Review

Apoptosis-Inducing TNF Superfamily Ligands for Cancer Therapy

Olivia A. Diaz Arguello and Hidde J. Haisma *

Citation: Diaz Arguello, O.A.; Haisma, H.J. Apoptosis-Inducing TNF Superfamily Ligands for Cancer Therapy. Cancers 2021, 13, 1543. https://doi.org/10.3390/cancers13071543

Academic Editor: Najoua Lalaoui

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 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

Cancer is one of the leading causes of death globally; it is a complex disease that involves bypassing growth suppressors, angiogenesis, metastasis, and apoptosis evasion [1]. The most common treatment courses include chemotherapy, surgery, and radiation [2], which, in many cases, are not effective and can cause multisystemic toxicity. Therefore, the need to improve cancer treatments necessitates the development of more specific and sophisticated treatments that can target cancer cells with high selectivity without harming the healthy ones.

Apoptosis evasion is one of the main hallmarks of cancer; its dysregulation is related to overexpression of antiapoptotic genes or survival signals and downregulation/mutation of proapoptotic genes [3]. Therefore, overcoming cell death resistance by triggering the apoptotic pathways has been an area of interest in the development of cancer treatments. The study of apoptotic mechanisms has brought to the spotlight a subgroup of the apoptosis-inducing ligands. Those ligands belong to the tumor necrosis factor (TNF) family [4]; members of this family are naturally expressed by the immune system and possess tumoricidal activity [5]. These ligands can potentially be used as a cancer treatment.

This review provides an overview of the TNF family of receptors and their apoptosis-inducing ligands as cancer treatments. Additionally, we describe several engineered death
receptor agonists and how gene therapy may improve the apoptosis-inducing ligands’ efficacy.

2. Apoptosis

Apoptosis is a regulated cell death process that keeps the cell population balanced in a living organism [6]. It is a mechanism that the immune system uses to prevent the accumulation of damaged or infected cells. Both the intrinsic pathway, also known as the mitochondria-dependent apoptosis pathway, and the extrinsic pathway can induce apoptosis (Figure 1) [6–8].

**Figure 1.** Simplified extrinsic signaling pathway from TNF-R1, Fas, DR4, and DR5.

The intrinsic pathway is activated when the outer membrane of the mitochondria is permeabilized, releasing cytochrome c. Then, it binds to the apoptotic protease activating factor 1 (APAF1), which leads to the formation of the apoptosome. The apoptosome recruits caspase-9, which activates caspases-3 and -7, leading to cell death. Besides cytochrome c, the mitochondria also release the second mitochondria-derived activator of caspase (SMAC/DIABLO) and the serine protease HtrA2/Omi. These factors impede the inhibitor of apoptosis proteins (IAPs), boosting the induction of apoptosis [6,9]. The mitochondrial outer membrane permeabilization (MOMP) may occur because of different factors such as the lack of growth factors, cytokines, or hormones; DNA damage; endoplasmic reticulum (ER) stress; toxins; radiation; and viruses [10].

The extrinsic pathway’s activation requires the stimulation of death receptors (DRs); these are transmembrane receptors that belong to the TNF receptor superfamily [8]. They have a death domain (DD) that has a six-helical bundle fold as a structural characteristic [11]; this intracellular domain transfers the signal from the extracellular domain to the cytosol, starting the recruitment of death-inducing signaling complex (DISC) [12]. This process results in the autoactivation of procaspase-8 to caspase-8, leading to the activation of caspases-3 and -7 and resulting in apoptosis [6,13].
Independently of which pathway is activated, both lead to the activation of caspases-3, -6, and -7, which induce apoptosis [14]. Several members of the TNF superfamily were brought to the spotlight as cancer treatments because of their capability of specific apoptosis induction in transformed cells [15].

Even though the DRs are well known for their apoptosis induction characteristics, they may also trigger necroptosis. A regulated necrosis process mediated by the RIP kinase family members can be induced, in some cell lines, under certain circumstances, such as the use of caspase inhibitors [16], downregulation of cIAPs [17], and acidic extracellular pH [18]. In this review, we will focus on apoptosis.

2.1. Apoptosis-Inducing Ligands and Their Receptors

In recent years, members of the TNF superfamily of cytokines, TNF-α, Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL), have been studied for their apoptosis induction capability when bound to their receptors that contain a DD [19]. The most studied DRs from the TNF superfamily (TNFSFR) (Scheme 1) are TNF-R1 (DR1), CD95 (DR2, Fas), TNF-related apoptosis-inducing ligand receptor 1 (TRAIL-R1, DR4), and TRAIL-R2 (DR5) [20].

![Scheme 1. Tumor necrosis factor (TNF) apoptosis-inducing ligands and their receptors.](image-url)

The death receptors are type I transmembrane proteins with cysteine-rich extracellular domains. The DRs have been detected in a wide variety of healthy [17,18] (Figure 2) and cancerous tissues either by assessing protein expression or RNA expression. It was found that in some cases of clear cell renal cell carcinoma (ccRCC), TNF-R1 is upregulated [19]; however, in ovarian cancer, the expression levels were similar to the ones in healthy tissues [20].
The analysis of Fas expression revealed its downregulation in some colon carcinomas [21], lung carcinomas [22], and gynecological cancer tissues [23,24]. At the same time, DR4 and DR5 were found to be upregulated in pancreatic [25], colorectal [26], and cervical [24] cancers. It is important to remark that level of receptor expression can vary among patients with the same type of cancer (Figure 3), as Kawasaki et al. [27] and Hwang et al. [28] found; those differences can be helpful in developing biomarkers of resistance.

Interestingly, the TNF apoptosis-inducing ligands also bind to decoy receptors (DcRs). These receptors are not able to trigger the apoptosis cascade, but they can activate survival and migration signaling [29,30]; the identified DcRs are DcR1 (also known as TRAIL-R3 or TRID), DcR2 (TRAIL-R4 or TRUNDD), DcR3, and osteoprotegerin (OPG) [31]. DcR1 and DcR2 are membrane proteins, while DcR3 and OPG are soluble receptors [32].

TNF-α, FasL, and TRAIL are expressed mainly in immune cells, including granulocytes, monocytes, T cells, B cells, dendritic cells, and NK cells [33]. Most apoptosis-inducing ligands (e.g., TNF-α, FasL, and TRAIL) are transmembrane proteins that can be proteolytically cleaved and released in a soluble form. Both forms are present in noncovalent trimeric forms [34].

| Tissues                        | TNF-R1 | Fas | DR4 | DR5 |
|--------------------------------|--------|-----|-----|-----|
| Brain                          |        |     |     |     |
| Eye                            |        |     |     |     |
| Endocrine tissue               |        |     |     |     |
| Lungs                          |        |     |     |     |
| Proximal digestive tract       |        |     |     |     |
| Gastrointestinal tract         |        |     |     |     |
| Liver and gallbladder          |        |     |     |     |
| Pancreas                       |        |     |     |     |
| Kidney and urinary bladder     |        |     |     |     |
| Male tissues                   |        |     |     |     |
| Female tissues                 |        |     |     |     |
| Adipose & soft tissue          |        |     |     |     |
| Skin                           |        |     |     |     |
| Bone marrow & lymphoid tissues |        |     |     |     |
| Blood                          |        |     |     |     |
| Muscle tissues                 |        |     |     |     |

**Figure 2.** Summary of the consensus normalized expression (NX) level of each death receptor in healthy tissue. Information based on The Human Protein Atlas (https://www.proteinatlas.org, accessed on 10th March 2021), which combines the data of three transcriptomic datasheets (HPA, GTEx, and FANTOM5).
2.1.1. Tumor Necrosis Factor-α (TNF-α)

TNF-α is a type II transmembrane protein with different biological roles: mediation of the inflammatory response, regulation of immune cells, and cytotoxicity. TNF-α binds to tumor necrosis factor receptor 1 (TNF-R1), also known as death receptor 1 (DR1), and tumor necrosis factor receptor 2 (TNF-R2); both receptors are involved in prosurvival signaling and proliferation by activating the NF-kB pathway. However, only TNF-R1 has a death domain that can trigger apoptosis through caspase cascade activation under certain conditions, such as the absence of the IAPs [35,36]. TNF-R1 can be found in basically all the cell types (Figure 3); in contrast, TNF-R2 is mainly expressed in immune cells and endothelial cells [37,38].

TNF-R1, TNF-R2, and TNF-α can be cleaved by the metalloprotease TNF-α converting enzyme (TACE or ADAM-17), resulting in the soluble forms of TNF-α (sTNF-α), sTNF-R1, and sTNF-R2 [39–42]; interestingly, sTNF-α can activate TNF-R1, but it is inferior in activating TNF-R2 [5].

Recombinant TNF-α has been studied as a cancer treatment. Unfortunately, in clinical trials, it showed systemic toxicity when administrated intravenously. Therefore, treatment directed to a specific organ or an area of the body seems to be a better option [43].

2.1.2. Fas Ligand (FasL)

FasL (CD95L) is a type II transmembrane protein expressed in diverse immune cells, like B, T, and NK cells [44]. It can interact with DcR3 and Fas receptors [45]. DcR3 is a soluble secreted receptor from the TNF superfamily; when FasL binds to DcR3, it inhibits FasL/Fas apoptotic activity, acting thus as a “decoy” [46]. However, when FasL binds to Fas (also known as CD95, DR2), a type I transmembrane receptor, it starts the clustering of the receptors and recruits the Fas-associated death domain (FADD)/caspase-8 complex, leading to apoptosis [20,47]. Fas is present in a wide variety of cells (Figure 2).

Under normal circumstances, DcR3 is expressed in the brain, gastrointestinal tract, ovary, kidney, and urinary bladder, but it is difficult to detect it in serum [48,49]. However, in inflammatory diseases and cancer (e.g., breast cancer [50], renal cell carcinoma [51], and pancreatic head carcinoma [52]), overexpression of DcR3 has been identified.

A soluble form of FasL (sFasL) can be produced either by proteolytic cleavage or alternative splicing [44]. However, sFasL is not capable of activating Fas as the membrane-bound FasL does because it is not able to induce DISC formation; instead, it can activate motility signaling, and sFasL can possibly compete with FasL for receptor binding [5,44,53].
So far, recombinant FasL (rFasL) has not reached clinical trials because of the liver toxicity induced by the systemic administration of rFasL in mice [54,55].

2.1.3. TNF-Related Apoptosis-Inducing Ligand (TRAIL)

TRAIL is a homotrimeric transmembrane protein type II. TRAIL is expressed on the surface of T cells, macrophages, and NK cells; its principal role is to modulate the immune response [56]. TRAIL binds to five receptors, three decoys (DcR1, DcR2, and OPG) and two death receptors (DR 4 and 5). DcR1, also known as TRAIL-R3, is a GPI-anchored protein lacking the intracellular and transmembrane domains [57], while DcR2 (TRAIL-R4) has an intracellular portion containing a truncated DD [58]; both receptors are unable to induce apoptosis after TRAIL binding. However, DcR2 activation by TRAIL triggers the NF-κB pathway [29,58]. Osteoprotegerin (OPG) is a soluble receptor that can be released by several types of tissues, including the cardiovascular system, gastrointestinal tract, lungs, kidney, bones, and immune cells [59,60]. OPG binds to TRAIL and many ligands, including another member of the TNF family, the receptor activator of nuclear factor-kB ligand (RANKL) [61]. Although DR4 and DR5 share 60% homology and both can trigger apoptosis [62], it has come to light that DR5 can trigger prosurvival, proliferative, and migration signaling in some cancer cell lines when they are treated with sTRAIL [63,64].

TRAIL is a homotrimeric protein; a zinc ion stabilizes the trimeric conformation in the Cys230 residue [65]. This conformation is essential for receptor recognition and apoptosis induction [66]. When TRAIL binds to the DRs, it induces receptor trimerization, which triggers the extrinsic apoptotic pathway [67,68]. TRAIL is known for apoptosis induction in transformed cells while sparing the nontransformed ones. This characteristic has brought TRAIL to the clinic as a cancer treatment [69].

Dulanermin is a recombinant human soluble TRAIL (sTRAIL, amino acids 114–281) protein that was tested in several clinical trials alone or in combination with chemotherapy. In phase I, it was tested as a treatment for advanced cancer [70] and metastatic colorectal cancer [71], where dulanermin was declared safe. In phase II, dulanermin was assessed as treatment of non-small-cell lung cancer (NSCLC) in combination with paclitaxel and carboplatin (PC) with or without bevacizumab (PCB), where dulanermin did not improve the efficacy of the treatment [72]. Later, in a phase III clinical trial, dulanermin was tested in combination with vinorelbine and cisplatin in the same type of cancer. There was an improvement in progression-free survival but not overall survival in the patients [73]. It is thought that the limited efficacy is related to the short half-life of dulanermin (around 1 h) [70].

A circular recombinant mutant of human TRAIL known as circularly permuted TRAIL (CPT) was developed; it consists of the N-terminal amino acids (121–135) fused with a flexible linker to the C-terminal amino acids (135–281) of TRAIL. It has a longer half-life compared to dulanermin. CPT has been tested in patients (phases Ib and II) with relapsed or refractory multiple myeloma (RRMM), where in general it was well tolerated, albeit elevation of liver enzymes was reported [74,75]. CPT has been tested in combination with thalidomide and dexamethasone (CPT + TD); it showed a median progression-free survival of 6.7 months in the CPT + TD group in comparison with the 3.1 months in the TD group [76].

3. Improving Receptor Specificity of the Apoptosis-Inducing Ligands

As mentioned before, the apoptosis-inducing ligands from the TNF superfamily interact with more than one receptor. This promiscuous interaction, in some cases, can block the activation of the apoptotic pathway [77], as in the case of TNF-α that induces apoptosis via TNF-R1 and TNF-R2 and can lead to tumor progression [40,78], and TRAIL also binds to decoy receptors (DcR1 and DcR2) that do not induce apoptosis [79]. Therefore, many TNF-α and TRAIL mutants have been engineered (Table 1) by mutating a few amino acids, thus improving the affinity towards one of their death receptors [80]. These proteins
have been investigated in preclinical studies showing promising results. However, their efficacy in clinical trials remains to be demonstrated.

