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# Therapeutic applications of TRAIL receptor agonists in cancer and beyond

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ABSTRACT

TRAIL/Apo-2L is a member of the TNF superfamily first described as an apoptosis-inducing cytokine in 1995. Similar to TNF and Fas ligand, TRAIL induces apoptosis in caspase-dependent manner following TRAIL death receptor trimerization. Because tumor cells were shown to be particularly sensitive to this cytokine while normal cells/tissues proved to be resistant along with being able to synthesize and release TRAIL, it was rapidly appreciated that TRAIL likely served as one of our major physiologic weapons against cancer. In line with this, a number of research laboratories and pharmaceutical companies have attempted to exploit the ability of TRAIL to kill cancer cells by developing recombinant forms of TRAIL or TRAIL receptor agonists (e.g., receptor-specific mAb) for therapeutic purposes. In this review article we will describe the biochemical pathways used by TRAIL to induce different cell death programs. We will also summarize the clinical trials related to this pathway and discuss possible novel uses of TRAIL-related therapies. In recent years, the physiological importance of TRAIL has expanded beyond being a tumoricidal molecule to one critical for a number of clinical settings — ranging from infectious disease and autoimmunity to cardiovascular anomalies. We will also highlight some of these conditions where modulation of the TRAIL/TRAIL receptor system may be targeted in the future.



**Contents**



## 1. Introduction

The quest for the so-called "magic bullet" of cancer therapy can be considered one of the oldest and foremost aspirations of the scientific community. During this long and fierce journey, scientists have struggled with financial, ethical, as well as biological obstacles. The work completed over the years has generated a lavish amount of information, including the discovery of novel biochemical pathways that regulate tumor cell growth and anti-tumor molecules, thereby improving considerably the way we currently treat cancer. Strikingly, much of this knowledge has also contributed to the development of strategies for fighting other diseases not related to cancer. No "magic bullet" has emerged so far, and the scientific community agrees that combined therapies are the best strategy to fight cancer and many other diseases.

The discovery of *T*NF-*r*elated *a*poptosis-*i*nducing *l*igand (TRAIL/Apo-2L) was well preceded by the description of *t*umor *n*ecrosis *f*actor

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(TNF)/Lymphotoxin (LT) in the late 1960's and early 1970's (Carswell et al., 1975; Granger & Kolb, 1968; Kolb & Granger, 1968) and cloning of TNF/LT in 1985 (Pennica et al., 1984; Aggarwal & Kohr, 1985). TNF-α is the prototype of a superfamily of proteins that are bioactive as a transmembrane protein and/or in soluble form. Initially, TNF-α was considered by many to be the first "magic bullet" against cancer, since it induced tumor cell death, as its name implies. Soon enough, however, it was realized that the major physiological property of TNF-α was to mediate immune/inflammatory responses, and pharmacological concentrations of TNF-α resulted in dramatic hepatotoxicity and a systemic inflammatory response syndrome (Kimura et al., 1987; Ciesielski & Modzelewski, 1995). The discovery and cloning of Fas (CD95) (Trauth et al., 1989; Yonehara et al., 1989; Itoh et al., 1991) and Fas Ligand (FasL/CD178) (Suda et al., 1993) led to the description of the pro-apoptotic Fas/FasL pathway and rekindled the expectations of finding a physiological "magic bullet" against tumor cells. But once again, disappointment emerged with the findings that the introduction of Fas agonists in mouse models rapidly resulted in acute lethal hepatotoxicity (Ogasawara et al., 1993). In mid-1990's two groups independently described a third member of the TNF family with potent tumoricidal activity, which soon proved to be relatively non-toxic to normal cells and tissues in vivo (Ashkenazi et al., 1999; Walczak et al., 1999). A group at Immunex, led by Raymond Goodwin and Craig Smith, named this protein TNF-related apoptosis-inducing ligand, or TRAIL (Wiley et al., 1995), while the group at Genentech, led by Avi Ashkenazi, called their molecule Apo-2 ligand, or Apo-2L (Pitti et al., 1996).

