-resistant bladder tumor cells. *Cancer Res.* **2006**, *66*, 499–507. [[CrossRef](#)] [[PubMed](#)]
75. Sayers, T.J.; Murphy, W.J. Combining proteasome inhibition with TNF-related apoptosis-inducing ligand (Apo2L/TRAIL) for cancer therapy. *Cancer Immunol. Immunother.* **2006**, *55*, 76–84. [[CrossRef](#)]
76. Merino, D.; Lalaoui, N.; Morizot, A.; Schneider, P.; Solary, E.; Micheau, O. Differential inhibition of TRAIL-mediated DR5-DISC formation by decoy receptors 1 and 2. *Mol. Cell Biol.* **2006**, *26*, 7046–7055. [[CrossRef](#)]
77. Sheikh, M.S.; Huang, Y.; Fernandez-Salas, E.A.; El-Deiry, W.S.; Friess, H.; Amundson, S.; Yin, J.; Meltzer, S.J.; Holbrook, N.J.; Fornace, A.J., Jr. The antiapoptotic decoy receptor TRID/TRAIL-R3 is a p53-regulated DNA damage-inducible gene that is overexpressed in primary tumors of the gastrointestinal tract. *Oncogene* **1999**, *18*, 4153–4159. [[CrossRef](#)]
78. Morizot, A.; Merino, D.; Lalaoui, N.; Jacquemin, G.; Granci, V.; Iessi, E.; Lanneau, D.; Bouyer, F.; Solary, E.; Chauffert, B.; et al. Chemotherapy overcomes TRAIL-R4-mediated TRAIL resistance at the DISC level. *Cell. Death Differ.* **2011**, *18*, 700–711. [[CrossRef](#)]

79. Lalaoui, N.; Morle, A.; Merino, D.; Jacquemin, G.; Iessi, E.; Morizot, A.; Shirley, S.; Robert, B.; Solary, E.; Garrido, C.; et al. TRAIL-R4 promotes tumor growth and resistance to apoptosis in cervical carcinoma HeLa cells through AKT. *PLoS ONE* **2011**, *6*, e19679. [\[CrossRef\]](#)
80. Yang, J.; LeBlanc, F.R.; Dighe, S.A.; Hamele, C.E.; Olson, T.L.; Feith, D.J.; Loughran, T.P., Jr. TRAIL mediates and sustains constitutive NF-kappaB activation in LGL leukemia. *Blood* **2018**, *131*, 2803–2815. [\[CrossRef\]](#)
81. Krueger, A.; Baumann, S.; Krammer, P.H.; Kirchhoff, S. FLICE-inhibitory proteins: Regulators of death receptor-mediated apoptosis. *Mol. Cell Biol.* **2001**, *21*, 8247–8254. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Humphreys, L.M.; Fox, J.P.; Higgins, C.A.; Majkut, J.; Sessler, T.; McLaughlin, K.; McCann, C.; Roberts, J.Z.; Crawford, N.T.; McDade, S.S.; et al. A revised model of TRAIL-R2 DISC assembly explains how FLIP(L) can inhibit or promote apoptosis. *EMBO Rep.* **2020**, *21*, e49254. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Li, M.; Wu, X.M.; Gao, J.; Yang, F.; Zhang, C.L.; Ke, K.; Wang, Y.C.; Zheng, Y.S.; Yao, J.F.; Guan, Y.Y.; et al. Mutations in the P10 region of procaspase-8 lead to chemotherapy resistance in acute myeloid leukemia by impairing procaspase-8 dimerization. *Cell Death Dis.* **2018**, *9*, 516. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Jager, R.; Zwacka, R.M. The enigmatic roles of caspases in tumor development. *Cancers* **2010**, *2*, 1952–1979. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Marin-Rubio, J.L.; Vela-Martin, L.; Fernandez-Piqueras, J.; Villa-Morales, M. FADD in Cancer: Mechanisms of Altered Expression and Function, and Clinical Implications. *Cancers* **2019**, *11*, 1462. [\[CrossRef\]](#)
86. Sun, S.Y.; Yue, P.; Zhou, J.-Y.; Wang, Y.; Kim, H.C.; Lotan, R.; Wu, G.S. Overexpression of bcl2 blocks TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in human lung cancer cells. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 788–797. [\[CrossRef\]](#)