### Table 1. TNF-α and TRAIL mutants.

| Based on | Protein | Format | Modification | Affinity | Ref. |
|----------|---------|--------|--------------|----------|------|
| TNF-α    | Mutant 471 | TRAIL (aa 95–281) | 1-7del + P8R/S9K/D10R | TNF-R1 | [81] |
|          | mutant R32w | TRAIL (aa 95–281) | R32w       | TNF-R1 | [82] |
|          | Mutant M3   | TRAIL (aa 95–281) | 1-7del + S52I, Y56F | TNF-R1 | [83] |
|          | RGD-V29     | TRAIL (aa 95–281) | includes cell adhesive sequence (R4, G5, D6) + R29V | TNF-R1 | [84] |
|          | rmhTNF      | TRAIL (aa 95–281) | 1-7del + P8R/S9K/D10R/L157F | TNF-R1 | [85] |
| TRAIL    | TRAIL-R1-5  | TRAIL (aa 95–281) | Q193S/N199 V/K201R/Y213 W/S215N | DR4 | [86] |
|          | 4c7         | TRAIL (aa 114–281) | G131R/R149I/S159R/N199R/K201H/S215D S159R | DR4 | [87] |
|          | FLAG-Apo2L.DR5–8 | TRAIL (aa 96–281) | Y189 N/R191 K/Q193R/H264R/I266L/D267Q | DR5 | [88] |
|          | DHER        | TRAIL (aa 114–281) | D269H and E195R | DR5 | [90] |
|          | DR5-A       | TRAIL (aa 114–281) | Y189 N/R191 K/Q193R/H264R/I266L/D267Q/D269H | DR5 | [91] |
|          | DR5-B       | TRAIL (aa 114–281) | Y189 N/R191 K/Q193R/H264R/I266L/D267Q/D269H | DR5 | [91] |
|          | TRAIL-Mu3   | TRAIL (aa 114–281) | aa 114–121 (VRERGPQR) were replaced by RRRRRRRR | DR4 and DR5 | [92] |

#### 3.1. DR-Targeting Antibodies

The study of antibody-mediated therapies to treat cancer, where monoclonal antibodies (mAbs) are designed to target a specific antigen [93], such as the death receptors [94,95], has been going on for years. A well-designed mAbs can have high specificity, fewer adverse effects than apoptosis-inducing ligands, and a half-life that can last weeks [95].

Although antibody targeting of TNF-R1 as cancer therapy seems promising due to its proinflammatory signaling [96], diverse TNF-R1 antagonist antibodies (e.g., Atrosab) have been designed as treatment of rheumatoid arthritis (RA) and multiple sclerosis, among other autoimmune diseases [97,98]. Therefore, TNF blockers have been used to ameliorate the immune-related adverse events in patients undergoing cancer treatment with immune checkpoint inhibitors (ICIs) [99]. In patients with melanoma (phase Ib), it was found that the TNF inhibitors (infliximab or certolizumab) can boost the antitumor effect of ICIs (ipilimumab and nivolumab) [100].

A monoclonal antibody against murine Fas developed in the early 1990s showed liver toxicity in mice after intraperitoneal administration [101]. Later, HFE7A, a mouse anti-human Fas mAb, was designed to induce apoptosis in lymphocytes without showing liver toxicity [102]. HFE7A was able to ameliorate the symptoms of lymphadenopathy in mice by inducing apoptosis in T cells. Moreover, it induced apoptosis in synovial cells from patients with rheumatoid arthritis [103]. However, the worrisome immunogenicity of HFE7A led to the development of humanized antibody designs with improvements in pharmacokinetics and lower immunogenicity [93].

Many DR4- and DR5-targeting antibodies have been designed for cancer treatment. Mapatumumab (HGS-ETR1) is a fully human agonist monoclonal antibody (mAb) that binds with high affinity to DR4 [104]. This monoclonal antibody was tested in several clinical trials alone or with chemotherapy, showing that it was well tolerated [105]. However, there was no response in advanced solid tumors [106], non-small-cell lung carcinoma [107], and hepatocellular carcinoma [108].
Of several monoclonal antibodies designed for targeting DR5, only five reached clinical trials: conatumumab [109–113], lexatumumab [114,115], tigatuzumab [116–118], drozitumab [119,120], and LBY135 [121]; however, none of them reached phase III clinical trials. These antibodies showed a prolonged half-life in serum. Still, they failed to show an antitumor effect, which may be related to their inability to induce the oligomerization of death receptors [45]. A new generation of DR-targeting antibodies has been designed to improve bioactivity, e.g., the bispecific antibody RG7386/RO6874813 that targets the fibroblast-activation protein (FAP) and DR5 [122]. This FAP-DR5 antibody was recently tested in a phase I study in patients with advanced or metastatic tumors (NCT02558140); the results have not been published yet. Another example is the hexabodies, engineered Fc fragments that boost IgG hexamers’ formation upon binding to the membrane-bound antigen [123]. HexaBody-DR5/DR5 (GEN1029) is a mixture of two noncompeting DR5 antibodies with a hexamerization-enhancing mutation [124]; this antibody is being tested in a phase I/II trial in patients with solid tumors (NCT03576131).

3.2. Trimer Conformation Plays a Crucial Role in Receptor Activation

As mentioned previously, the TNF apoptosis-inducing ligands are present as trimeric transmembrane proteins that can be cleaved, resulting in soluble proteins [34]. These proteins can interact with their respective receptors but sometimes cannot activate them [125], as in the case of sFasL. Studies have shown that the re-enforcement or improvement of the trimeric conformation improves the soluble protein’s activity [125]; the most common approaches use leucine zippers, His-tagged proteins, and covalent trimerization domains [125].

The activation of Fas by sFasL requires the oligomerization of sFasL [126]; with this in mind, APO010 (MegaFasL) was designed. APO010 is a hexameric fusion protein created by the fusion of the collagen domain adiponectin to two FasL extracellular domain trimers [127]. This fusion protein has reached clinical trials (NCT00437736); the results have not been published [128].

In the case of TRAIL, different approaches have been followed to help stabilize the trimer conformation, from using a fused leucine zipper motif, the N-terminal of sTRAIL (LZ-TRAIL) [129], to using a FLAG-tag that also helps in TRAIL purification [130]. Although these TRAIL-tagged forms have stable trimeric conformations, they can form aggregates that can induce toxicity to healthy human cells [131,132]. Another option is to stabilize the trimeric conformation by covalent linkage of the monomers, creating a single-chain TRAIL (scTRAIL) protein conferring an increase in activity and decrease in aggregates [133].

3.3. Fusion Proteins Improve Receptor Activation and Half-Life

Fusion proteins have been used to improve the activity of the soluble form of the apoptosis-inducing ligands. For years, it has been known that soluble ligands have a binding activity differing from that of membrane-bound ligands [134]. As described previously, sFasL barely activates Fas [126], and sTRAIL can efficiently activate DR4 but is not very efficient in DR5 activation [135]. Research has shown that genetically fusing the soluble form of apoptosis-inducing ligands to Fc-domains of antibodies or single-chain variable fragments (scFv) in a fusion protein overcomes the lack of activity [133,134].

Fusion proteins are molecules with multifunctional properties depending on the moieties that conform to them [136]. A common approach is to fuse the apoptosis-inducing ligands to an scFv. The scFv is a small fusion protein that contains the variable region of heavy (V\textsubscript{H}) and light (V\textsubscript{L}) of the immunoglobulin; these two regions are bonded together by a small and flexible linker [137]. This engineered antibody keeps the full antigen-binding capacity, making it useful for cancer therapy because it can target specific antigens expressed in transformed cells [138].
3.3.1. TNF-α

Several fusion proteins containing TNF-α have been designed as a cancer treatment (Table 2). A fusion protein composed of an scFv antibody from the high-molecular-weight melanoma-associated glycoprotein gp240 (ScFvMEL) and TNF-α known as scFvMEL/TNF was tested in murine models to assess the therapeutic effect, toxicity, and pharmacokinetics [139]. The results showed a therapeutic effect at a dose of 2.5 mg/kg in athymic mice with melanoma xenograft tumors; the maximum tolerated dose (MTD) was 4 mg/kg, and the terminal-phase half-life was 17.6 h after IV administration [139].

| Name              | Fusion Domain                                      | Format               | Ref.     |
|-------------------|----------------------------------------------------|----------------------|----------|
| anti-FAP-TNF      | FAP-positive tumor stroma                          | humanized anti-FAP Fab + TNF | [140]    |
| sFv23/TNF         | HER2/neo                                           | scFv23 + TNF         | [141]    |
| MFE-23:TNF-α      | carcinoembryonic antigen (CEA)                     | scFvMFe-23 + TNF     | [142]    |
| IL-12-L19-TNF-α   | T-cell-stimulating factor and scFv (L19) against the EDB domain of fibronectin | Triple fusion protein: IL-12 + scFvL19 + TNF-α | [143]    |
| scFvMEL/TNF       | gp240 antigen on human melanoma cells              | scFvMEL + TNF-α      | [139,144]|
| L19-TNF*          | EDB domain of fibronectin                         | hmAb L19 + TNF-α     | [145,146]|

Another TNF-α fusion protein is the IL-12-L19-TNFα a triple fusion protein containing interleukin-12 (IL-12), an antibody fragment specific to fibronectin (L19) extra-domain B, and TNF-α. It was tested in vitro, where it showed cytotoxic activity in murine L-M fibroblast [143]. However, when tested in mice for biodistribution assessment, it was found that the triple fusion protein was rapidly cleared from kidneys and liver, thus failing to localize the tumor and showing no activity whatsoever. Its lack of effectiveness is related to the large size of the protein (340 kDa), which affects its biodistribution [143]. In the same study, the fusion protein L19-TNFα was tested, showing a better tumor uptake than the triple fusion protein.

The fusion protein L19-TNFα was designed for localized administration and reached clinical trials. L19 has an affinity for the EB-D domain of fibronectin, which is considered a marker of angiogenesis in cancer patients [147]. It was tested in phase I/II clinical trials as monotherapy for advanced solid cancer patients, where it showed its safety; however, it did not show an objective tumor response [145]. Later, the fusion protein safety was tested in combination with melphalan in isolated limb perfusions (ILP) in extremity melanoma patients, showing promising results [146].

3.3.2. FasL

Although membrane-bound FasL can induce apoptosis, sFasL lacks the apoptotic activity, which can be regained by fusing sFasL with an scFv. Different FasL fusion proteins have been designed to improve FasL apoptotic activity. Those proteins (Table 3) have been tested in preclinical studies showing promising results; for instance, the fusion protein sc40-FasL was tested against tumor stroma in mice by intravenous administration, showing no signs of systemic toxicity and preventing the growth of FAP-positive cells [148]. Moreover, scFvRit:sFasL was studied as a treatment for B-cell leukemia. It triggered CD20 and Fas apoptotic signaling in malignant B cells in samples from patients without showing systemic toxicity in mice [149].
Table 3. FasL fusion proteins.

| Name                      | Fusion Domain                        | Format           | Ref. |
|---------------------------|--------------------------------------|------------------|------|
| sc40-FasL                 | scFv against fibroblast activation protein (FAP) | CD152 + FasL     | [148]|
| scFvCD7:sFasL             | scFv against CD7 (T-cell leukemia-associated antigen) | scFv40 + FasL    | [150]|
| scFvRitz:sFasL            | scFv against CD20 (Rituximab)        | scFvCD7 + sFasL  | [149]|
| CTLA-4-FasL               | Extracellular domain of receptor CTLA4 (B/) |                  | [151]|
| cc49scFv-FasLext          | scFv against human tumor-associated glycoprotein (TAG-72) | scFvRituximab + sFasL | [152]|

3.3.3. TRAIL

The use of engineered antibodies in the format of an scFv is an explored approach to create TRAIL fusion proteins with high stability and the capacity to activate the apoptotic pathway [45]. The fusion of sTRAIL (20 kDa) with an scFv (30 kDa) helps to overcome the short half-life of sTRAIL because the molecular weight of the homotrimeric scFv:sTRAIL fusion protein, around 150 kDa [134,153], decreases the renal clearance and increases the time in the circulation. TRAIL has been linked to different scFvs and antibodies (Table 4) over the years. Most of the fusion proteins are in preclinical studies, like scFv-scTRAIL and CD19-sTRAIL. scFv-scTRAIL is a fusion protein composed of an scFv against the extracellular domain of ErbB2 genetically fused to three TRAIL protomers expressed as a single polypeptide chain (scTRAIL). This fusion protein showed, in vivo, an increase in the half-life and a higher apoptotic activity when compared with scTRAIL [154]. CD19L-sTRAIL is a fusion protein that contains the ligand of the human CD19 receptor (CD19L) genetically fused to sTRAIL. CD19 is a receptor expressed in B-cell precursor acute lymphoblastic leukemia. This protein was well tolerated in mice at doses between 32 fmol/kg and 3.2 pmol/kg; it also showed apoptotic activity in C19+ xenograft mouse models at doses in the fmol/kg range [155]. The TRAIL fusion proteins mentioned showed potential as cancer treatments.

