

The Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand and Lung Cancer

Still Following the Right TRAIL?

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Abstract: Tumor necrosis factor-related apoptosis-inducing ligand is a type II membrane-bound protein whose C-terminal extracellular domain shows clear homology to other tumor necrosis factor family members. It is constitutively expressed on macrophages, T cells, natural killer cells, and dendritic cells and selectively kills transformed cells leaving most of the normal cells alone. This selectivity has led to great interest in its use as a therapeutic agent for the treatment of malignancy. In this review, this critical pathway is described, highlighting its mechanistic manipulation for therapeutic benefit and the recent phase I and II trials in lung cancer that have been performed or are currently ongoing are also discussed.

Key Words: TNF-related apoptosis-inducing ligand (TRAIL), Non-small cell lung cancer, Small cell lung cancer, Apoptosis, Clinical trials.

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In 1995, Wiley et al. characterized tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) as a new member of the tumor necrosis factor (TNF) family that induces apoptosis. The protein, designated TRAIL, consists of 281 and 291 amino acids in the human and murine forms, respectively, which share 65% amino acid identity.¹ The TRAIL gene is located on chromosome 3 at position *3q26* and is isolated from other known TNF ligand family members. TRAIL also exists in a soluble form (sTRAIL) generated through enzymatic shedding or released in microvesicles.^{2,3} TRAIL transcripts are detected in a variety of human tissues, most predominantly in spleen, lung, and prostate. Originally, one TRAIL receptor was discovered, which was widely found on transformed and normal cells. Further studies quickly progressed and three more receptors (one death receptor and two decoy receptors) on the cell surface and one soluble receptor named osteoprotegerin were discovered. The

cell surface death receptors (DRs) are designated Trail-DR4 (TRAIL-R1) and DR5 (TRAIL-R2). The decoy receptors are identified as Dc-1 (TRAIL-R3) and Dc-R2 (TRAIL-R4). Osteoprotegerin is a soluble decoy receptor for TRAIL, which exists as a dimer in its active form.⁴

The death receptors have cytoplasmic death domains, which can activate both caspases and nuclear factor kappa-light-chain-enhancer of activated B cells. The decoy receptors have truncated death domains, which do not conduct a stimulating signal and thus divert TRAIL away from the competing functional death receptor. TRAIL is constitutively expressed on macrophages, natural killer cells, T cells, and dendritic cells.⁴

Animal studies have greatly enhanced our understanding of TRAIL. In mice, a single membrane TRAIL death receptor has been identified, which shows the highest sequence homology with human DR5. Like humans, mice have two decoy receptors that do not have transmembrane domains.⁵

In 2002, Cretney et al. and Sedger et al. published the first results from TRAIL-deficient mice. Both studies reported that the mice were viable and fertile, showed no gross phenotype, and did not display any developmental defects indicating that TRAIL has no crucial role in embryonic development.^{5,6} Research on TRAIL-R-deficient mice revealed similar findings.^{7,8} Later work suggested that these mice had a tendency to spontaneous tumor formation and the development of autoimmune disease.⁹

Major roles for the TRAIL/TRAIL-R system were subsequently identified in the immune system. TRAIL has been shown to influence infections and inflammatory diseases while also playing a role in the immune surveillance of tumors and metastasis.⁹ Both full-length cell surface expressed TRAIL and picomolar concentrations of soluble TRAIL rapidly induce apoptosis in a wide variety of transformed cells, but importantly, unlike other members of the TNF superfamily (TNF- α and FAS ligand) that tend to leave normal cells alone. TRAIL's ability to selectively kill transformed cells made it an attractive therapeutic target for disease such as cancer.

TRAIL-induced target cell apoptosis begins with the binding of TRAIL to a death receptor (TRAIL-DR4/5) (Figure 1). The death receptor forms a trimer with two other bound death receptors (these trimers are associated through preligand assem-