87. Burns, T.F.; El-Deiry, W.S. Identification of inhibitors of TRAIL-induced death (ITIDs) in the TRAIL-sensitive colon carcinoma cell line SW480 using a genetic approach. *J. Biol. Chem.* **2001**, *276*, 37879–37886. [\[CrossRef\]](#)
88. Hao, J.H.; Yu, M.; Liu, F.T.; Newland, A.C.; Jia, L. Bcl-2 inhibitors sensitize tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by uncoupling of mitochondrial respiration in human leukemic CEM cells. *Cancer Res.* **2004**, *64*, 3607–3616. [\[CrossRef\]](#)
89. Fulda, S.; Wick, W.; Weller, M.; Debatin, K.M. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. *Nat. Med.* **2002**, *8*, 808–815. [\[CrossRef\]](#)
90. Li, L.; Thomas, R.M.; Suzuki, H.; De Brabander, J.K.; Wang, X.; Harran, P.G. A small molecule Smac mimic potentiates TRAIL- and TNFalpha-mediated cell death. *Science* **2004**, *305*, 1471–1474. [\[CrossRef\]](#)
91. Montinaro, A.; Walczak, H. Harnessing TRAIL-induced cell death for cancer therapy: A long walk with thrilling discoveries. *Cell Death Differ.* **2022**, *30*, 237–249. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Kundu, M.; Greer, Y.E.; Dine, J.L.; Lipkowitz, S. Targeting TRAIL Death Receptors in Triple-Negative Breast Cancers: Challenges and Strategies for Cancer Therapy. *Cells* **2022**, *11*, 3717. [\[CrossRef\]](#) [\[PubMed\]](#)
93. de Miguel, D.; Lemke, J.; Anel, A.; Walczak, H.; Martinez-Lostao, L. Onto better TRAILs for cancer treatment. *Cell Death Differ.* **2016**, *23*, 733–747. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Ganten, T.M.; Koschny, R.; Sykora, J.; Schulze-Bergkamen, H.; Buchler, P.; Haas, T.L.; Schader, M.B.; Untergasser, A.; Stremmel, W.; Walczak, H. Preclinical differentiation between apparently safe and potentially hepatotoxic applications of TRAIL either alone or in combination with chemotherapeutic drugs. *Clin. Cancer Res.* **2006**, *12*, 2640–2646. [\[CrossRef\]](#) [\[PubMed\]](#)
95. Jo, M.; Kim, T.H.; Seol, D.W.; Esplen, J.E.; Dorko, K.; Billiar, T.R.; Strom, S.C. Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. *Nat. Med.* **2000**, *6*, 564–567. [\[CrossRef\]](#)
96. Pollack, I.F.; Erff, M.; Ashkenazi, A. Direct stimulation of apoptotic signaling by soluble Apo2L/tumor necrosis factor-related apoptosis-inducing ligand leads to selective killing of glioma cells. *Clin. Cancer Res.* **2001**, *7*, 1362–1369. [\[PubMed\]](#)
97. Ouyang, X.; Shi, M.; Jie, F.; Bai, Y.; Shen, P.; Yu, Z.; Wang, X.; Huang, C.; Tao, M.; Wang, Z.; et al. Phase III study of dulanermin (recombinant human tumor necrosis factor-related apoptosis-inducing ligand/Apo2 ligand) combined with vinorelbine and cisplatin in patients with advanced non-small-cell lung cancer. *Investig. New Drugs* **2018**, *36*, 315–322. [\[CrossRef\]](#)
98. Tuthill, M.H.; Montinaro, A.; Zinngrebe, J.; Prieske, K.; Draber, P.; Prieske, S.; Newsom-Davis, T.; von Karstedt, S.; Graves, J.; Walczak, H. TRAIL-R2-specific antibodies and recombinant TRAIL can synergise to kill cancer cells. *Oncogene* **2015**, *34*, 2138–2144. [\[CrossRef\]](#)