Table 4. TRAIL fusion proteins.

| Name                  | Target Antigen | Combination                             | TRAIL Format | Ref. |
|-----------------------|----------------|-----------------------------------------|--------------|------|
| scFvC54-sTRAIL        | EGP2           | -                                       | sTRAIL       | [156]|
| scFv425-sTRAIL        | EGFR           | Iressa                                  | sTRAIL       | [157]|
| scFv425-sTRAILmR1-5   | EGFR           | Cisplatin, valproic acid                | DR4-specific sTRAIL mutant three sTRAIL monomers (aa 95–281) | [158]|
| scFv-scTRAIL          | ErbB2          | -                                       | sTRAIL       | [154]|
| Anti-MCSP:TRAIL       | MCSP           | Rimcazole                               | TNC-TRAIL (95–281); TNC-sTRAIL monomer (aa 99–281) | [159]|
| scFv:G28-TRAIL        | CD40           | -                                       | TNC-TRAIL    | [160]|
| scFv:CD70-TRAIL       | CD27           | -                                       | wt, DR4, and DR5-specific sTRAIL | [161]|
| scFvM58-sTRAIL        | MRP3           | -                                       | sTRAIL       | [162]|
| CD19L-sTRAIL          | CD19           | Radiation                               | sTRAIL (aa 114–281) | [163]|
| scFv62-TRAIL          | Kv10.1         | Doxorubicin                             | Full-length TRAIL | [164]|
| ss-TR3                | Mesothelin     | -                                       | Covalent linked-TRAIL trimer (Monomer aa 91–281) | [165]|
| ABBV-621              | Human IgG1-Fc  | Venetoclax (DLBCL, AML only), FOLFIRI + bevacizumab (KRAS-mutant CRC) | scTRAIL-RBD | NCT03082209 |

TCN, trimerization domain; DLBCL, diffuse large B-cell lymphoma; AML, acute myeloid leukemia; CRC, colorectal cancer; RBD, receptor-binding domain.

Another TRAIL fusion protein in clinical trials is ABBV-621. It is now in phase I study as a therapy for patients with previously treated solid tumors and hematologic malignancies (NCT03082209). ABBV-621 is a fusion protein that contains an immunoglobulin
G1 (IgG1)-fragment crystallizable region (Fc) portion fused to a single chain trimer of TRAIL subunits. This fusion protein was designed to maximize the clustering of TRAIL receptors [94,166].

4. Gene Therapy

Gene therapy may deliver proapoptotic genes to express these proteins to induce cell apoptosis [167,168]. The gene delivery can be through viral and nonviral vectors. The viral vectors possess the natural advantage of gene delivery into a wide range of host cells, promoting high transgene expression levels. The vectors can be genetically modified to alter their cell tropism, modify or remove the ability to replicate, and deliver a transgene with therapeutic properties [80,169–171]. Moreover, the transgene local effect can be achieved with tissue-specific or tumor-specific and inducible promoters [172]. Hence, gene therapy can be used for a local production of the apoptosis-inducing ligands, thus avoiding systemic toxicity; moreover, it can improve the ligands’ pharmacokinetics by the continuous production of the transgene [40].

Many viral vectors have been designed to deliver apoptosis-inducing ligands throughout the years, but only a few reached clinical trials, such as the adenoviral vectors TNFerade [173,174] and VB-111 [175,176].

TNFerade is a second-generation replication-defective adenovirus armed with human TNF-α cDNA. The vector includes the radiation-inducible promoter early growth response (Egf-1) upstream of the transcriptional start site of human TNF-α [177]. The Egf-1 promoter is activated by radiotherapy, which is usually applied in the tumor’s localized area; this helps regulate TNF-α’s transcription, keeping its activity restricted to a location and avoiding systemic toxicity [178].

TNFerade was used in clinical trial phases I and II to treat different types of cancers, where the patients tolerated the treatment well. However, in phase III, TNFerade was used to treat advanced pancreatic cancer but was not effective [174,177,179].

VB-111 (ofranergene obadenovec) is a replication-defective adenovirus serotype 5 vector armed with a modified murine pre-proendothelin promoter (PPE-1) and human Fas-chimera transgene. The modified PPE-1 promoter has a hypoxia-responsive factor and three copies of the endothelium-specific element that increase the specificity for angiogenic vessels—the vector was designed to target Fas-chimera transgene expression in angiogenic blood vessels to induce apoptosis [180]. In a phase I clinical trial, VB-111 was tested in patients with solid tumors, and safety and tolerability were established [175,180,181]. Then, VB-111 was then tested in patients with recurrent glioblastoma (rGBM) in a phase I/II (NCT01260506) study. The results showed that the patients primed with VB-111 alone and then treated with bevacizumab (a monoclonal antibody against the vascular endothelial growth factor (VEGF)) and VB-111 had significantly better survival and progression-free survival [182].

Later, VB-111 was tested in combination with bevacizumab in a phase III study (NCT02511405), where it did not show efficacy. It is essential to consider that the treatment setting was different from the previous phase I/II study. In this trial, the patients had a combinational treatment of VB-111 and bevacizumab. Coughesy et al. thought that the lack of effectiveness in this trial was because bevacizumab antagonized VB-111 [176]. VB-111 is still a matter of study in combination with different drugs as treatment for colorectal cancer (NCT04166383) and ovarian cancer (NCT03398655).

The adenovirus vectors can also be engineered as oncolytic viruses; they can replicate selectively in cancer cells and kill them, releasing new viral particles that can infect neighboring and distant cancer cells [172]. An example of an oncolytic adenoviral vector is the H5CmTERT-Ad/TRAIL. This vector has six copies of hypoxia-responsive elements (HER) upstream of a cancer-specific modified human telomerase reverse transcriptase (5CmTERT) promoter, and it is armed with sTRAIL. (114–281 aa). H5CmTERT-Ad/TRAIL was tested in subcutaneous and orthotopic xenograft models of glioblastoma. It showed the capability to replicate efficiently in normoxic and hypoxic conditions and a strong antitumor effect
potentiated by sTRAIL, which helps with the viral distribution and apoptotic induction in TRAIL-resistant glioblastomas [183]. H5CmTERT-Ad/TRAIL is a good candidate for further investigation as glioblastoma treatment.

Another example of an oncolytic vector is the NDV/Anh-TRAIL [184]. This vector was designed using the Newcastle disease virus (NDV) from the Anhinga strain, a single-stranded nonsegmented negative-sense RNA virus with natural oncolytic activity. This characteristic gives the NDV the capability to replicate in interferon (IFN)-deficient cells, i.e., cancer cells [185]. NDV/Anh-TRAIL contains sTRAIL, which improves the apoptotic capacity of the vector. The vector was tested as a treatment for hepatocellular carcinoma in vivo and in vitro. It affected the cell viability of HepG2 cells, and it was well tolerated without significant toxicity in the hepatocellular carcinoma mouse model leading to tumor regression [184].

In the case of TRAIL, no gene therapy has reached clinical trials yet. However, several viral vectors have been designed to increase TRAIL effectiveness and avoid repeated administration [69]; those vectors are being studied in preclinical models (Table 5). The most common viral vector used is the adenovirus (Ad). It can be engineered as a replication-defective vector like the Ad-scFv425:sTRAIL; this vector contains the anti-EGFR single antibody chain fragment (scFv425) fused to sTRAIL. In an in vitro test, the vector showed potent apoptotic activity in transformed infected cells and noninfected EGFR-positive cancer cells.

Table 5. Viral vectors armed with TRAIL protein for cancer treatment.

| Vector Type | TRAIL format | Target/Aim | Ref. |
|-------------|-------------|------------|------|
| Ad/TRAIR-F/RGD | Replication-defective adenovirus | Pancreatic cancer, NSCLC | [186] |
| Ad/TRAIR-E1 | Oncolytic adenovirus | Colon cancer, Gastric cancer | [188-190] |
| Ad-TRAIR | Replication-defective adenovirus | Glioblastoma, Lung cancer cells AML | [191,192] |
| Ad-sTRAIL | Replication-defective adenovirus | SS-ILZ-TRAIL (114–281 aa) | Lung cancer, AML | [193,194] |
| Ad-TRAIL | Oncolytic adenovirus | Renal carcinoma | [196] |
| AAV-hTERT-TRAIR | Oncolytic adenovirus | Full-length TRAIL | Prostate cancer, HCC, HNSCC, Glioma | [197,198,199] |
| AAV-TRAIR | Adeno-associated virus | Adeno-associated virus | GFP-TRAIR, soluble TRAIL | [200] |
| AAV-TRAIR and Ad-gTRAIR | Replication-defective adenovirus | TRAIL-(IETD)-Smac | Hepatoma | [202] |
| ZDD5-TRAIR-(IETD)-Smac | Oncolytic adenovirus | scFv against VEGF + sTRAIL (114–281 aa) | Solid tumors | [203] |
| Ad-KDRscFv:sTRAIL | Oncolytic adenovirus | | Bladder cancer, Uveal melanoma, Breast cancer, Esophageal cancer, Prostate cancer, Lung cancer | [204,205,206,207,208,209] |
| Ad-TRAIR-MRE | Replication-defective adenovirus | | | |
| Ad-AB/TRAIR plus | Oncolytic adenovirus | HCC | [210] |
| AAV9-NSE-sTRAIL | Adeno-associated virus | | | |
| CD55-TRAIR-(IETD)-MnSOD | Oncolytic adenovirus | TRAIL-(IETD)-MnSOD | Glioblastoma | [212] |
| NDV/Anh-TRAIR | Newcastle disease virus/oncolytic virus | Soluble TRAIL | HCC | [184] |
| rAAV2-sTRAIL 95-281 | Adeno-associated virus | | Solid tumors, Glioblastoma, Lung cancer | [214,183,215] |
| H5CmTERT-Ad/TRAIR | Oncolytic adenovirus | sTRAIL (114–281 aa) | Glioblastoma, Lung cancer | [183,215] |
Additionally, Ad-scFv425:sTRAIL was tested in an established renal carcinoma xenograft in nude mice. After an intraocular administration of $10^{10}$ viral particles (vp), the tumor size decreased by around 90%. The fusion protein was detected in plasma 60 days after infection with a concentration of around 200 µg/mL. It did not show liver toxicity, thus proving to be safe for systemic administration [196].

5. Conclusions

As a cancer treatment, induction of cell death by apoptosis appears an interesting pathway to follow. In search of apoptosis-inducing ligands, targeting the death receptors (DRs) from the TNF superfamily seems the right approach. The cytokines from the TNF superfamily, TNF-α, FasL, and TRAIL, can induce apoptosis by activating the extrinsic pathway when they bind to DR1, DR2, DR4, or DR5 [216].

Although TNF-α was identified as promising cancer therapeutic and several agonists were developed for that purpose, TNF-α antagonists have a greater value in treating inflammatory and autoimmune diseases (rheumatoid arthritis) [96,217]. Recently, in a phase Ib study, it was found that the TNF blockers (infliximab or certolizumab) helped to mitigate the immune-related adverse events in melanoma patients undergoing treatment with immune checkpoint inhibitors (ICls) while enhancing their antitumor effect [100]. Out of the TNF apoptosis-inducing ligands, TRAIL is a promising anticancer agent, mainly because of its characteristic of selectively inducing apoptosis in cancer cells while sparing the nontransformed cells. However, TRAIL’s short half-life has been a limitation for its use in the clinic.

In clinical trials, some of the recombinant death ligands and agonists developed as cancer treatments showed limited activity and, in some cases, systemic toxicity [43,70]. Therefore, gene therapy may be a useful tool to improve the activity of the apoptosis-inducing ligands and eliminate systemic toxicity.

The use of viral vectors can improve the apoptosis-inducing ligands’ effectiveness; using tumor-specific promoters can give a localized effect, thus averting systemic toxicity. The transgene’s continuous production can help overcome the short half-life of the ligands and avoid the need for multiple administrations. For instance, the use of Fas agonists in a systemic administration can lead to liver toxicity, which may be prevented by localized administration with adenoviral vectors and tumor-specific promoters. The drawback of TRAIL’s short half-life can be overcome using an adenoviral vector, as Bremer et al. demonstrated [196].

Although TNF apoptosis-inducing ligands have been designed as cancer therapies and analyzed in preclinical studies showing promising results, only a few have reached clinical trials. The majority of them faltered in the first stages of the studies, showing a lack of effectiveness. Therefore, the use of gene therapy as a tool to improve the pharmacokinetics and meet the need for better biomarkers of resistance, which help screen and select the target population to design tailored anticancer approaches from single to combination agents, may help overcome the lack of effectiveness during the clinical trials. Additionally, the TNF apoptosis-inducing ligands’ effectiveness may also be boosted by using them in combination with other anticancer therapeutics.

Considering that the gene therapy techniques are being developed and improved to such an extent that vaccines against SARS-CoV-2 were developed in record time using Ad5 [218] and lipid nanoparticles [219,220] as vectors, we expect a major step forward in the field of cancer gene therapy based on all the developments over the last few decades.

Author Contributions: O.A.D.A. and H.J.H. performed the literature search, analyzed the data, and wrote the manuscript. O.A.D.A. created the figures. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no funding.