Since its discovery, numerous reports have provided strong evidence showing that TRAIL plays a major role as a tumor suppressor protein. First, a variety of tumor cell lines exhibit exquisite sensitivity to TRAIL, compared to primary cells (Wiley et al., 1995; Griffith & Lynch, 1998; Walczak et al., 1999). Second, administration of recombinant TRAIL protein (or TRAIL cDNA using a recombinant adenovirus) was extremely effective in eliminating tumor cells in vivo (Walczak et al., 1997, 1999; Ashkenazi et al., 1999; Griffith & Broghammer, 2001). Third, stimulation of a variety of hematopoietic cells, including T cells, NK cells, B cells and monocytes, with types I and II IFN induces TRAIL expression and endows these cells with a potent anti-tumor activity (Zamai et al., 1998; Fanger et al., 1999; Griffith et al., 1999; Kayagaki et al., 1999; Sedger et al., 1999; Smyth et al., 2001; Takeda et al., 2001; Kemp et al., 2003a; Kemp et al., 2004). In addition, neutrophils can release bioactive TRAIL from granule stores upon proper stimulation (Kamohara et al., 2004; Ludwig et al., 2004; Tecchio et al., 2004; Kemp et al., 2005; Cassatella et al., 2006; Simons et al., 2007, 2008). Forth, TRAIL deficiency in mice was associated with increased carcinogen-induced tumorigenesis and metastasis, particularly to the liver (Cretney et al., 2002; Sedger et al., 2002). Fifth, TRAIL expression is down regulated in a variety of human cancers and restoration of TRAIL expression enhances in vitro tumor sensitivity to chemotherapeutic drugs (De Carvalho et al., 2011, 2013).

## 2. TRAIL and TRAIL receptor signaling to apoptosis

TRAIL is a 281 amino acid type II transmembrane protein that shares homology with other members of the TNF superfamily via the so-called *T*NF *h*omology *d*omain (THD), a conserved sequence of approximately 150 residues located at the extracellular, carboxy terminal end of the molecules (Wiley et al., 1995; Pitti et al., 1996). Unlike FasL and TNF-α, TRAIL is widely distributed and constitutively expressed in many tissues, such as small intestine, colon, placenta, and in most cells of the hematopoietic tissue (Wiley et al., 1995). Interestingly enough, murine and human forms of TRAIL are 65% identical at the amino acid level and completely cross-reactive. TRAIL interacts with five different receptors (Fig. 1) that may act as transducers of signaling information into the target cells to induce cell death, or as regulators/decoys to preventing the signaling events that lead to death (Wajant et al., 2002). The TRAIL death receptors (DRs), DR4/TRAIL-R1 and DR5/TRAIL-R2, have an intracellular *death domain* (DD), a homodimerization module responsible for the aggregation of proteins that promote signaling transduction. DR4/TRAIL-R1 (Pan et al., 1997b) and DR5/TRAIL-R2 (Chaudhary et al., 1997; MacFarlane et al., 1997; Pan et al., 1997a; Schneider et al., 1997a; Screaton et al., 1997; Sheridan et al., 1997; Walczak et al., 1997; Wu et al., 1997) are Type I transmembrane proteins with 58% identity. Tissue distribution of DR4/TRAIL-R1 and DR5/TRAIL-R2 mRNA by Northern blot analysis is broad, with expression in most normal human tissues (colon, esophagus, heart, kidney, liver, lung, ovary, pancreas, placenta, prostate, skeletal muscle, small intestine, spleen, stomach, testis, thymus, uterus) (Pan et al., 1997a,b; Walczak et al., 1997). Interestingly, all vertebrates, with the exception of human and chimpanzees, present only one TRAIL death receptor. Thus, the question whether DR4/TRAIL-R1 and DR5/TRAIL-R2 in humans and chimpanzees serve redundant function or have arisen in the need for fundamentally distinct signal transduction pathways and/or biological consequences may ultimately have implications for future development of receptor-specific targeting reagents (van Roosmalen et al., 2014).