99. von Karstedt, S.; Montinaro, A.; Walczak, H. Exploring the TRAILs less travelled: TRAIL in cancer biology and therapy. *Nat. Rev. Cancer* **2017**, *17*, 352–366. [\[CrossRef\]](#)
100. Herbst, R.S.; Eckhardt, S.G.; Kurzrock, R.; Ebbinghaus, S.; O'Dwyer, P.J.; Gordon, M.S.; Novotny, W.; Goldwasser, M.A.; Tohny, T.M.; Lum, B.L.; et al. Phase I dose-escalation study of recombinant human Apo2L/TRAIL, a dual proapoptotic receptor agonist, in patients with advanced cancer. *J. Clin. Oncol.* **2010**, *28*, 2839–2846. [\[CrossRef\]](#)
101. Soria, J.C.; Smit, E.; Khayat, D.; Besse, B.; Yang, X.; Hsu, C.P.; Reese, D.; Wiezorek, J.; Blackhall, F. Phase 1b study of dulanermin (recombinant human Apo2L/TRAIL) in combination with paclitaxel, carboplatin, and bevacizumab in patients with advanced non-squamous non-small-cell lung cancer. *J. Clin. Oncol.* **2010**, *28*, 1527–1533. [\[CrossRef\]](#) [\[PubMed\]](#)
102. Guo, Y.; Roohullah, A.; Xue, J.; Zhao, W.; Aghmesheh, M.; Martin, D.; Zhou, Y.; Gao, C.; Yang, Y.; Xu, D.-Z.; et al. First-in-human (FIH) phase I studies of SCB-313, a novel TNF-related apoptosis-inducing ligand TRAIL-TrimerTM fusion protein, for treatment of patients (pts) with malignant ascites (MA). *Cancer Res.* **2022**, *82* (Suppl. 12), 6180. [\[CrossRef\]](#)

103. LoRusso, P.; Ratain, M.J.; Doi, T.; Rasco, D.W.; de Jonge, M.J.A.; Moreno, V.; Carneiro, B.A.; Devriese, L.A.; Petrich, A.; Modi, D.; et al. Eftozanermin alfa (ABBV-621) monotherapy in patients with previously treated solid tumors: Findings of a phase 1, first-in-human study. *Investig. New Drugs* **2022**, *40*, 762–772. [\[CrossRef\]](#)
104. Kim, T.H.; Jiang, H.H.; Youn, Y.S.; Park, C.W.; Lim, S.M.; Jin, C.H.; Tak, K.K.; Lee, H.S.; Lee, K.C. Preparation and characterization of Apo2L/TNF-related apoptosis-inducing ligand-loaded human serum albumin nanoparticles with improved stability and tumor distribution. *J. Pharm. Sci.* **2011**, *100*, 482–491. [\[CrossRef\]](#)
105. Bae, S.; Ma, K.; Kim, T.H.; Lee, E.S.; Oh, K.T.; Park, E.S.; Lee, K.C.; Youn, Y.S. Doxorubicin-loaded human serum albumin nanoparticles surface-modified with TNF-related apoptosis-inducing ligand and transferrin for targeting multiple tumor types. *Biomaterials* **2012**, *33*, 1536–1546. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Choi, S.H.; Byeon, H.J.; Choi, J.S.; Thao, L.; Kim, I.; Lee, E.S.; Shin, B.S.; Lee, K.C.; Youn, Y.S. Inhalable self-assembled albumin nanoparticles for treating drug-resistant lung cancer. *J. Control. Release* **2015**, *197*, 199–207. [\[CrossRef\]](#)
107. Jiang, H.H.; Kim, T.H.; Lee, S.; Chen, X.; Youn, Y.S.; Lee, K.C. PEGylated TNF-related apoptosis-inducing ligand (TRAIL) for effective tumor combination therapy. *Biomaterials* **2011**, *32*, 8529–8537. [\[CrossRef\]](#)
108. Kim, H.; Jeong, D.; Kang, H.E.; Lee, K.C.; Na, K. A sulfate polysaccharide/TNF-related apoptosis-inducing ligand (TRAIL) complex for the long-term delivery of TRAIL in poly(lactic-co-glycolic acid) (PLGA) microspheres. *J. Pharm. Pharmacol.* **2013**, *65*, 11–21. [\[CrossRef\]](#)
109. Guo, L.; Fan, L.; Pang, Z.; Ren, J.; Ren, Y.; Li, J.; Chen, J.; Wen, Z.; Jiang, X. TRAIL and doxorubicin combination enhances anti-glioblastoma effect based on passive tumor targeting of liposomes. *J. Control. Release* **2011**, *154*, 93–102. [\[CrossRef\]](#)