Acknowledgments: O.A.D.A. received a PhD research fellowship from the Mexican National Council for Science and Technology (CONACyT).
Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The Next Generation. Cell 2011, 144, 646–674. [CrossRef] [PubMed]
2. Holohan, C.; Van Schaeybroeck, S.; Longley, D.B.; Johnston, P.G. Cancer drug resistance: An evolving paradigm. Nat. Rev. Cancer 2013, 13, 714–726. [CrossRef]
3. Kroemer, G.; Pouyssegur, J. Tumor Cell Metabolism: Cancer’s Achilles’ Heel. Cancer Cell 2008, 13, 472–482. [CrossRef] [PubMed]
4. Jia, L.T.; Chen, S.Y.; Yang, A.G. Cancer gene therapy targeting cellular apoptosis machinery. Cancer Treat. Rev. 2012, 38, 868–876. [CrossRef]
5. Bremer, E.; de Bruyn, M.; Wajant, H.; Helfrich, W. Targeted Cancer Immunotherapy Using Ligands of the Tumor Necrosis Factor Super-Family. Curr. Drug Targets 2009, 10, 94–103. [CrossRef]
6. Elmore, S. Apoptosis: A Review of Programmed Cell Death. Toxicol. Pathol. 2007, 35, 495–516. [CrossRef]
7. Kiraz, Y.; Adan, A.; Kartal Yandim, M.; Baran, Y. Major apoptotic mechanisms and genes involved in apoptosis. Tumor Biol. 2016, 37, 8471–8486. [CrossRef] [PubMed]
8. Mahmoud, Z.; Shukla, Y. Death receptors: Targets for cancer therapy. Exp. Cell Res. 2010, 316, 887–899. [CrossRef]
9. Tait, S.W.G.; Green, D.R. Mitochondria and cell death: Outer membrane permeabilization and beyond. Nat. Rev. Mol. Cell Biol. 2010, 11, 621–632. [CrossRef]
10. Ichim, G.; Tait, S.W.G. A fate worse than death: Apoptosis as an oncogenic process. Nat. Rev. Cancer 2016, 16, 539–548. [CrossRef]
11. Park, H.H. Domain swapping of death domain superfamily: Alternative strategy for dimerization. Int. J. Biol. Macromol. 2019, 138, 565–572. [CrossRef]
12. MacKenzie, S.H.; Clark, A.C. Targeting cell death in tumors by activating caspases. Curr. Cancer Drug Targets 2008, 8, 98–109. [CrossRef]
13. D’Arcy, M.S. Cell death: A review of the major forms of apoptosis, necrosis and autophagy. Cell Biol. Int. 2019, 43, 582–592. [CrossRef]
14. Taylor, R.C.; Cullen, S.P.; Martin, S.J. Apoptosis: Controlled demolition at the cellular level. Nat. Rev. Mol. Cell Biol. 2008, 9, 231–241. [CrossRef] [PubMed]
15. Walczak, H. Death receptor-ligand systems in cancer, cell death, and inflammation. Cold Spring Harb. Perspect. Biol. 2013, 5, 1–18. [CrossRef]
16. Nikoletopoulou, V.; Markaki, M.; Palikaras, K.; Tavernarakis, N. Crosstalk between apoptosis, necrosis and autophagy. Biochim. Biophys. Acta Mol. Cell Res. 2013, 1833, 3448–3459. [CrossRef] [PubMed]
17. Jain, M.V.; Paczulla, A.M.; Klonisch, T.; Dimgba, F.N.; Rao, S.B.; Roberg, K.; Schweizer, F.; Lengerke, C.; Davoodpour, P.; Palicharla, V.R.; et al. Interconnections between apoptotic, autaphagic and necrotic pathways: Implications for cancer therapy development. J. Cell. Mol. Med. 2013, 17, 12–29. [CrossRef]
18. Jouan-Lanhouet, S.; Arshad, M.I.; Piquet-Pellorce, C.; Martin-Chouly, C.; Le Moigne-Muller, G.; Van Herreweghe, F.; Takahashi, N.; Sergent, O.; Lagadic-Gossman, D.; Vandenabeele, P.; et al. TRAIL induces necroptosis involving RIPK1/RIPK3-dependent PARP-1 activation. Cell Death Diff. 2012, 19, 2003–2014. [CrossRef] [PubMed]
19. Yi, F.; Frazzette, N.; Cruz, A.C.; Klebanoff, C.A.; Siegel, R.M. Beyond Cell Death: New Functions for TNF Family Cytokines in Autoimmunity and Tumor Immunotherapy. Trends Mol. Med. 2018, 1–12. [CrossRef]
20. Paperfuss, K.; Cordier, S.M.; Walczak, H. Death receptors as targets for anti-cancer therapy. J. Cell. Mol. Med. 2008, 12, 2566–2585. [CrossRef]
21. Möller, P.; Koretz, K.; Leithäuser, F.; Brüderlein, S.; Henne, C.; Quentmeier, A.; Krammer, P.H. Expression of APO-1 (CD95), a member of the NGF/TNF receptor superfamily, in normal and neoplastic colon epithelium. Int. J. Cancer 1994, 57, 371–377. [CrossRef] [PubMed]
22. Viard-Leveuge, I.; Veyrenc, S.; French, L.E.; Brambilia, C.; Brambilia, E. Frequent loss of Fas expression and function in human lung tumours with overexpression of FasL in small cell lung carcinoma. J. Pathol. 2003, 201, 268–277. [CrossRef] [PubMed]
23. Das, H.; Koizumi, T.; Sugimoto, T.; Chakraborty, S.; Ichimura, T.; Hasegawa, K.; Nishimura, R. Quantiﬁcation of Fas and Fas ligand gene expression in human ovarian, cervical and endometrial carcinomas using real time quantitative RT-PCR. Br. J. Cancer 2000, 82, 1682–1688. [CrossRef] [PubMed]
24. Reesink-Peters, N.; Hougardy, B.M.T.; Van Den Heuvel, F.A.J.; Ten Hoor, K.A.; Hollema, H.; Boezen, H.M.; De Vries, E.G.E.; De Jong, S.; Van Der Zee, A.G.J. Death receptors and ligands in cervical carcinogenesis: An immunohistochemical study. Gynecol. Oncol. 2005, 96, 705–713. [CrossRef]
25. Ozawa, F.; Friess, H.; Kleeff, J.; Xu, Z.; Zimmermann, A.; Sheikh, M.; Büchler, M. Effects and expression of apoptosis-promoting receptors in human pancreatic cancer. Cancer Lett. 2012, 163, 71–81. [CrossRef]
26. Koornstra, J.J.; Kleibeuker, J.H.; van Geelen, C.M.M.; Rijken, E.E.M.; Hollema, H.; De Vries, E.G.E.; De Jong, S. Expression of TRAIL (TNF-related apoptosis-inducing ligand) and its receptors in normal colon mucosa, adenomas, and carcinomas. J. Pathol. 2003, 200, 327–335. [CrossRef]
27. Kawasaki, M.; Kuwano, K.; Nakanishi, Y.; Hagimoto, N.; Takayama, K.; Pei, X.-H.; Maeyama, T.; Yoshimi, M.; Har, N. Analysis of Fas and Fas ligand expression and function in lung cancer cell lines. Eur. J. Cancer 2000, 36, 656–663. [CrossRef]
Cancers 2021, 13, 1543

28. Hwang, H.S.; Park, Y.Y.; Shin, S.J.; Go, H.J.; Park, J.M.; Yoon, S.Y.; Lee, J.L.; Cho, Y.M. Involvement of the tnf-α pathway in thioretinase resistance and suggestion of ftnr1 as a predictive biomarker for tki responsiveness in clear cell renal cell carcinoma. J. Korean Med. Sci. 2020, 35, 1–12. [CrossRef]

29. Yang, J.; LeBlanc, F.R.; Dighe, S.A.; Hamele, C.E.; Olson, T.L.; Feith, D.J.; Loughran, T.P. TRAIL mediates and sustains constitutive NF-κB activation in LGL leukemia. Blood 2018, 131, 2803–2815. [CrossRef]

30. TODA, M.; KAWAMOTO, T.; UEHA, T.; KISHIMOTO, K.; HARA, H.; FUKASE, N.; ONISHI, Y.; HARADA, R.; MINODA, M.; KUROSAKA, M.; et al. ‘Decoy’ and non-decoy’ functions of DcR3 promote malignant potential in human malignant fibrous histiocytoma cells. Int. J. Oncol. 2013, 43, 703–712. [CrossRef] [PubMed]

31. Wu, H.; Hymowitz, S.G. Structure and Function of Tumor Necrosis Factor (TNF) at the Cell Surface. In Handbook of Cell Signaling; Bradshaw, R.A., Dennis, E.A., Eds.; Academic Press: San Diego, CA, USA, 2010; ISBN 9780123741455.

32. Sheik, M.; Fornace, A. Death and decoy receptors and p53-mediated apoptosis. Leukemia 2000, 14, 1509–1513. [CrossRef] [PubMed]

33. Aggarwal, B.B. Signalling pathways of the TNF superfamily: A double-edged sword. Nat. Rev. Immunol. 2003, 3, 745–756. [CrossRef] [PubMed]

34. Locksley, R.M.; Killeen, N.; Lenardo, M.J. The TNF and TNF Receptor Superfamilies. Cell 2001, 104, 487–501. [CrossRef]

35. Vinic, J.E.; Wong, W.W.L.; Khan, N.; Feltham, R.; Chau, D.; Ahmed, A.U.; Benetatos, C.A.; Chunduru, S.K.; Condon, S.M.; Pitti, R.M.; Marsters, S.A.; Lawrence, D.A.; Roy, M.; Kischkel, F.C.; Dowd, P.; Huang, A.; Donahue, C.J.; Sherwood, S.W.; Baldwin, A.; et al. IAP Antagonists Target cIAP1 to Induce TNFα-Dependent Apoptosis. Cell 2007, 131, 682–693. [CrossRef] [PubMed]

36. Varfolomeev, E.; Vucic, D. Intracellular regulation of TNF activity in health and disease. Cytokine 2018, 101, 26–32. [CrossRef] [PubMed]

37. Medler, J.; Wajant, H. Tumor necrosis factor receptor-2 (TNFR2): An overview of an emerging drug target. Expert Opin. Ther. Targets 2019, 23, 295–307. [CrossRef] [PubMed]

38. Martinez-Reza, I.; Diaz, L.; Garcia-Becerra, R. Preclinical and clinical aspects of TNF-α and its receptors TNFR1 and TNFR2 in breast cancer. J. Biomed. Sci. 2017, 24, 90. [CrossRef] [PubMed]

39. Black, R.A.; Rauch, C.T.; Kozlosky, C.J.; Peschon, J.J.; Slack, J.L.; Wolfson, M.F.; Castner, B.J.; Stolking, K.L.; Reddy, P.; Srinivasan, S.; et al. A metalloproteinase disintegrin that releases tumour-necrosis factor-α from cells. Nature 1997, 385, 729–733. [CrossRef] [PubMed]

40. Josephs, S.F.; Ichim, T.E.; Prince, S.M.; Kesari, S.; Marincola, F.M.; Escobedo, A.R.; Jafri, A. Unleashing endogenous TNF-α-alpha as a cancer immunotherapeutic. J. Transl. Med. 2018, 16, 242. [CrossRef] [PubMed]

41. Hikita, A.; Tanaka, N.; Yamane, S.; Ikeda, Y.; Furukawa, H.; Tohma, S.; Suzuki, R.; Tanaka, S.; Mitomi, H.; Fukui, N. Involvement of a disintegrin and metalloproteinase 10 and 17 in shedding of tumor necrosis factor-α. Biochem. Biophys. Res. Commun. 2009, 381, 581–593. [PubMed]

42. Schwartz, J.; Broder, C.; Helmstetter, A.; Schmidt, S.; Yan, I.; Muller, M.; Schmidt-Arras, D.; Becker-Pauly, C.; Koch-Nolte, F.; Mittrucker, H.-W.; et al. Short-term TNFα shedding is independent of cytoplasmic phosphorylation or furin cleavage of ADAM17. Biochim. Biophys. Acta Mol. Cell Res. 2013, 1833, 3355–3367. [CrossRef] [PubMed]

43. Balkwill, F. Tumour necrosis factor and cancer. Nat. Rev. Cancer 2009, 9, 361–371. [CrossRef] [PubMed]

44. Ehrenschwender, M.; Wajant, H. The Role of FasL and Fas in Health and Disease. Adv. Exp. Med. Biol. 2009, 647, 64–93. [PubMed]

45. Vanamee, E.S.; Faustman, D.L. Structural principles of tumor necrosis factor superfamily signaling. Sci. Signal. 2018, 11, eaao4910. [CrossRef] [PubMed]

46. Pitti, R.M.; Marsters, S.A.; Lawrence, D.A.; Roy, M.; Kischkel, F.C.; Dowd, P.; Huang, A.; Donahue, C.J.; Sherwood, S.W.; Baldwin, D.T.; et al. Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. Neoplasia 1998, 396, 699–703. [PubMed]

47. Wajant, H. Principles and mechanisms of CD95 activation. Biol. Chem. 2014, 395, 1401–1416. [CrossRef] [PubMed]

48. Hsieh, S.-L.; Lin, W.-W. Decoy receptor 3: An endogenous immunomodulator in cancer growth and inflammatory reactions. J. Biomed. Sci. 2017, 24, 39. [CrossRef] [PubMed]

49. Ge, Z.; Sanders, A.J.; Ye, L.; Jiang, W.G. Aberrant expression and function of death receptor-3 and death decoy receptor-3 in human cancer. Exp. Ther. Mol. Biol. 2011, 2, 167–172. [CrossRef] [PubMed]

50. Wu, Q.; Zheng, Y.; Chen, D.; Li, X.; Lu, C.; Zhang, Z. Aberrant expression of decoy receptor 3 in human breast cancer: Relevance to lymphangiogenesis. J. Surg. Res. 2014, 188, 454–465. [CrossRef] [PubMed]

51. Macher-Goeppinger, S.; Aulmann, S.; Wagener, N.; Funke, B.; Tagscherer, K.E.; Haferkamp, A.; Hohenfellner, M.; Kim, S.; Autschbach, F.; Schirmacher, P.; et al. Decoy Receptor 3 Is a prognostic Factor in Renal Cell Carcinoma. Neoplasia 2008, 10, 1049–IN2. [CrossRef] [PubMed]

52. Zhou, J.; Song, S.; Li, D.; He, S.; Zhang, B.; Wang, Z.; Zhu, X. Decoy receptor 3 (DcR3) overexpression predicts the prognosis and pN2 in pancreatic head carcinoma. World J. Surg. Oncol. 2014, 12, 52. [CrossRef] [PubMed]