In contrast to the TRAIL DRs, DcR1/TRAIL-R3 completely lacks an intracellular tail (suggesting it has no intracellular signaling ability) and is expressed on the cell surface via glycosyl-phosphatidylinositol linkage (Degli-Esposti et al., 1997b; MacFarlane et al., 1997; Pan et al., 1997a; Schneider et al., 1997a; Sheridan et al., 1997; Mongkolsapaya et al., 1998). DcR2/TRAIL-R4, on the other hand, has a truncated intracellular domain missing 52 of the 76 amino acids found in the DDs of DR4/TRAIL-R1 and DR5/TRAIL-R2 (Degli-Esposti et al., 1997a; Marsters et al., 1997; Pan et al., 1998). These two TRAIL-binding proteins are unable to transduce signaling events that lead to cell death, and have subsequently been defined as TRAIL decoy receptors (DcR). Despite being unable to signal for apoptosis, TRAIL ligation of DcR2/TRAIL-R4 does activate NF-κB (Degli-Esposti et al., 1997a). As NF-κB activation has been linked to increased resistance to apoptosis-inducing cytokines, including TRAIL (Beg & Baltimore, 1996; Van Antwerp et al., 1996; Keane et al., 2000; Ravi et al., 2001; Karacay et al., 2004), it is possible that DcR2/TRAIL-R4 protects against TRAIL-induced apoptosis through ligand sequestration and induction of proteins with anti-apoptotic activity. Tissue distribution of DcR1/TRAIL-R3 is much more restricted than DR4/TRAIL-R1 and DR5/TRAIL-R2, with mRNA found only in the heart, kidney, liver, lung, placenta, peripheral blood leukocytes, and spleen (Degli-Esposti et al., 1997a,b; Pan et al., 1997a). DcR2/TRAIL-R4 mRNA, in contrast to DcR1/TRAIL-R3, is found in a much broader range of human tissues, with it expressed in most of the same tissues as DR4/TRAIL-R1 and DR5/TRAIL-R2 (Degli-Esposti et al., 1997a,b; Pan et al., 1998). The tissues where DcR1/TRAIL-R3 mRNA was detected are heavily vascularized, suggesting the possibility the mRNA present was coming from "blood contamination" and the actual organ tissue does not normally express DcR1/TRAIL-R3 mRNA. Interestingly, the genes for all four human TRAIL receptors map to chromosome 8p21 (Degli-Esposti et al., 1997a, b; Walczak et al., 1997), suggesting that they are the result of recent gene duplications. A fifth receptor, osteoprotegerin (OPG), is a soluble protein that interacts with TRAIL with low affinity (Emery et al., 1998), but the in vivo functional relevance of this event remains unclear.

In general terms, the signaling cascade initiated by TRAIL binding to its death receptors is similar to the signal generated after Fas/FasL interaction, and is known as the extrinsic pathway of apoptosis (Fig. 1) (Schulze-Osthoff et al., 1998; Amarante-Mendes & Green, 1999; Barnhart et al., 2003). Binding of TRAIL to either DR4/TRAIL-R1 or DR5/TRAIL-R2 results in receptor trimerization and further aggregation, allowing the recruitment of the death domain-containing protein *F*AS-*a*ssociated *d*eath *d*omain (FADD) to the receptors. FADD has a second domain called *death effector domain* (DED) capable of binding to the DED domains present on caspases-8 or -10. Recruitment of caspase-8 and/or -10 results in the formation of the *d*eath-*i*nducing signaling complex (DISC) and activation a proteolytic signaling cascade. Depending on the cell type, high or low amount of caspase activation