110. De Miguel, D.; Basanez, G.; Sanchez, D.; Malo, P.G.; Marzo, I.; Larrad, L.; Naval, J.; Pardo, J.; Anel, A.; Martinez-Lostao, L. Liposomes decorated with Apo2L/TRAIL overcome chemoresistance of human hematologic tumor cells. *Mol. Pharm.* **2013**, *10*, 893–904. [\[CrossRef\]](#)
111. Di Cristofano, F.; George, A.; Tajiknia, V.; Ghandali, M.; Wu, L.; Zhang, Y.; Srinivasan, P.; Strandberg, J.; Hahn, M.; Sanchez Sevilla Uruchurtu, A.; et al. Therapeutic targeting of TRAIL death receptors. *Biochem. Soc. Trans.* **2023**, *51*, 57–70. [\[CrossRef\]](#) [\[PubMed\]](#)
112. Hotte, S.J.; Hirte, H.W.; Chen, E.X.; Siu, L.L.; Le, L.H.; Corey, A.; Iacobucci, A.; MacLean, M.; Lo, L.; Fox, N.L.; et al. A phase 1 study of mapatumumab (fully human monoclonal antibody to TRAIL-R1) in patients with advanced solid malignancies. *Clin. Cancer Res.* **2008**, *14*, 3450–3455. [\[CrossRef\]](#) [\[PubMed\]](#)
113. Herbst, R.S.; Kurzrock, R.; Hong, D.S.; Valdivieso, M.; Hsu, C.P.; Goyal, L.; Juan, G.; Hwang, Y.C.; Wong, S.; Hill, J.S.; et al. A first-in-human study of conatumumab in adult patients with advanced solid tumors. *Clin. Cancer Res.* **2010**, *16*, 5883–5891. [\[CrossRef\]](#) [\[PubMed\]](#)
114. Plummer, R.; Attard, G.; Pacey, S.; Li, L.; Razak, A.; Perrett, R.; Barrett, M.; Judson, I.; Kaye, S.; Fox, N.L.; et al. Phase 1 and pharmacokinetic study of lexatumumab in patients with advanced cancers. *Clin. Cancer Res.* **2007**, *13*, 6187–6194. [\[CrossRef\]](#)
115. Forero-Torres, A.; Shah, J.; Wood, T.; Posey, J.; Carlisle, R.; Copigneaux, C.; Luo, F.R.; Wojtowicz-Praga, S.; Percent, I.; Saleh, M. Phase I trial of weekly tigatuzumab, an agonistic humanized monoclonal antibody targeting death receptor 5 (DR5). *Cancer Biother. Radiopharm.* **2010**, *25*, 13–19. [\[CrossRef\]](#)
116. Camidge, D.R.; Herbst, R.S.; Gordon, M.S.; Eckhardt, S.G.; Kurzrock, R.; Durbin, B.; Ing, J.; Tohny, T.M.; Sager, J.; Ashkenazi, A.; et al. A phase I safety and pharmacokinetic study of the death receptor 5 agonistic antibody PRO95780 in patients with advanced malignancies. *Clin. Cancer Res.* **2010**, *16*, 1256–1263. [\[CrossRef\]](#) [\[PubMed\]](#)
117. Sharma, S.; de Vries, E.G.; Infante, J.R.; Oldenhuis, C.N.; Gietema, J.A.; Yang, L.; Bilic, S.; Parker, K.; Goldbrunner, M.; Scott, J.W.; et al. Safety, pharmacokinetics, and pharmacodynamics of the DR5 antibody LBY135 alone and in combination with capecitabine in patients with advanced solid tumors. *Investig. New Drugs* **2014**, *32*, 135–144. [\[CrossRef\]](#)
118. Lemke, J.; von Karstedt, S.; Zinngrebe, J.; Walczak, H. Getting TRAIL back on track for cancer therapy. *Cell Death Differ.* **2014**, *21*, 1350–1364. [\[CrossRef\]](#)
119. Micheau, O.; Shirley, S.; Dufour, F. Death receptors as targets in cancer. *Br. J. Pharmacol.* **2013**, *169*, 1723–1744. [\[CrossRef\]](#)
120. Chawla, S.P.; Wasp, G.T.; Shepard, D.R.; Blay, J.-Y.; Jones, R.L.; Stacchiotti, S.; Reichardt, P.; Gelderblom, H.; Martin-Broto, J.; Eckelman, B.; et al. A randomized, placebo-controlled, phase 2 trial of INBRX-109 in unresectable or metastatic conventional chondrosarcoma. *J. Clin. Oncol.* **2022**, *40* (Suppl. 16). [\[CrossRef\]](#)