53. Malleter, M.; Tautzin, S.; Bessed, A.; Castellano, R.; Goubard, A.; Godey, F.; Levêque, J.; Jézéquel, P.; Campon; L.; Campone, M.; et al. Card45 cell surface cleavage triggers a prometastatic signaling pathway in triple-negative breast cancer. Cancer Res. 2013, 73, 6711–6721. [CrossRef] [PubMed]

54. ElOjeimy, S.; McKillop, J.C.; El-Zawahry, A.M.; Holman, D.H.; Liu, X.; Schwartz, D.A.; Day, T.A.; Dong, J.Y.; Norris, J.S. Fasl gene therapy: A new therapeutic modality for head and neck cancer. Cancer Gene Ther. 2006, 13, 739–745. [CrossRef] [PubMed]

55. Timmer, T.; de Vries, E.G.E.; de Jong, S. Fas receptor-mediated apoptosis: A clinical application? J. Pathol. 2002, 196, 125–134. [CrossRef] [PubMed]
56. Trivedi, R.; Mishra, D.P. Trailing TRAIL Resistance: Novel Targets for TRAIL Sensitization in Cancer Cells. Front. Oncol. 2015, 5, 69. [CrossRef] [PubMed]

57. Degli-Esposti, M.A.; Smolak, P.J.; Walczak, H.; Waugh, J.; Huang, C.-P.; Dubose, R.F.; Goodwin, R.G.; Smith, C.A. Cloning and Characterization of TRAIL-R3, a Novel Member of the Emerging TRAIL Receptor Family. J. Exp. Med. 1997, 186, 1165–1170. [CrossRef]

58. Falschlehner, C.; Emmerich, C.H.; Gerlach, B.; Walczak, H. TRAIL signalling: Decisions between life and death. Int. J. Biochem. Cell Biol. 2007, 39, 1462–1475. [CrossRef] [PubMed]

59. Bernardi, S.; Voltan, R.; Rimondi, E.; Melloni, E.; Milani, D.; Cervellati, C.; Gemmati, D.; Celeghini, C.; Secchiero, P.; Zauli, G.; et al. TRAIL, OPG, and TWEAK in kidney disease: Biomarkers or therapeutic targets? Clin. Sci. 2019, 133, 1145–1166. [CrossRef]

60. Candido, R. The osteoprotegerin/tumor necrosis factor related apoptosis-inducing ligand axis in the kidney. Curr. Opin. Nephrol. Hypertens. 2014, 23, 69–74. [CrossRef]

61. Deligiorgi, M.V.; Panayiotidis, M.I.; Griniatsos, J.; Trafalis, D.T. Harnessing the versatile role of OPG in bone oncology: Counterbalancing RANKL and TRAIL signaling and beyond. Clin. Exp. Metastasis 2020, 37, 13–30. [CrossRef]

62. 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. Sect. F. Struct. Biol. Commun. 2015, 71, 1273–1281. [CrossRef]

63. Aziiji, K.; Yuvaraj, S.; Peppelenbosch, M.P.; Würdinger, T.; Dekker, H.; Dekker, H.; Ben Mabrouk, H.; Humblin, E.; Jacquemin, G.; Szegedi, E.; Delacote, F.; et al. TRAIL receptor gene editing unveils TRAIL-R1 as a master player of apoptosis induced by TRAIL and ER stress. Oncotarget 2017, 8, 9974–9985. [CrossRef]

64. Dufour, F.; Rattier, T.; Constantinescu, A.A.; Zischler, H.; Morlé, A.; Ben Mabrouk, H.; Humblin, E.; Jacquemin, G.; Szegedi, E.; Delacote, F.; et al. TRAIL receptor gene editing unveils TRAIL-R1 as a master player of apoptosis induced by TRAIL and ER stress. Oncotarget 2017, 8, 9974–9985. [CrossRef]

65. Bodmer, J.L.; Meier, P.; Tschopp, J.; Schneider, P. Cysteine 230 is essential for the structure and activity of the cytotoxic ligand TRAIL. Cell Biol. 2000, 275, 20632–20637. [CrossRef] [PubMed]

66. Kim, M.H.; Billiar, T.R.; Seol, D.W. The secretable form of trimeric TRAIL, a potent inducer of apoptosis. Biochem. Biophys. Res. Commun. 2004, 321, 930–935. [CrossRef]

67. Amarante-Mendes, G.P.; Griffith, T.S. Therapeutic applications of TRAIL receptor agonists in cancer and beyond. Pharmacol. Ther. 2015, 155, 117–131. [CrossRef]

68. Lemke, J.; von Karstedt, S.; Zinninger, J.; Walczak, H. Getting TRAIL back on track for cancer therapy. Cell Death Differ. 2014, 21, 1350–1364. [CrossRef]

69. Stuckey, D.W.; Shah, K. TRAIL on trial: Preclinical advances in cancer therapy. Trends Mol. Med. 2013, 19, 685–694. [CrossRef]

70. Herbst, R.S.; Eckhardt, S.G.; Kurzrock, R.; Ebbinghaus, S.; O’Dwyer, P.J.; Gordon, M.S.; Novotny, W.; Goldwasser, M.A.; Tohnya, 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]

71. Wainberg, Z.A.; Peddi, P.F.; Kapp, A.V.; Ashkenazi, A.; Royer-Joo, S.; Portera, C.C.; Kozloff, M.F. A Phase 1B Study of Dulanermin in Combination With Modified FOLFOX6 Plus Bevacizumab in Patients With Metastatic Colorectal Cancer. Clin. Colorectal Cancer 2013, 12, 248–254. [CrossRef]

72. Soria, J.C.; Márk, Z.; Zatloukal, P.; Szirma, B.; Albert, I.; Juhasz, E.; Pujol, J.-L.; Kozielski, J.; Baker, N.; Smethurst, D.; et al. Randomized Phase II Study of Dulanermin in Combination With Paclitaxel, Carboplatin, and Bevacizumab in Advanced Non–Small-Cell Lung Cancer. J. Clin. Oncol. 2011, 29, 4442–4451. [CrossRef]

73. 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/Ipo2 ligand) combined with vinorelbine and cisplatin in patients with advanced non-small-cell lung cancer. Invest. New Drugs 2018, 36, 315–322. [CrossRef]

74. Hou, J.; Qiu, L.; Zhao, Y.; Zhang, X.; Liu, Y.; Zhou, F.; Leng, Y.; Yang, S.; Xi, H.; et al. A Phase Ib Dose Escalation Study of Recombinant Circularly Permuted TRAIL in Patients With Relapsed or Refractory Multiple Myeloma. Am. J. Clin. Oncol. 2018, 41, 1008–1014. [CrossRef]

75. Leng, Y.; Qiu, L.; Hou, J.; Zhao, Y.; Zhang, X.; Yang, S.; Xi, H.; Huang, Z.; Pan, L.; Chen, W. Phase II open-label study of recombinant circularly permuted TRAIL as a single-agent treatment for relapsed or refractory multiple myeloma. Chin. J. Cancer 2016, 35, 86. [CrossRef]

76. Leng, Y.; Hou, J.; Jin, J.; Zhang, M.; Ke, X.; Jiang, B.; Pan, L.; Yang, L.; Zhou, F.; Wang, J.; et al. Circularly permuted TRAIL plus thalidomide and dexamethasone versus thalidomide and dexamethasone for relapsed/refractory multiple myeloma: A phase 2 study. Cancer Chemother. Pharmacol. 2017, 79, 1141–1149. [CrossRef] [PubMed]

77. WAJANT, H.; GERSPACH, J.; PFIZENMAIER, K. Tumor therapeutics by design: Targeting and activation of death receptors. Cytokine Growth Factor Rev. 2005, 16, 55–76. [CrossRef]

78. Sheng, Y.; Li, F.; Qin, Z. TNF Receptor 2 Makes Tumor Necrosis Factor a Friend of Tumors. Front. Immunol. 2018, 9, 1–9. [CrossRef]

79. Holland, P.M. Death receptor agonist therapies for cancer, which is the right TRAIL? Cytokine Growth Factor Rev. 2014, 25, 185–193. [CrossRef]
80. Wajant, H.; Gerschler, J.; Pfizenmaier, K. Engineering death receptor ligands for cancer therapy. Cancer Lett. 2013, 332, 163–174. [CrossRef]
81. Nakamura, S.; Kato, A.; Masugi, T.; Fukuoka, M.; Kitai, K.; Ogawa, H.; Ichikawa, Y.; Maeda, M.; Watanabe, N.; Kohgo, Y.; et al. A novel recombinant tumor necrosis factor-alpha mutant with increased anti-tumor activity and lower toxicity. Int. J. Cancer 1991, 48, 744–748. [CrossRef]
82. Van Ostade, X.; Vandenebeeke, P.; Everaertd, B.; Loetscher, H.; Gentz, R.; Brockhaus, M.; Lesslauer, W.; Tavernier, J.; Brouckaert, P.; Fiers, W. Human TNF mutants with selective activity on the p55 receptor. Nature 1993, 361, 266–269. [CrossRef]
83. Shin, N.-K.; Lee, I.; Chang, S.-G.; Shin, H.-C. A novel tumor necrosis factor alpha mutant with significantly enhanced cytotoxicity and receptor binding affinity. IUBMB Life 1998, 44, 1075–1082. [CrossRef]
84. Kuroda, K.; Miyata, K.; Fujita, F.; Koike, M.; Fujita, M.; Nomura, M.; Nakagawa, S.; Tsutsuki, Y.; Kawagoe, T.; Mitsuishi, Y.; et al. Human tumor necrosis factor alpha mutant RGD-V2 (F4614) shows potent antitumor activity and reduced toxicity against human tumor xenografted nude mice. Cancer Lett. 2000, 159, 33–41. [CrossRef]
85. Yan, Z.; Zhao, N.; Wang, Z.; Li, B.; Bao, C.; Shi, J.; Han, W.; Zhang, Y. A mutated human tumor necrosis factor-alpha improves the therapeutic index in vitro and in vivo. Cytoteraphy 2006, 8, 415–423. [CrossRef]
86. MacFarlane, M.; Kohlihas, S.L.; Sutcliffe, M.J.; Dyer, M.J.S.; Cohen, G.M. TRAIL receptor-selective mutants signal to apoptosis via TRAIL-R1 in primary lymphoid malignancies. Cancer Res. 2005, 65, 11265–11270. [CrossRef]
87. Reis, C.R.; van der Sloot, A.M.; Natoni, A.; Szegedi, E.; Setroikromo, R.; Meijer, M.; Sjollema, K.; Stricher, F.; Cool, R.H.; Samali, A.; et al. Rapid and efficient cancer cell killing mediated by high-affinity death receptor homotrimerizing TRAIL variants. Cell Death Dis. 2010, 1, e83. [CrossRef]
88. Yu, R.; Albarenesque, S.M.; Cool, R.H.; Quax, W.J.; Mohr, A.; Zwacka, R.M. DR4 specific TRAIL variants are more efficacious than wild-type TRAIL in pancreatic cancer. Cancer Biol. Ther. 2014, 15, 1658–1666. [CrossRef]
89. 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]
90. Duiker, E.W.; De Vries, E.G.E.; Mahalingam, D.; Meersma, G.J.; Van EK, W.B.; Hollem, H.; De Hooge, M.N.L.; Van Dam, G.M.; Cool, R.H.; Quax, W.J.; et al. Enhanced antitumor efficacy of a DR5-specific TRAIL variant over recombinant human TRAIL in a bioluminescent ovarian cancer xenograft model. Clin. Cancer Res. 2009, 15, 2048–2057. [CrossRef]
91. Gasparian, M.E.; Chernyak, B.V.; Dolgikh, D.A.; Yagolovich, A.V.; Popova, E.N.; Sycheva, A.M.; Moshkovskii, S.A.; Kiritchenkov, M.P. Generation of new TRAIL mutants DR5-A and DR5-B with improved selectivity to death receptor 5. Apoptosis 2009, 14, 778–787. [CrossRef] [PubMed]
92. Huang, M.; Zhu, H.; Yi, C.; Yan, J.; Wei, L.; Yang, X.; Chen, S.; Huang, Y. A novel TRAIL mutant-TRAIL-Mu3 enhances the antitumor effects by the increased affinity and the up-expression of DR5 in pancreatic cancer. Cancer Chemother. Pharmacol. 2018, 82, 829–838. [CrossRef]
93. Scott, A.M.; Wolchok, J.D.; Old, L.J. Antibody therapy of cancer. Nat. Rev. Cancer 2012, 12, 278–287. [CrossRef] [PubMed]
94. Lim, B.; Greer, Y.; Lipkowitz, S.; Takebe, N. Novel apoptosis-inducing agents for the treatment of cancer, a new arsenal in the toolbox. Cancers 2019, 11, 1087. [CrossRef]
95. Castelli, M.S.; McGonigle, P.; Hornby, P.J. The pharmacology and therapeutic applications of monoclonal antibodies. Pharmacol. Res. Perspect. 2019, 7, e00535. [CrossRef] [PubMed]
96. Fischer, R.; Kontermann, R.E.; Pfizenmaier, K. Selective Targeting of TNF Receptors as a Novel Therapeutic Approach. Front. Cell Dev. Biol. 2020, 8, 1–21. [CrossRef]
97. Zettlitz, K.A.; Lorenz, V.; Landauer, K.; Münsel, K.; Herrmann, A.; Scheurich, P.; Pfizenmaier, K.; Kontermann, R.E. ATROSAB, a humanized antagonistic anti-tumor necrosis factor receptor one-specific antibody. MAbs 2010, 2, 639–647. [CrossRef]
98. Williams, S.K.; Fairless, R.; Maier, O.; Liehr, M.; Setroikromo, R.; Fischer, R.; Eisell, U.L.M.; Kontermann, R.E.; Herrmann, A.; Weksler, B.; et al. Anti-TNFFR1 targeting in humanized mice ameliorates disease in a model of multiple sclerosis. Sci. Rep. 2018, 8, 1–14. [CrossRef] [PubMed]
99. Badran, Y.R.; Cohen, J.V.; Brastianos, P.K.; Parikh, A.R.; Hong, T.S.; Dougan, M. Concurrent therapy with immune checkpoint inhibitors and TNFα blockade in patients with gastrointestinal immune-related adverse events. J. Immunother. Cancer 2019, 7, 226. [CrossRef]
100. Montfort, A.; Filleron, T.; Virazels, M.; Dufau, C.; Milhès, J.; Pages, C.; Olivier, P.; Ayyoub, M.; Mounier, M.; Lusque, A.; et al. Combining Nivolumab and Ipilimumab with Infliximab or Certolizumab in Patients with Advanced Melanoma: First Results of a Phase Ib Clinical Trial. Clin. Cancer Res. 2021, 27, 1037–1047. [CrossRef] [PubMed]
101. Ogasawara, J.; Watanabe-Fukunaga, R.; Adachi, M.; Matsuzawa, A.; Kasugai, T.; Kitamura, Y.; Itoh, N.; Suda, T.; Nagata, S. Lethal effect of the anti-Fas antibody in mice. Nature 1993, 364, 806–809. [CrossRef]
102. Yonehara, S. Death receptor Fas and autoimmune disease: From the original generation to therapeutic application of agonistic anti-Fas monoclonal antibody. Cytkine Growth Factor Rev. 2002, 13, 393–402. [CrossRef]
103. Ichikawa, K.; Yoshida-Kato, H.; Ohtsuki, M.; Ohsumi, J.; Yamaguchi, J.; Takahashi, S.; Tani, Y.; Watanabe, M.; Shiraishi, A.; Nishioka, K.; et al. A novel murine anti-human Fas mAb which mitigates lymphadenopathy without hepatotoxicity. Int. Immunol. 2000, 12, 555–562. [CrossRef] [PubMed]
104. Pukac, L.; Kanakaraj, P.; Humphreys, R.; Alderson, R.; Bloom, M.; Sung, C.; Riccobene, T.; Johnson, R.; Fiscella, M.; Mahoney, A.; et al. HGS-ETR1, a fully human TRAIL-receptor 1 monoclonal antibody, induces cell death in multiple tumour types in vitro and in vivo. *Br. J. Cancer* 2005, 92, 1430–1441. [CrossRef]