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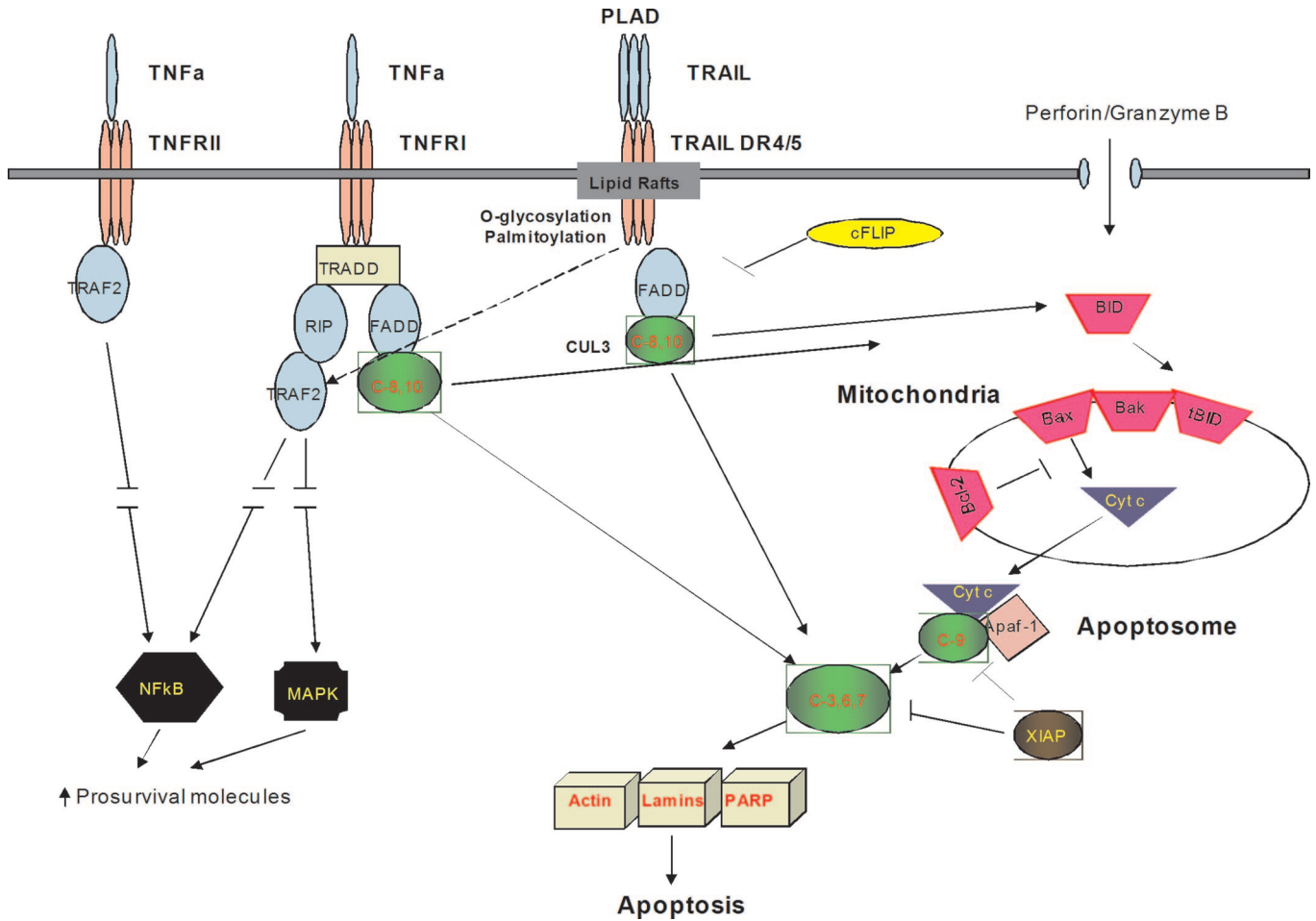


FIGURE 1. TRAIL induces apoptosis of transformed or malignant cells through both the intrinsic (mitochondrial) and extrinsic (death receptor/caspase) pathway. TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

bly domain and preligand-binding assembly domains) leading to the recruitment of FAS-associated death domain to the receptor complex (through DD interactions). *O*-glycosylation of DR4 and DR5 and palmitoylation of DR4 facilitate ligand-induced receptor clustering (extrinsic pathway). This process results in the formation of the death-inducing signalling complex (DISC), which is made up of oligomerized receptors, the adaptor molecule FAS-associated death domain, the initiator proteases caspase-8 and/or caspase-10, and, depending on the context, the cellular FADD-like interleukin-1 beta-converting enzyme-like inhibitory protein (c-FLIP).

The DISC moves into a lipid-rich membrane compartment where caspase-8 comes into contact with a CUL3/Rbx1-based E3 ligase complex, which results in the polyubiquitylation of caspase-8 on its C-terminal region. Polyubiquitylated caspase-8 associates with the ubiquitin-binding protein p62/sequestosome-1, thus promoting the translocation of caspase-8 from the DISC into ubiquitin-rich intracellular foci.¹⁰ This process increases the focal concentration of caspase-8, facilitating its full activation.

c-FLIP, an apoptosis inhibitor, can also be recruited to the DISC, inhibiting caspase-8 recruitment and activation,

exerting an antiapoptotic effect. Caspases-8 and -10 then cleave and activate the effector caspases-3, -6, and -7, which execute the apoptotic death program. Although this process operates effectively in type I cells, amplification of apoptosis in type II cells requires activation of the mitochondrial intrinsic pathway.¹⁰

The intrinsic pathway involves TRAIL receptor binding-induced apoptosis through the cleavage of B-cell lymphoma (BCL)-2 interacting domain by caspase-8, which then translocates to the mitochondrial membrane causing a conformational change in associated X protein (Bax), which along with the Bcl-2 homologous antagonist/killer (Bak) forms a pore in the membrane allowing the release of cytochrome *c*. This step can be inhibited by BCL-2. Cytochrome *c* and apoptotic protease-activating factor 1 form the apoptosome, which in turn recruits procaspase-9, activating it and allowing it to cleave caspase-3 into an active form. Caspase-3 and -9 may be inhibited by X-linked inhibitor of apoptosis protein (XIAP).