121. Wang, B.T.; Kothambawala, T.; Wang, L.; Matthew, T.J.; Calhoun, S.E.; Saini, A.K.; Kotturi, M.F.; Hernandez, G.; Humke, E.W.; Peterson, M.S.; et al. Multimeric Anti-DR5 IgM Agonist Antibody IGM-8444 Is a Potent Inducer of Cancer Cell Apoptosis and Synergizes with Chemotherapy and BCL-2 Inhibitor ABT-199. *Mol. Cancer Ther.* **2021**, *20*, 2483–2494. [\[CrossRef\]](#) [\[PubMed\]](#)
122. Overdijk, M.B.; Strumane, K.; Beurskens, F.J.; Ortiz Buijsse, A.; Vermot-Desroches, C.; Vuillermoz, B.S.; Kroes, T.; de Jong, B.; Hoevenaars, N.; Hibbert, R.G.; et al. Dual Epitope Targeting and Enhanced Hexamerization by DR5 Antibodies as a Novel Approach to Induce Potent Antitumor Activity Through DR5 Agonism. *Mol. Cancer Ther.* **2020**, *19*, 2126–2138. [\[CrossRef\]](#)
123. Garcia-Martinez, J.M.; Wang, S.; Weishaupl, C.; Wernitznig, A.; Chetta, P.; Pinto, C.; Ho, J.; Dutcher, D.; Gorman, P.N.; Kroe-Barrett, R.; et al. Selective Tumor Cell Apoptosis and Tumor Regression in CDH17-Positive Colorectal Cancer Models using BI 905711, a Novel Liver-Sparing TRAILR2 Agonist. *Mol. Cancer Ther.* **2021**, *20*, 96–108. [\[CrossRef\]](#) [\[PubMed\]](#)
124. Dubuisson, A.; Micheau, O. Antibodies and Derivatives Targeting DR4 and DR5 for Cancer Therapy. *Antibodies* **2017**, *6*, 16. [\[CrossRef\]](#) [\[PubMed\]](#)

125. Allen, J.E.; Kringsfeld, G.; Mayes, P.A.; Patel, L.; Dicker, D.T.; Patel, A.S.; Dolloff, N.G.; Messaris, E.; Scata, K.A.; Wang, W.; et al. Dual inactivation of Akt and ERK by TIC10 signals Foxo3a nuclear translocation, TRAIL gene induction, and potent antitumor effects. *Sci. Transl. Med.* **2013**, *5*, 171ra117. [\[CrossRef\]](#)
126. Allen, J.E.; Kline, C.L.; Prabhu, V.V.; Wagner, J.; Ishizawa, J.; Madhukar, N.; Lev, A.; Baumeister, M.; Zhou, L.; Lulla, A.; et al. Discovery and clinical introduction of first-in-class imipridone ONC201. *Oncotarget* **2016**, *7*, 74380–74392. [\[CrossRef\]](#)
127. Prabhu, V.V.; Allen, J.E.; Dicker, D.T.; El-Deiry, W.S. Small-Molecule ONC201/TIC10 Targets Chemotherapy-Resistant Colorectal Cancer Stem-like Cells in an Akt/Foxo3a/TRAIL-Dependent Manner. *Cancer Res.* **2015**, *75*, 1423–1432. [\[CrossRef\]](#)
128. Yuan, X.; Kho, D.; Xu, J.; Gajan, A.; Wu, K.; Wu, G.S. ONC201 activates ER stress to inhibit the growth of triple-negative breast cancer cells. *Oncotarget* **2017**, *8*, 21626–21638. [\[CrossRef\]](#)
129. Prabhu, V.V.; Morrow, S.; Rahman Kawakibi, A.; Zhou, L.; Ralff, M.; Ray, J.; Jhaveri, A.; Ferrarini, I.; Lee, Y.; Parker, C.; et al. ONC201 and imipridones: Anti-cancer compounds with clinical efficacy. *Neoplasia* **2020**, *22*, 725–744. [\[CrossRef\]](#)
130. Greer, Y.E.; Porat-Shliom, N.; Nagashima, K.; Stuelten, C.; Crooks, D.; Koparde, V.N.; Gilbert, S.F.; Islam, C.; Ubaldini, A.; Ji, Y.; et al. ONC201 kills breast cancer cells in vitro by targeting mitochondria. *Oncotarget* **2018**, *9*, 18454–18479. [\[CrossRef\]](#)
131. Swann, J.B.; Smyth, M.J. Immune surveillance of tumors. *J. Clin. Investig.* **2007**, *117*, 1137–1146. [\[CrossRef\]](#) [\[PubMed\]](#)