105. Tolcher, A.W.; Mita, M.; Meropol, N.J.; Von Mehren, M.; Patnaik, A.; Padavick, K.; Hill, M.; Mays, T.; McCoy, T.; Fox, N.L.; et al. Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor-related apoptosis-inducing ligand receptor-1. *J. Clin. Oncol.* 2007, 25, 1390–1395. [CrossRef]

106. Rader, C.; Wiestner, A. Six-packed antibodies punch better. *Clin. Cancer Res.* 2008, 14, 3450–3455. [CrossRef]

107. Von Pawel, J.; Harvey, J.H.; Spigel, D.R.; Dediu, M.; Reck, M.; Cebotaru, C.L.; Humphreys, R.C.; Gribbin, M.J.; Fox, N.L.; Camidge, D.R. Phase II Trial of Mapatumumab, a Fully Human Agonist Monoclonal Antibody to Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptor 1 (TRAIL-R1), in Combination With Paclitaxel and Carboplatin in Patients With Advanced Non–Small-Cell Lung Cancer. *Clin. Lung Cancer* 2014, 15, 188–196.e2. [CrossRef] [PubMed]

108. Ciuleanu, T.; Bazin, I.; Lungulescu, D.; Miron, L.; Bondarenko, I.; Deptala, A.; Rodriguez-Torres, M.; Giantonio, B.; Fox, N.L.; Wissel, P.; et al. A randomized, double-blind, placebo-controlled phase II study to assess the efficacy and safety of mapatumumab with sorafenib in patients with advanced hepatocellular carcinoma. *Ann. Oncol.* 2016, 27, 680–687. [CrossRef]

109. 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]

110. Demetri, G.D.; Le Cesne, A.; Chawla, S.P.; Brodowicz, T.; Maki, R.G.; Bach, B.A.; Smethurst, D.P.; Bray, S.; Hei, Y.; Blay, J.-Y. First-line treatment of metastatic or locally advanced unresectable soft tissue sarcomas with conatumumab in combination with doxorubicin or doxorubicin alone: A Phase I/II open-label and double-blind study. *Eur. J. Cancer* 2012, 48, 547–563. [CrossRef]

111. Paz-Ares, L.; Béjar, B.; van Meerbeeck, J.P.; Wierzbicki, R.; Souza, P.; Galimi, F.; Haddad, V.; Sabin, T.; Hei, Y.; et al. A Randomized Phase 2 Study of Paclitaxel and Carboplatin with or without Conatumumab for First-Line Treatment of Advanced Non–Small-Cell Lung Cancer. *J. Thorac. Oncol.* 2013, 8, 329–337. [CrossRef]

112. Kindler, H.L.; Richards, D.A.; Garbo, L.E.; Gordon, J.G.; Stephenson, J.J.; Chim, C.; Safran, H.; Chan, D.; Kocs, D.M.; Galimi, F.; et al. A randomized, placebo-controlled phase 2 study of ganitumab (AMG 479) or conatumumab (AMG 655) in combination with gemcitabine in patients with metastatic pancreatic cancer. *Ann. Oncol.* 2012, 23, 2834–2842. [CrossRef]

113. Fuchs, C.S.; Fakhir, M.; Schwartzberg, L.; Cohn, A.L.; Yee, L.; Dreisbach, L.; Kozloff, M.F.; Hei, Y.; Galimi, F.; Pan, Y.; et al. TRAIL receptor agonist conatumumab with modified FOLFOX6 plus bevacizumab for first-line treatment of metastatic colorectal cancer. *Cancer* 2013, 119, 4290–4298. [CrossRef]

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. Merchant, M.S.; Geller, J.I.; Baird, K.; Chou, A.J.; Galli, S.; Charles, A.; Amaoko, M.; Maclennan, M.; Lo, L.; Fox, N.L.; et al. Safety, pharmacokinetics, and pharmacodynamics of the DR5 antibody LBY135 alone and in combination with capecitabine in patients with metastatic colorectal cancer. *Clin. Cancer Res.* 2012, 18, 30–39. [CrossRef]

116. Forero-Torres, A.; Shah, J.; Wood, T.; Posey, J.; Carlisle, R.; Copigneaux, C.; Luo, F. (Roger); Wojtowicz-Praga, S.; Percent, I.; Saleh, M.; Camidge, D.R. Phase II Trial of Mapatumumab, a Fully Human Monoclonal Antibody to TRAIL-R1 in Patients with Advanced Solid Malignancies. *Clin. Lung Cancer* 2008, 9, 169–177. [CrossRef]

117. Forero-Torres, A.; Infante, J.R.; Waterhouse, D.; Wong, L.; Vickers, S.; Arrowsmith, E.; Hart, L.; Trent, D.; Wade, J.; et al. Phase 2, multicenter, open-label study of tigatuzumab (CS-1008), a humanized monoclonal antibody targeting death receptor 5, in combination with gemcitabine in chemotherapy-naïve patients with unresectable or metastatic pancreatic cancer. *Cancer Med.* 2013, 2, 925–932. [CrossRef]

118. Camidge, D.R.; Herbst, R.S.; Gordon, M.S.; Eckhardt, S.G.; Kurzrock, R.; Durbin, B.; Ing, J.; Tohma, T.; 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]

119. Cheng, A.L.; Kang, Y.K.; He, A.R.; Lim, H.Y.; Ryoo, B.Y.; Hung, C.H.; Sheen, I.S.; Izumi, N.; Austin, T.; Wang, Q.; et al. Safety and efficacy of tigatuzumab plus sorafenib as first-line therapy in subjects with advanced hepatocellular carcinoma: A Phase 2 randomized study. *J. Hepatol.* 2015, 63, 896–904. [CrossRef]

120. Forero-Torres, A.; Infante, J.R.; Waterhouse, D.; Wong, L.; Vickers, S.; Arrowsmith, E.; Hart, L.; Trent, D.; Wade, J.; et al. Phase 2, multicenter, open-label study of tigatuzumab (CS-1008), a humanized monoclonal antibody targeting death receptor 5, in combination with gemcitabine in chemotherapy-naïve patients with unresectable or metastatic pancreatic cancer. *Cancer Med.* 2013, 2, 925–932. [CrossRef]

121. 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. *Clin. Cancer Res.* 2014, 20, 135–144. [CrossRef]

122. Brünker, P.; Wartha, K.; Friess, T.; Grau-Richards, S.; Waldhauer, I.; Koller, C.F.; Weiser, B.; Majeti, M.; Runza, V.; Niu, H.; et al. RG7386, a novel tetravalent FAP-DR5 antibody, effectively triggers FAP-dependent, avidity-driven DR5 hyperclustering and tumor cell apoptosis. *Mol. Cancer Ther.* 2016, 15, 946–957. [CrossRef]

123. Rader, C.; Wiestner, A. Six-packed antibodies punch better. *Haematologica* 2019, 104, 1696–1699. [CrossRef]
124. Overdijk, M.B.; Strumane, K.; Buijsse, A.O.; Vermot-Desroches, C.; Kroes, T.; de Jong, B.; Hoevenaars, N.; Beurskens, F.J.; de Jong, R.N.; Lingnau, A.; et al. Abstract 2391: DR5 agonist activity of HexaBody ®-DR5/DR5 (GEN1029) is potentiated by C1q and independent of Fc-gamma receptor binding in preclinical tumor models. In Proceedings of the Immunology; American Association for Cancer Research: Atlanta, GA, USA, 2019; Volume 5, p. 2391.

125. Berg, D.; Lehne, M.; Müller, N.; Siegmund, D.; Münkel, S.; Sebald, W.; Pfizenmaier, K.; Wajant, H. Enforced covalent trimerization increases the activity of the TNF ligand family members TRAIL and CD95L. Cell Death Differ. 2007, 14, 2021–2034. [CrossRef] [PubMed]

126. Schneider, P.; Holler, N.; Bodmer, J.-L.; Hahnne, M.; Frei, K.; Fontana, A.; Tschopp, J. Conversion of Membrane-bound Fas(CD95) Ligand to Its Soluble Form Is Associated with Downregulation of Its Proapoptotic Activity and Loss of Liver Toxicity. J. Exp. Med. 1998, 187, 1205–1213. [CrossRef] [PubMed]

127. Eisele, G.; Roth, P.; Hasenbach, K.; Aulwurm, S.; Wolpert, F.; Tabatabai, G.; Wick, W.; Weller, M. APO010, a synthetic hexameric CD95 ligand, induces human glioma cell death in vitro and in vivo. Neuro Oncol. 2011, 13, 155–164. [CrossRef] [PubMed]

128. Onxeo A Phase I Dose Finding Study of APO010 in Patients With Solid Tumors (AP1001). Available online: https://clinicaltrials.gov/ct2/show/NCT01043773?term=apo010&draw=2&rank=2 (accessed on 2 September 2020).

129. Walczak, H.; Miller, R.E.; Ariail, K.; Gliniak, B.; 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]

130. Schneider, P. Production of Recombinant TRAIL and TRAIL Receptor: Fc Chimeric Proteins. In Methods in Enzymology; Academic Press: Cambridge, MA, USA, 2000; Volume 322, pp. 325–345.

131. Ganten, T.M. 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]

132. Koschny, R.; Walczak, H.; Ganten, T.M. The promise of TRAIL—potential and risks of a novel anticancer therapy. J. Mol. Med. 2007, 85, 925–935. [CrossRef] [PubMed]

133. Hutt, M.; Marquardt, L.; Seifert, O.; Siegemund, M.; Müller, I.; Kulms, D.; Pfizenmaier, K.; Kontermann, R.E. Superior properties of Fc-containing scTRAIL fusion proteins. Mol. Cancer Ther. 2017, 16, 2792–2802. [CrossRef] [PubMed]

134. De Bruyn, M.; Bremer, E.; Helfrich, W. Antibody-based fusion proteins to target death receptors in cancer. Cancer Lett. 2013, 332, 175–183. [CrossRef] [PubMed]

135. Wajant, H.; Moosmayer, D.; Wüest, T.; Bartke, T.; Gerlach, E.; Schönherr, U.; Peters, N.; Scheurich, P.; Pfizenmaier, K. Differential activation of TRAIL-R1 and -2 by soluble and membrane TRAIL allows selective surface antigen-directed activation of TRAIL-R2 by a soluble TRAIL derivative. Oncogene 2001, 20, 4101–4106. [CrossRef] [PubMed]

136. Chen, X.; Zaro, J.L.; Shen, W.C. Fusion protein linkers: Property, design and functionality. Adv. Drug Deliv. Rev. 2013, 65, 1357–1369. [CrossRef] [PubMed]

137. Ahmad, Z.A.; Yeap, S.K.; Ali, A.M.; Ho, W.Y.; Alitheen, N.B.M.; Hamid, M. ScFv antibody: Principles and clinical application. Clin. Dev. Immunol. 2012, 2012. [CrossRef] [PubMed]

138. Monnier, P.; Vigouroux, R.; Tassew, N. In Vivo Applications of Single Chain Fv (Variable Domain) (scFv) Fragments. Antibodies 2013, 2, 193–208. [CrossRef] [PubMed]

139. Liu, Y.; Zhang, W.; Cheung, L.H.; Niu, T.; Wu, Q.; Li, C.; Van Pelt, C.S.; Rosenblum, M.G. The antimelanoma immunocytokine scFvMEL/TNF shows reduced toxicity and potent antitumor activity against human tumor xenografts. Neoplasia 2006, 8, 384–393. [CrossRef] [PubMed]

140. Bauer, S.; Adrian, N.; Williamson, B.; Panousis, C.; Fadle, N.; Smerd, J.; Fettah, I.; Scott, A.M.; Pfreundschuh, M.; Renner, C. Targeted Bioactivity of Membrane-Anchored TNF by an Antibody-Derived TNF Fusion Protein. J. Immunol. 2004, 172, 3930–3939. [CrossRef] [PubMed]

141. Rosenblum, M.G.; Horn, S.A.; Cheung, L.H. A novel recombinant fusion toxin targeting HER-2/NEU-over-expressing cells and containing human tumor necrosis factor. Int. J. Cancer 2000, 88, 267–273. [CrossRef]

142. Cooke, S.P.; Pedley, R.B.; Boden, R.; Begent, R.H.J.; Chester, K.A. In Vivo Tumor Delivery of a Recombinant Single-Chain Fv: Tumor Necrosis Factor: A Fusion Protein. Bioconjug. Chem. 2002, 13, 7–15. [CrossRef] [PubMed]

143. Hallin, C.; Gafner, V.; Villani, M.K.; Borsi, L.; Berndt, A.; Kosmehl, H.; Zardi, L.; Neri, D. Synergistic therapeutic effects of a tumor targeting antibody fragment, fused to interleukin 12 and to tumor necrosis factor alpha. Cancer Res. 2003, 63, 3202–3210.