TRAIL binding can also activate pro-survival signaling pathway with the recruitment of TNF receptor-associated factor 2, which can activate multiple kinase pathways and

nuclear factor kappa-light-chain-enhancer of activated B cells leading to cell proliferation and to the transcription of anti-apoptotic genes promoting the expression of antiapoptotic proteins such as c-FLIP and XIAP.^{11–13}

Both the intrinsic and extrinsic pathways are closely regulated at different levels, balancing the pro- and antiapoptotic signals. Apoptosis inhibitors such as c-FLIP, BCL-2 family, and XIAP can inhibit TRAIL's function. These inhibitors can be up-regulated in many tumor types rendering the tumors resistant to TRAIL-induced death.^{14,15} Moreover, mutations in the TRAIL death receptors have also been found in tumors including non-small cell lung cancer (NSCLC) rendering them resistant to TRAIL as a therapeutic agent.

Targeting the TRAIL Pathway

The obvious advantage of targeting this pathway is that TRAIL selectively kills tumor cells, leaving normal cells alone.¹⁶ The exact reason for this is still not completely understood but is probably because of a number of factors including increased expression of decoy receptors on normal cells and increased expression of DR4 and DR5 on tumor cells.¹⁷

Importantly, TRAIL-induced tumor killing is independent of protein 53 (p53) status and therefore TRAIL can bypass an inactivated P53 pathway, often a reason for drug therapy resistance.¹⁴

Therapeutic agents directed at the TRAIL pathway have been developed and include recombinant human soluble TRAIL (rhTRAIL), which has a half-life of 20 to 30 minutes, and agonistic monoclonal antibodies directed at DR4 and/or DR5, which have a half-life of 18 to 21 days.^{14,18}

NSCLC seems to be a particularly suitable target for a TRAIL-based therapy. These tumors express large amounts of TRAIL DR4 and 5 in the majority of specimens tested.¹⁹ An inactive P53 pathway also occurs in 50% of these tumors conferring chemo/radiotherapy resistance. TRAIL pathway stimulation allows this p53 pathway to be bypassed.¹⁴ Although the DNA damage-induced caspase-9 activation pathway is disrupted in many patients with NSCLC, the caspase-8 mitochondrial pathway is fully operational allowing TRAIL to effectively kill the tumor cells in these cases.^{20,21}

Nevertheless, preclinical work on NSCLC revealed that the tumors were not always susceptible to TRAIL-mediated killing as first thought. This resistance to TRAIL-mediated death in NSCLC can occur at various levels in the pathway with up-regulation of decoy receptors, c-FLIP, and BCL-2 proteins well described.¹⁵

Further work has revealed that this resistance is overcome when combination therapy with other established or newer agents is introduced.¹⁵

For example, standard chemo- or radiotherapy can thwart this resistance by both p53-dependent and independent mechanisms resulting in up-regulation of TRAIL receptor expression and increased intrinsic pathway efficiency.¹⁵

Alternatively, Bortezomib, a proteasome inhibitor, blocks the activity of antiapoptotic proteins including BCL-XL, XIAP, and c-FLIP at the transcriptional level by inhibiting NF- κ B activity.^{14,15,22,23}

Unfortunately, unlike NSCLC, small cell lung cancer (SCLC) is inherently resistant to TRAIL-mediated killing as these tumor cells lack components of the DISC complex including caspase-8, a phenomenon attributed to methylation of the caspase-8 gene.²⁴ Moreover, Belyanskaya et al.²⁵ demonstrated that TRAIL augmented SCLC cell proliferation and survival through DR5-mediated extracellular signal regulated kinase 1/2 activation.