132. Smyth, M.J.; Cretney, E.; Takeda, K.; Wiltroft, R.H.; Sedger, L.M.; Kayagaki, N.; Yagita, H.; Okumura, K. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) contributes to interferon gamma-dependent natural killer cell protection from tumor metastasis. *J. Exp. Med.* **2001**, *193*, 661–670. [\[CrossRef\]](#) [\[PubMed\]](#)
133. Takeda, K.; Hayakawa, Y.; Smyth, M.J.; Kayagaki, N.; Yamaguchi, N.; Kakuta, S.; Iwakura, Y.; Yagita, H.; Okumura, K. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nat. Med.* **2001**, *7*, 94–100. [\[CrossRef\]](#) [\[PubMed\]](#)
134. Falschlehner, C.; Schaefer, U.; Walczak, H. Following TRAIL's path in the immune system. *Immunology* **2009**, *127*, 145–154. [\[CrossRef\]](#)
135. Kemp, T.J.; Moore, J.M.; Griffith, T.S. Human B cells express functional TRAIL/Apo-2 ligand after CpG-containing oligodeoxynucleotide stimulation. *J. Immunol.* **2004**, *173*, 892–899. [\[CrossRef\]](#)
136. Halaas, O.; Vik, R.; Ashkenazi, A.; Espevik, T. Lipopolysaccharide induces expression of APO2 ligand/TRAIL in human monocytes and macrophages. *Scand. J. Immunol.* **2000**, *51*, 244–250. [\[CrossRef\]](#)
137. Kayagaki, N.; Yamaguchi, N.; Nakayama, M.; Eto, H.; Okumura, K.; Yagita, H. Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs. *J. Exp. Med.* **1999**, *189*, 1451–1460. [\[CrossRef\]](#)
138. Sato, K.; Hida, S.; Takayanagi, H.; Yokochi, T.; Kayagaki, N.; Takeda, K.; Yagita, H.; Okumura, K.; Tanaka, N.; Taniguchi, T.; et al. Antiviral response by natural killer cells through TRAIL gene induction by IFN- α /beta. *Eur. J. Immunol.* **2001**, *31*, 3138–3146. [\[CrossRef\]](#)
139. Kemp, T.J.; Ludwig, A.T.; Earel, J.K.; Moore, J.M.; Vanoosten, R.L.; Moses, B.; Leidal, K.; Nauseef, W.M.; Griffith, T.S. Neutrophil stimulation with Mycobacterium bovis bacillus Calmette-Guerin (BCG) results in the release of functional soluble TRAIL/Apo-2L. *Blood* **2005**, *106*, 3474–3482. [\[CrossRef\]](#)
140. Cassatella, M.A.; Huber, V.; Calzetti, F.; Margotto, D.; Tamassia, N.; Peri, G.; Mantovani, A.; Rivoltini, L.; Tecchio, C. Interferon-activated neutrophils store a TNF-related apoptosis-inducing ligand (TRAIL/Apo-2 ligand) intracellular pool that is readily mobilizable following exposure to proinflammatory mediators. *J. Leukoc. Biol.* **2006**, *79*, 123–132. [\[CrossRef\]](#)
141. Ehrlich, S.; Infante-Duarte, C.; Seeger, B.; Zipp, F. Regulation of soluble and surface-bound TRAIL in human T cells, B cells, and monocytes. *Cytokine* **2003**, *24*, 244–253. [\[CrossRef\]](#) [\[PubMed\]](#)
142. Fanger, N.A.; Maliszewski, C.R.; Schooley, K.; Griffith, T.S. Human dendritic cells mediate cellular apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *J. Exp. Med.* **1999**, *190*, 1155–1164. [\[CrossRef\]](#) [\[PubMed\]](#)
143. Griffith, T.S.; Wiley, S.R.; Kubin, M.Z.; Sedger, L.M.; Maliszewski, C.R.; Fanger, N.A. Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL. *J. Exp. Med.* **1999**, *189*, 1343–1354. [\[CrossRef\]](#) [\[PubMed\]](#)
144. Liu, S.; Yu, Y.; Zhang, M.; Wang, W.; Cao, X. The involvement of TNF- α -related apoptosis-inducing ligand in the enhanced cytotoxicity of IFN- β -stimulated human dendritic cells to tumor cells. *J. Immunol.* **2001**, *166*, 5407–5415. [\[CrossRef\]](#)