144. Liu, Y.; Cheung, L.H.; Marks, J.W.; Rosenblum, M.G. Recombinant single-chain antibody fusion construct targeting human melanoma cells and containing tumor necrosis factor. Int. J. Cancer 2004, 108, 549–557. [CrossRef] [PubMed]

145. Spitaleri, G.; Berardi, R.; Pierantoni, C.; De Pas, T.; Noberasco, C.; Libbra, C.; González-Iglesias, R.; Giovannoni, L.; Tasciotti, A.; Neri, D.; et al. Phase I/II study of the tumour-targeting human monoclonal antibody–cytokine fusion protein L19-TNF in patients with advanced solid tumours. J. Cancer Res. Clin. Oncol. 2013, 139, 447–455. [CrossRef] [PubMed]

146. Papadia, F.; Basso, V.; Patuzzo, R.; Maurichi, A.; Di Florio, A.; Zardi, L.; Ventura, E.; González-Iglesias, R.; Lovato, V.; Giovannoni, L.; et al. Isolated limb perfusion with the tumor-targeting human monoclonal antibody-cytokine fusion protein L19-TNF plus melphalan and mild hyperthermia in patients with locally advanced extremity melanoma. J. Surg. Oncol. 2013, 107, 173–179. [CrossRef] [PubMed]

147. Danielli, R.; Patuzzo, R.; Ruffini, P.A.; Maurichi, A.; Giovannoni, L.; Elia, G.; Neri, D.; Santinami, M. Armed antibodies for cancer treatment: A promising tool in a changing era. Cancer Immunol. Immunother. 2015, 64, 113–121. [CrossRef] [PubMed]
148. Samel, D.; Müller, D.; Gerspach, J.; Assouhou-Luty, C.; Sass, G.; Tieg, G.; Pfizenmaier, K.; Wajant, H. Generation of a FasL-based proapoptotic fusion protein devoid of systemic toxicity due to cell-surface antigen-restricted activation. J. Biol. Chem. 2003, 278, 32077–32082. [CrossRef]

149. Bremer, E.; ten Cate, B.; Samplonius, D.F.; Mueller, N.; Wajant, H.; Stel, A.J.; Chamuleau, M.; van de Loosdrecht, A.A.; Stieglmaier, J.; Fey, G.H.; et al. Superior Activity of Fusion Protein scFvRitsFasL over Cotreatment with Ritusimab and Fas Agonists. Cancer Res. 2008, 68, 597–604. [CrossRef]

150. Bremer, E.; ten Cate, B.; Samplonius, D.F.; de Leij, L.F.M.H.; Helfrich, W. CD7-restricted activation of Fas-mediated apoptosis: A novel therapeutic approach for acute T-cell leukemia. Blood 2006, 107, 2863–2870. [CrossRef]

151. Huang, J.-H.; Tykocinski, M.L. CTLA-4-Fas ligand functions as a trans signal converter protein in bridging antigen-presenting cells and T cells. Int. Immunol. 2001, 13, 529–539. [CrossRef]

152. Chan, D.V.; Sharma, R.; Ju, C.-Y.A.; Roffler, S.R.; Ju, S.-T. A recombinant scFv-FasLext as a targeting cytotoxic agent against human Jurkat-Ras cancer. J. Biomed. Sci. 2013, 20, 16. [CrossRef]

153. Ahamadi-Fesharaki, R.; Fateh, A.; Vaziri, F.; Solgi, G.; Siadat, S.D.; Mahboudi, F.; Rahimi-Jamnani, F. Single-Chain Variable Fragment-Based Bispecific Antibodies: Hitting Two Targets with One Sophisticated Arrow. Mol. Ther. Oncol. 2019, 14, 38–56. [CrossRef]

154. Schneider, B.; Münk, S.; Krippner-Heidenreich, A.; Grunwald, I.; Wels, W.S.; Wajant, H.; Pfizenmaier, K.; Gerspach, J. Potent antitumoral activity of TRAIL through generation of tumor-targeted single-chain fusion proteins. Cell Death Dis. 2010, 1, e68. [CrossRef]

155. Uckun, F.M.; Myers, D.E.; Qazi, S.; Ozer, Z.; Rose, R.; Cruz, O.J.D.; Ma, H. Recombinant human CD19L-sTRAIL effectively targets B cell precursor acute lymphoblastic leukemia. J. Clin. Invest. 2014, 125, 1–13. [CrossRef]

156. Bremer, E.; Kuilen, J.; Samplonius, D.F.; Walczak, H.; de Leij, L.; Helfrich, W. Target cell-restricted and -enhanced apoptosis induction by a scFv:sTRAIL fusion protein with specificity for the pancarcinoma-associated antigen EGP2. Int. J. Cancer 2004, 109, 281–290. [CrossRef]

157. Bremer, E.; Samplonius, D.F.; Van Genne, L.; Dijkstra, M.H.; Kroesen, B.J.; De Leij, L.F.M.H.; Helfrich, W. Simultaneous Inhibition of Epidermal Growth Factor Receptor (EGFR) Signaling and Enhanced Activation of Tumor Necrosis mediated Apoptosis Induction by an scFv:sTRAIL Fusion Protein with Specificity for Human EGFR*. Biochemistry 2005, 280, 10025–10033. [CrossRef]

158. Bremer, E.; de Bruyn, M.; Samplonius, D.F.; Bijma, T.; ten Cate, B.; de Leij, L.F.M.H.; Helfrich, W. Targeted delivery of a designed sTRAIL mutant results in superior apoptotic activity towards EGFR-positive tumor cells. J. Mol. Med. 2008, 86, 909–924. [CrossRef]

159. De Bruyn, M.; Rybczynska, A.A.; Wei, Y.; Schwenkert, M.; Fey, G.H.; Dierckx, R.A.J.O.; van Waarde, A.; Helfrich, W.; Bremer, E.; De Bruyn, M.; et al. Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP) -targeted delivery of soluble TRAIL potently inhibits melanoma outgrowth in vitro and in vivo. Mol. Cancer 2010, 9, 301. [CrossRef]

160. El-Mesery, M.; Trebing, J.; Schäfer, V.; Weisenberger, D.; Siegmund, D.; Wajant, H. CD40-directed scFv-TRAIL fusion proteins induce CD40-restricted tumor cell death and activate dendritic cells. Cell Death Dis. 2013, 4, e916. [CrossRef]

161. Breting, J.; El-Mesery, M.; Schäfer, V.; Weisenberger, D.; Siegmund, D.; Silence, K.; Wajant, H. CD70-restricted specific activation of TRAILR1 or TRAILR2 using scFv-targeted TRAIL mutants. Cell Death Dis. 2014, 5, e1035. [CrossRef]

162. Wang, L.-H.; Ni, C.-W.; Lin, Y.-Z.; Yin, L.; Jiang, C.-B.; Lv, C.-T.; Le, Y.; Lang, Y.; Zhao, C.-Y.; Yang, K.; et al. Targeted induction of apoptosis in glioblastoma multiforme cells by an MRP3-specific TRAIL fusion protein in vitro. J. Biol. Chem. 2019, 294, 32077–32082. [CrossRef]

163. De Bruyn, M.; et al. Superior Activity of Fusion Protein scFvRitsFasL over Cotreatment with Ritusimab and Fas Agonists. Cancer Res. 2008, 68, 597–604. [CrossRef]

164. Hartung, F.; Pardo, L.A. Guiding TRAIL to cancer cells through Kv10.1 potassium channel overcomes resistance to doxorubicin. Eur. J. Pharmacol. 2016, 745, 709–719. [CrossRef]

165. Tatzel, K.; Kuroki, L.; Dmitriev, I.; Kashentseva, E.; Curiel, D.T.; Goedegebuure, S.P.; Powell, M.A.; Mutch, D.G.; Hawkins, W.G.; Spitzer, D. Membrane-proximal TRAIL species are incapable of inducing short circuit apoptosis signaling: Implications for drug development and basic cytokine biology. Sci. Rep. 2016, 6, 1–13. [CrossRef]

166. Kretz, A.; Trauzold, A.; Hillenbrand, A.; Knippischild, U.; Henne-Bruns, D.; von Karstedt, S.; Lemke, J. TRAILblazing Strategies for Cancer Treatment. Cancers 2019, 11, 456. [CrossRef]

167. Haisma, H.J.; Bellu, A.R. Pharmacological interventions for improving adenovirus usage in gene therapy. Mol. Pharm. 2011, 8, 50–55. [CrossRef]

168. Khaligheinjad, N.; Hariri, H.; Behnamfar, O.; Yousefi, A.; Momeni, A. Adenoviral gene therapy in gastric cancer: A review. World J. Gastroenterol. 2008, 14, 180–184. [CrossRef]

169. Chen, Y.H.; Keiser, M.S.; Davidson, B.L. Viral Vectors for Gene Transfer. Curr. Protoc. Mol. Biol. 2018, 8, e58. [CrossRef]

170. Lee, C.S.; Bishop, E.S.; Zhang, R.; Yu, X.; Farina, E.M.; Yan, S.; Zhao, C.; Zeng, Z.; Shu, Y.; Wu, X.; et al. Adenovirus-mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine. Genes Dis. 2017, 4, 43–63. [CrossRef]

171. Chulpanova, D.; Solovyeva, V.; Kitaeva, K.; Dunham, S.; Khaiboullina, S.; Rizvanov, A. Recombinant Viruses for Cancer Therapy. Biomedicines 2018, 6, 94. [CrossRef]
172. Hardcastle, J.; Kurozumi, K.; Antonio Chiocca, E.; Kaur, B. Oncolytic Viruses Driven by Tumor-Specific Promoters. *Curr. Cancer Drug Targets* **2007**, *7*, 181–189. [CrossRef]

173. Seiwert, T.Y.; Darga, T.; Haraf, D.; Blair, E.A.; Stenson, K.; Cohen, E.E.W.; Salama, J.K.; Villaflor, V.; Witt, M.E.; Lingen, M.W.; et al. A phase I dose escalation study of Ad GVEGR.TNF.F11D (TNFerade™ Biologic) with concurrent chemoradiotherapy in patients with recurrent head and neck cancer undergoing reirradiation. *Ann. Oncol.* **2013**, *24*, 769–776. [CrossRef]

174. Herman, J.M.; Wild, A.T.; Wang, H.; Tran, P.T.; Chang, K.J.; Taylor, G.E.; Donehower, R.C.; Pawlik, T.M.; Ziegler, M.A.; Cai, H.; et al. Randomized phase iii multi-institutional study of infereon biologic with fluorouracil and radiotherapy for locally advanced pancreatic cancer: Final results. *J. Clin. Oncol.* **2013**, *31*, 886–894. [CrossRef]

175. Brenner, A.J.; Cohen, Y.C.; Breitbart, E.; Bangio, L.; Sarantopoulos, J.; Giles, F.J.; Borden, E.C.; Harats, D.; Triozzi, P.L. Phase i dose-escalation study of VB-111, an antiangiogenic vireotheray, in patients with advanced solid tumors. *Clin. Cancer Res.* **2013**, *19*, 3996–4007. [CrossRef]

176. Cloughesy, T.F.; Brenner, A.; de Groot, J.F.; Butowski, N.A.; Zach, L.; Campian, J.J.; Ellingson, B.M.; Freedman, L.S.; Cohen, Y.C.; Lowenton-Spier, N.; et al. A randomized controlled phase III study of VB-111 combined with bevacizumab vs bevacizumab monotherapy in patients with recurrent glioblastoma (GLOBE). *Neuro. Oncol.* **2020**, *22*, 705–717. [CrossRef]

177. Senzer, N.; Mani, S.; Rosemurgy, A.; Nemunaitis, J.; Cunningham, C.; Guha, C.; Bayol, N.; Gillen, M.; Chu, K.; Rasmussen, C.; et al. TNFerade biologic, an adenovector with a radiation-inducible promoter, carrying the human tumor necrosis factor alpha gene: A phase I study in patients with solid tumors. *J. Clin. Oncol.* **2004**, *22*, 592–601. [CrossRef] [PubMed]

178. Weichselbaum, R.R.; Kufe, D. Translation of the radio- and chemo-inducible TNFerade vector to the treatment of human cancers. *Cancer Gene Ther.* **2009**, *16*, 609–619. [CrossRef] [PubMed]

179. Moradi Marjaneh, R.; Hassanian, S.M.; G hobadi, N.; Ferns, G.A.; Karimi, A.; Jazayeri, M.H.; Nasiri, M.; Avan, A.; Khazaei, M. Targeting the death receptor signaling pathway as a potential therapeutic target in the treatment of colorectal cancer. *J. Cell. Physiol.* **2018**, *233*, 6538–6549. [CrossRef]