Approaches to demethylate caspase-8 using interferon- γ have increased SCLC tumor cells to TRAIL-mediated killing.²⁶

Histone deacetylase inhibitors such as valproic acid and trichostatin A have been shown to sensitize tumors to TRAIL-mediated death. In general, these agents elevate death receptors and suppress apoptotic inhibitors.²⁷ In the case of NSCLC, these agents increase TRAIL-induced caspase activity, although their use in SCLC has not been reported.^{28,29}

Other studies investigating TRAIL resistance have shown that the use of TRAIL in combination with etoposide or doxorubicin on SCLC cell lines leads to up-regulation of TRAIL-R2 and down-regulation of c-FLIP.³⁰

Recent Clinical Trials

Phase I and II clinical trials using recombinant TRAIL or agonistic monoclonal antibodies to DR4 or DR5 are running currently.¹⁴ Soria et al.³¹ demonstrated that rhTRAIL in combination with paclitaxel, carboplatin, and bevacizumab had a therapeutic benefit in patients with advanced NSCLC. The primary objective of this study was to determine the maximum tolerated dose of rhTRAIL; the secondary objective was to determine pharmacokinetics of this agent. The sample size was 24 patients. Eligibility criteria included patients \geq 18 years, stage IIIB/IV nonsquamous NSCLC, Eastern Cooperative Oncology Group performance status 0 to 1, normal organ function, no brain metastases, and no prior therapy for NSCLC.³¹ rhTRAIL was administered intravenously (IV) at 4 or 8 mg/kg/d on days 1 to 5, or at 15 or 20 mg/kg/d on days 1 and 2 of each 21-day cycle. This phase Ib study in patients with advanced NSCLC showed no dose-limiting toxicities and an overall response rate of 56%.^{31,32}

A phase II multicenter, open label, randomized study of AMG 951 (rhTRAIL) in subjects with previously untreated stage IIIB/IV NSCLC treated with chemotherapy with or without bevacizumab is currently underway.^{14,32}

Numerous anti-DR5 studies have been performed. Three of these analyzed the use of this monoclonal antibody in the treatment of solid tumors.^{33–35} These studies revealed that lexatumumab (anti-DR5) therapy while having dose-related liver toxicity is generally safe when administered at a dose of 10 mg/kg every 2 weeks.^{14,33–35} Phase II trials with this agent in NSCLC as part of combination therapy with agents such as paclitaxel and carboplatin are ongoing.¹³ These include a multicenter, randomized, double-blind, placebo-controlled trial looking at anti-DR5 therapy in combination with these two agents for first-line treatment of advanced NSCLC. The study is due for completion in 2013.³² Another is a randomized, double-blind, phase II, placebo-controlled trial adding on to a background of effective treatment designed to evaluate the efficacy, safety, and pharmacokinetics of PRO95780 (anti-DR5) combined with paclitaxel +

carboplatin + bevacizumab therapy in patients with previously untreated stage IIIB, stage IV, or recurrent NSCLC. Approximately 120 patients have been randomized to one of two treatment arms, and results are expected later this year.³²

Studies have also been published on the use of monoclonal agonistic anti-DR4 antibodies.^{36–39} The majority of these studies have been phase I clinical trials analyzing the effect of anti-DR4 alone or in combination with chemotherapeutic agents in the treatment of malignancy. Mom et al. enrolled patients with advanced solid tumors to receive gemcitabine 1250 mg/m² IV on days 1 and 8 and cisplatin 80 mg/m² IV on day 1 of each 21-day cycle. Escalating mapatumumab (anti-DR4) doses were administered IV every 21 days. Forty-nine patients received therapy. Adverse events included diarrhea, peripheral neuropathy, and reduced appetite.³⁸

A phase II multicenter study was designed to evaluate the efficacy, safety, and tolerability of mapatumumab in patients with NSCLC previously treated with at least one platinum-based regimen. Each patient received mapatumumab (anti-DR4) at a dose of 10 mg/kg administered IV every 21 days in the absence of disease progression. A total of 32 patients with relapsed or refractory stage IIIB or IV or recurrent NSCLC were enrolled. Patients had received a median of three previous therapeutic regimens (range 1–7). This study published in 2008 demonstrated no response to therapy.³⁷ A phase II, randomized, multicenter, open-label study to evaluate the efficacy and safety of mapatumumab in combination with carboplatin and paclitaxel as first-line treatment in advanced NSCLC has recently been completed but results are not yet available.^{14,32}

Data from the ongoing studies (details of which can be found at www.clinicaltrials.gov) are eagerly awaited as they may have an important impact on future therapeutic strategies in the treatment of malignant lung disease.

The manipulation of the TRAIL pathway offers great therapeutic potential in the treatment of life-threatening illnesses such as lung cancer. Research continues in not only the field of malignancy but also in areas of infection, inflammation, and immunity. Understanding the subtleties of the TRAIL system will help us manipulate potential sites in a pathway that fortunately specifically targets transformed cells leaving most normal cells alone. Understanding the balance between the different TRAIL receptors and intrinsic pathway proteins can only offer further opportunities to render tumors susceptible to this clean agent.

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