145. Poh, A.R.; Ernst, M. Targeting Macrophages in Cancer: From Bench to Bedside. *Front. Oncol.* **2018**, *8*, 49. [\[CrossRef\]](#) [\[PubMed\]](#)
146. de Looft, M.; de Jong, S.; Kruij, F.A.E. Multiple Interactions between Cancer Cells and the Tumor Microenvironment Modulate TRAIL Signaling: Implications for TRAIL Receptor Targeted Therapy. *Front. Immunol.* **2019**, *10*, 1530. [\[CrossRef\]](#) [\[PubMed\]](#)
147. Klimp, A.H.; de Vries, E.G.; Scherphof, G.L.; Daemen, T. A potential role of macrophage activation in the treatment of cancer. *Crit. Rev. Oncol. Hematol.* **2002**, *44*, 143–161. [\[CrossRef\]](#)
148. Lecoultre, M.; Dutoit, V.; Walker, P.R. Phagocytic function of tumor-associated macrophages as a key determinant of tumor progression control: A review. *J. Immunother. Cancer* **2020**, *8*, e001408. [\[CrossRef\]](#) [\[PubMed\]](#)
149. Zou, W.; Wolchok, J.D.; Chen, L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. *Sci. Transl. Med.* **2016**, *8*, 328rv324. [\[CrossRef\]](#)
150. Topalian, S.L.; Drake, C.G.; Pardoll, D.M. Immune checkpoint blockade: A common denominator approach to cancer therapy. *Cancer Cell* **2015**, *27*, 450–461. [\[CrossRef\]](#)
151. Sharma, P.; Hu-Lieskovan, S.; Wargo, J.A.; Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* **2017**, *168*, 707–723. [\[CrossRef\]](#) [\[PubMed\]](#)

152. Takeda, K.; Smyth, M.J.; Cretney, E.; Hayakawa, Y.; Kayagaki, N.; Yagita, H.; Okumura, K. Critical role for tumor necrosis factor-related apoptosis-inducing ligand in immune surveillance against tumor development. *J. Exp. Med.* **2002**, *195*, 161–169. [[CrossRef](#)]
153. Cretney, E.; Takeda, K.; Yagita, H.; Glaccum, M.; Peschon, J.J.; Smyth, M.J. Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. *J. Immunol.* **2002**, *168*, 1356–1361. [[CrossRef](#)] [[PubMed](#)]
154. Zhang, H.; Qin, G.; Zhang, C.; Yang, H.; Liu, J.; Hu, H.; Wu, P.; Liu, S.; Yang, L.; Chen, X.; et al. TRAIL promotes epithelial-to-mesenchymal transition by inducing PD-L1 expression in esophageal squamous cell carcinomas. *J. Exp. Clin. Cancer Res.* **2021**, *40*, 209. [[CrossRef](#)] [[PubMed](#)]
155. Lv, J.; Guo, T.; Qu, X.; Che, X.; Li, C.; Wang, S.; Gong, J.; Wu, P.; Liu, Y.; Liu, Y.; et al. PD-L1 Under Regulation of miR-429 Influences the Sensitivity of Gastric Cancer Cells to TRAIL by Binding of EGFR. *Front. Oncol.* **2020**, *10*, 1067. [[CrossRef](#)] [[PubMed](#)]
156. Mondal, T.; Shivange, G.N.; Tihagam, R.G.; Lyster, E.; Battista, M.; Talwar, D.; Mosavian, R.; Urbanek, K.; Rashid, N.S.; Harrell, J.C.; et al. Unexpected PD-L1 immune evasion mechanism in TNBC, ovarian, and other solid tumors by DR5 agonist antibodies. *EMBO Mol. Med.* **2021**, *13*, e12716. [[CrossRef](#)]
157. Hendriks, D.; He, Y.; Koopmans, I.; Wiersma, V.R.; van Ginkel, R.J.; Samplonius, D.F.; Helfrich, W.; Bremer, E. Programmed Death Ligand 1 (PD-L1)-targeted TRAIL combines PD-L1-mediated checkpoint inhibition with TRAIL-mediated apoptosis induction. *Oncoimmunology* **2016**, *5*, e1202390. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.