180. Triozzi, P.L.; Borden, E.C. VB-111 for cancer. *Expert Opin. Biol. Ther.* **2011**, *11*, 1669–1676. [CrossRef]

181. Grusola, A.; Cavazos, D.A.; Miller, J.R.; Breitbart, E.; Cohen, Y.C.; Bangio, L.; Yakov, N.; Soundararajan, A.; Floyd, J.R.; Brenner, A.J. VB-111: A novel anti-vascular therapeutic for glioblastoma multiforme. *J. Neurooncol.* **2015**, *124*, 365–372. [CrossRef]

182. Brenner, A.J.; Peters, K.V.; Vredenburgh, J.; Bokstein, F.; Blumenthal, D.T.; Wu, S.; Marini, F.C.; Fang, B. Suppressing orthotopic pancreatic tumor growth with a novel vascular-targeting agent for the treatment of patients with recurrent pancreatic adenocarcinoma. *J. Clin. Oncol.* **2013**, *31*, 181–189. [CrossRef]

183. Herman, J.M.; Wild, A.T.; Wang, H.; Tran, P.T.; Chang, K.J.; Taylor, G.E.; Donehower, R.C.; Pawlik, T.M.; Ziegler, M.A.; Cai, H.; et al. Randomized phase iii multi-institutional study of infereon biologic with fluorouracil and radiotherapy for locally advanced pancreatic cancer: Final results. *J. Clin. Oncol.* **2013**, *31*, 886–894. [CrossRef]

184. Zhou, W.; Dai, S.; Zhu, H.; Song, Z.; Cai, Y.; Lee, J.B.; Li, Z.; Hu, X.; Fang, B.; He, C.; et al. Telomerase-specific oncolytic adenovirus expressing secretable TRAIL suppresses peritoneal dissemination of gastric cancer. *Gene Ther.* **2017**, *24*, 199–207. [CrossRef]

185. Ganar, K.; Das, M.; Sinha, S.; Kumar, S. Newcastle disease virus: Current status and our understanding. *Virus Res.* **2014**, *184*, 71–81. [CrossRef]

186. Bogani, D.; Davis, J.; Zhu, H.; Zhang, L.; Teraishi, F.; Wu, S.; Marini, F.C.; Fang, B. Suppression of orthotopic pancreatic tumor growth with a fiber-modified adenovector expressing the TRAIL gene from the human telomerase reverse transcriptase promoter. *Clin. Cancer Res.* **2004**, *10*, 3535–3541. [CrossRef]

187. Chung, X. Radiotherapy Sensitization by Tumor-Specific TRAIL Gene Targeting Improves Survival of Mice Bearing Human Non-Small Cell Lung Cancer. *Clin. Cancer Res.* **2005**, *11*, 6657–6668. [CrossRef]

188. Dong, F. Eliminating Established Tumor in nu/nu Nude Mice by a Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand-Armored Oncolytic Adenovirus. *Clin. Cancer Res.* **2006**, *12*, 5224–5230. [CrossRef]

189. Zhou, W.; Zhu, H.; Chen, W.; Hu, X.; Pang, X.; Zhang, J.; Huang, X.; Fang, B.; He, C. Treatment of patient tumor-derived colon cancer xenografts by a TRAIL-armed adenoviral vector. *Cancer Gene Ther.* **2011**, *18*, 336–345. [CrossRef]

190. Zhou, W.; Dai, S.; Zhu, H.; Song, Z.; Cai, Y.; Lee, J.B.; Li, Z.; Hu, X.; Fang, B.; He, C.; et al. Telomerase-specific oncolytic adenovirus expressing TRAIL suppresses peritoneal dissemination of gastric cancer. *Gene Ther.* **2017**, *24*, 199–207. [CrossRef]

191. Yang, F.; Shi, P.; Xi, X.; Yi, S.; Li, H.; Sun, Q.; Sun, M. Recombinant Adenoviruses Expressing TRAIL Demonstrate Antitumor Effects on Non-Small Cell Lung Cancer (NSCLC). *Med. Oncol.* **2006**, *23*, 191–204. [CrossRef] [PubMed]

192. Chen, J.; Sun, X.; Yang, W.; Jiang, G.; Li, X. Cisplatin-enhanced sensitivity of glioblastoma multiforme U251 cells to adenovirus-delivered TRAIL in vitro. *Tumor Biol.* **2010**, *31*, 613–622. [CrossRef]

193. Kim, D.R.; Park, M.-Y.; Lee, C.-S.; Shim, S.-H.; Yoon, H.-I.; Lee, J.H.; Sung, M.-W.; Kim, Y.-S.; Lee, C.-T. Combination of vorinostat and adenovirus-TRAIL exhibits a synergistic antitumor effect by increasing transduction and transcription of TRAIL in lung cancer cells. *Cancer Gene Ther.* **2011**, *18*, 467–477. [CrossRef]

194. Yang, T.; Lan, J.; Huang, Q.; Chen, X.; Sun, X.; Liu, X.; Yang, P.; Jin, T.; Wang, S.; Mou, X. Embelin Sensitizes Acute Myeloid Leukemia Cells to TRAIL through XIAP Inhibition and NF-kB Inactivation. *Cell Biochem. Biophys.* **2015**, *71*, 291–297. [CrossRef]

195. Bremer, E.; van Dam, G.M.; de Bruyn, M.; van Riezen, M.; Dijkstra, M.; Kamps, G.; Helfrich, W.; Haisma, H. Potent systemic anticancer activity of adenovirally expressed EGFR-selective TRAIL fusion protein. *Mol. Ther.* **2008**, *16*, 1919–1926. [CrossRef]
197. Jiménez, J.A.; Li, X.; Zhang, Y.-P.; Bae, K.H.; Mohammadi, Y.; Pandya, P.; Kao, C.; Gardner, T.A. Antitumor activity of Ad-IU2, a prostate-specific replication-competent adenovirus encoding the apoptosis inducer, TRAIL. Cancer Gene Ther. 2010, 17, 180–191. [CrossRef] [PubMed]

198. Wang, Y.; Huang, F.; Cai, H.; Wu, Y.; He, G.; Tan, W.-S. The efficacy of combination therapy using adeno-associated virus-TRAIL targeting to telomerase activity and cisplatin in a mouse model of hepatocellular carcinoma. J. Cancer Res. Clin. Oncol. 2010, 136, 1827–1837. [CrossRef] [PubMed]

199. Jiang, M.; Liu, Z.; Xiang, Y.; Ma, H.; Liu, S.; Liu, Y.; Zheng, D. Synergistic antitumor effect of AAV-mediated TRAIL expression combined with cisplatin on head and neck squamous cell carcinoma. BMC Cancer 2011, 11, 54. [CrossRef]

200. Li, J.T.; Bian, K.; Zhang, A.L.; Kim, D.H.; Ashley, W.; Rath, N.; McCutcheon, I.; Fang, B.; Murad, F. Targeting different types of human meningioma and glioma cells using a novel adenoviral vector expressing GFP-TRAIL fusion protein from hTERT promoter. Cancer Cell Int. 2011, 11, 1–14. [CrossRef]

201. Zhang, R.; Zhang, X.; Ma, B.; Xiao, B.; Huang, F.; Huang, P.; Ying, C.; Liu, T.; Wang, Y. Enhanced antitumor effect of combining xenograft hepatoma by oncolytic adenovirus ZDS5 harboring TRAIL-ED3-smac gene with broad antitumor effect. Hum. Gene Ther. 2012, 23, 992–1002. [CrossRef]

202. Ru, Q.; Li, W.; Wang, X.; Zhang, S.; Chen, L.; Zhang, Y.; Ge, Y.; Zu, Y.; Liu, Y.; Zheng, D. Preclinical study of rAAV2-sTRAIL: Systemically administered AAV9-sTRAIL combats invasive glioblastoma in a patient-derived orthotopic xenograft model. J. Neurooncol. 2018, 143, 16017. [CrossRef] [PubMed]

203. Crommentuijn, M.H.W.; Kantar, R.; Noske, D.P.; Vandertop, W.P.; Badr, C.E.; Würdinger, T.; Maguire, C.A.; Tannous, B.A. Systemically administered AAV9-sTRAIL combats invasive glioblastoma in a patient-derived orthotopic xenograft model. Mol. Ther. Oncol. 2016, 3, 16017. [CrossRef] [PubMed]

204. Zhang, R.; Zhang, X.; Ma, B.; Xiao, B.; Huang, F.; Huang, P.; Ying, C.; Liu, T.; Wang, Y. Enhanced antitumor effect of combining TRAIL and MnSOD mediated by CEA-controlled oncolytic adenovirus in lung cancer. Cancer Gene Ther. 2016, 23, 168–177. [CrossRef] [PubMed]

205. Liu, J.; Ma, L.; Li, C.; Zhang, Z.; Yang, G.; Zhang, W. Tumor-targeting TRAIL expression mediated by miRNA response elements suppressed growth of uveal melanoma cells. Mol. Oncol. 2013, 7, 1043–1055. [CrossRef] [PubMed]

206. El-Shemi, A.G.; Ashshi, A.M.; Na, Y.; Li, Y.; Basalamah, M.; Al-Allaf, F.A.; Oh, E.; Jung, B.-K.; YUN, C.-O. Combined therapy with ING4 and TRAIL genes in cancer-targeting gene virotherapy strategy: First evidence in preclinical hepatocellular carcinoma. Gene Ther. 2018, 25, 54–65. [CrossRef]

207. Zhou, K.; Yan, Y.; Zhao, S. Esophageal cancer-selective expression of TRAIL mediated by MREs of miR-143 and miR-122. Tumor Biol. 2014, 35, 5787–5795. [CrossRef]

208. Wu, G.; Ji, Z.; Fan, L.; Wang, W. MiRNA regulation of TRAIL expression exerts selective cytotoxicity to prostate carcinoma cells. Mol. Cell. Biochem. 2014, 388, 123–133. [CrossRef]

209. Wu, G.; Ji, Z.; Li, H.; Lei, Y.; Jin, X.; Yu, Y.; Sun, M. Selective TRAIL-induced cytotoxicity to lung cancer cells mediated by miRNA response elements. Cell Biochem. Funct. 2014, 32, 547–556. [CrossRef]

210. El-Shemi, A.G.; Ashshi, A.M.; Na, Y.; Li, Y.; Basalamah, M.; Al-Allaf, F.A.; Oh, E.; Jung, B.-K.; YUN, C.-O. Combined therapy with oncolytic adenoviruses encoding TRAIL and IL-12 genes markedly suppressed human hepatocellular carcinoma both in vitro and in an orthotopic transplanted mouse model. J. Exp. Clin. Cancer Res. 2016, 35, 74. [CrossRef] [PubMed]

211. Galal El-Shemi, A.; Mohammed Ashshi, A.; Oh, E.; Jung, B.-K.; Basalamah, M.; Alsaegh, A.; Yun, C.-O. Efficacy of combining ING4 and TRAIL genes in cancer-targeting gene virotherapy strategy: First evidence in preclinical hepatocellular carcinoma. Gene Ther. 2018, 25, 54–65. [CrossRef]

212. Crommentuijn, M.H.W.; Kantar, R.; Noske, D.P.; Vandertop, W.P.; Badr, C.E.; Würdinger, T.; Maguire, C.A.; Tannous, B.A. Systemically administered AAV9-sTRAIL combats invasive glioblastoma in a patient-derived orthotopic xenograft model. Mol. Ther. Oncol. 2016, 3, 16017. [CrossRef] [PubMed]

213. Zhang, R.; Zhang, X.; Ma, B.; Xiao, B.; Huang, F.; Huang, P.; Ying, C.; Liu, T.; Wang, Y. Enhanced antitumor effect of combining TRAIL and MnSOD mediated by CEA-controlled oncolytic adenovirus in lung cancer. Cancer Gene Ther. 2016, 23, 168–177. [CrossRef] [PubMed]

214. Ru, Q.; Li, W.; Wang, X.; Zhang, S.; Chen, L.; Zhang, Y.; Ge, Y.; Zu, Y.; Liu, Y.; Zheng, D. Preclinical study of rAAV2-sTRAIL: Pharmacological efficacy, biodistribution and safety in animals. Cancer Gene Ther. 2017, 24, 251–258. [CrossRef]

215. Hu, J.; Wang, H.; Gu, J.; Liu, X.; Zhou, X. Trail armed oncolytic poxvirus suppresses lung cancer cell by inducing apoptosis. Acta Biochim. Biophys. Sin. 2018, 50, 1018–1027. [CrossRef]

216. Micheau, O.; Shirley, S.; Dufour, F. Death receptors as targets in cancer. Br. J. Pharmacol. 2013, 169, 1723–1744. [CrossRef]

217. Tansey, M.G.; Szymkowski, D.E. The TNF superfamily in 2009: New pathways, new indications, and new drugs. Drug Discov. Today 2009, 14, 1082–1088. [CrossRef] [PubMed]

218. Zhu, F.C.; Li, Y.H.; Guan, X.H.; Hou, L.H.; Wang, W.J.; Li, J.X.; Wu, S.P.; Sen Wang, B.; Wang, Z.; Wang, L.; et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectorized COVID-19 vaccine: A dose-escalation, open-label, non-randomised, first-in-human trial. Lancet 2020, 395, 1845–1854. [CrossRef]

219. Samaridou, E.; Heyes, J.; Lutwyche, P. Lipid nanoparticles for nucleic acid delivery: Current perspectives. Adv. Drug Deliv. Rev. 2020, 154–155, 37–63. [CrossRef]

220. Baden, L.R.; El Sahly, H.M.; Essink, B.; Kotloff, K.; Frey, S.; Novak, R.; Diemert, D.; Spector, S.A.; Rouphael, N.; Creech, C.B.; et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N. Engl. J. Med. 2021, 384, 403–416. [CrossRef]