
Biological Significance of Apoptosis in Ovarian Cancer: TRAIL Therapeutic Targeting

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Additional information is available at the end of the chapter

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1. Introduction

An apoptotic program is present in almost all cell types. Functional characterization of the apoptotic cascade has revealed how the apoptotic program is activated in response to diverse stresses such as DNA damage, signaling imbalance provoked by oncogene activation, survival factors insufficiency or hypoxia. One of the hallmarks of tumor cells is their ability to resist apoptosis. The concept that apoptosis serves as a barrier to cancer development has been well established (Evan and Littlewood, 1998; Hengartner, 2000; Lowe et al., 2004; Adams and Cory, 2007). This is especially relevant for ovarian cancer (OC) where most patients presenting with advanced OC (most commonly high grade serous OC) will respond to the initial chemotherapy treatment suggesting that most tumor cells present are sensitive to chemotherapy. However, only 10-15% of these patients maintain a complete response to the initial therapy implying that a fraction of the tumor cells escaped apoptosis induced by chemotherapeutic drugs. Thus, one of the main obstacles to an effective treatment in OC is the failure of the initial chemotherapy to eradicate a sufficient number of tumor cells to prevent disease recurrence. Attenuation of apoptosis in those tumor cells contributes to the resistance to subsequent therapy and likely plays an important role in OC progression.

This chapter focuses on the molecular pathways that lead to apoptotic resistance and the need to move towards new targeted treatment in OC. Particular attention will be given to the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling cascade. TRAIL is a cytokine that triggers apoptosis in a wide variety of tumor cells with apparent little effect on normal cells. We will discuss the various mechanisms that OC cells may develop to suppress TRAIL cytotoxicity. Furthermore, we will review the emerging TRAIL-tar-

getting strategies for treating OC and provide information about the latest clinical studies of TRAIL agonists that are being conducted for the treatment of OC.

2. Treatment for ovarian cancer

Because of the limited efficacy of current treatments for advanced OC, novel and more effective therapies are being investigated. An emerging option for the treatment of OC is the targeting of the TRAIL signaling cascade. Because of its unique ability to trigger apoptosis in cancer cells and spare normal cells, in contrast to other cytokines such as FasL and TNF α , TRAIL is an attractive and promising treatment for cancer therapy. Preclinical studies in mice provided the first evidence that the soluble recombinant TRAIL suppresses the growth of human tumor xenografts with no apparent systemic toxicity (Walczak et al., 1999; Ashkenazi et al., 1999). More recently, recombinant TRAIL has entered clinical trials for the treatment of various malignancies (Ashkenazi, 2008; Ashkenazi et al., 2008; Abdulghani and El-Deiry, 2010; Hellwig and Rehm, 2012). In addition to soluble TRAIL, several agonistic antibodies targeting TRAIL R1 or TRAIL R2 death receptors have been developed and entered into clinical trials that included OC patients (Ashkenazi et al., 2008; Hellwig and Rehm, 2012). As for standard chemotherapy, tumor cells have developed various mechanisms to escape the apoptosis induced by TRAIL. This underscores the need to understand the mechanisms of TRAIL resistance, and based on this knowledge, identify and validate novel combinations that could be used with TRAIL to potentiate its therapeutic efficacy. For example, TRAIL resistance has been often associated with overexpression of anti-apoptotic proteins. Therefore, the identification of combination treatments that abrogate anti-apoptotic protein function is promising.

3. Apoptosis overview

Deregulation of the apoptotic cascade not only plays a key role in the pathogenesis and progression of cancer, but also leads to resistance to chemotherapy. There are two major cellular death pathways that transduce the effects of various death inducers: the extrinsic and the intrinsic pathway (Figure 1). The extrinsic pathway is triggered when TRAIL binds to TRAIL R1 or TRAIL R2. Receptor trimerization, along with the subsequent oligomerization and clustering of the receptors, leads to the recruitment of the adaptor protein Fas-associated protein with death domain (FADD). FADD allows the recruitment of the inactive procaspase-8 or -caspase-10 via a shared death effector domain (DED) leading to the formation of the death-inducing signaling complex (DISC). Depending on the cell type, apoptosis activation through the extrinsic pathways may or may not depend on the intrinsic pathway. For example, in type I cells, upon DISC activation, sufficient caspase-8 is activated and, in turn, directly activates the effector caspases (caspase-3, -6, -7) leading to the execution of apoptosis (Abdulghani and El-Deiry, 2010). FLICE-inhibitory protein (c-FLIP) shares structural homology with pro-caspase-8 and possesses a death effector domain that lacks protease

activity. In specific conditions, its structure allows c-FLIP to be recruited to the DISC where it inhibits the processing and activation of pro-caspase-8. Although many isoforms of c-FLIP have been identified, only three are expressed in human cells (Djerbi et al., 2001). They consist of two short variants, c-FLIP_S and c-FLIP_R, and a long splice variant, c-FLIP_L. Both c-FLIP_L and c-FLIP_S contain two DEDs and compete with pro-caspase-8 for association with FADD (Bagnoli et al., 2010). Depending on the level of c-FLIP_L expression, its function at the DISC will vary. When present in high amounts, c-FLIP_L will exert an anti-apoptotic effect at the DISC (Krueger et al., 2001). When present in low amounts, it may heterodimerize with caspase-8 at the DISC and promotes apoptosis (Chang et al., 2002). c-FLIP is thus seen as a major inhibitor of the extrinsic pathway of apoptosis. In so-called type II cells, less caspase-8 is activated at the DISC and efficient apoptosis requires further signal amplification via the intrinsic or mitochondrial pathway. This is achieved by caspase-8-mediated Bid cleavage to generate a truncated form of Bid (tBid) which subsequently engages Bax/Bak to activate the mitochondria.

The intrinsic pathway is usually triggered in response to DNA damage, hypoxia or oncogene overexpression. As a sensor of cellular stress, p53 is a critical initiator of the intrinsic pathway. In response to cellular damage, p53 translocates from the cytoplasm to the nucleus where it promotes the transcription of pro-apoptotic members of the Bcl-2 family. Pro-apoptotic Bcl-2 family members Bax and Bak form pores in the outer mitochondrial membrane causing the release of cytochrome c and other apoptogenic factors such as apoptosis inducing factor (AIF) and SMAC/DIABLO into the cytoplasm. The released of cytochrome c, along with apoptosis protease activating factor-1 (APAF-1) and pro-caspase-9 form the apoptosome. Within the apoptosome, clustered pro-caspase-9 gets activated and cleaves downstream effector caspases, leading to the hallmark of apoptosis (Youle and Strasser, 2008; Brunelle and Letai, 2009). The release of SMAC/DIABLO from the mitochondria promotes apoptosis by binding to and neutralizing members of the family of inhibitor of apoptosis proteins (IAPs), which can block caspase-3 activity through its baculovirus IAP repeat domains. Although the extrinsic and intrinsic pathways are activated by different mechanisms, these two pathways are interconnected (Figure 1). In type II cells, activated caspase-8 cleaves pro-apoptotic Bcl-2 family member Bid to form truncated Bid (tBid), which can then interact with Bax/Bak. This interaction increases the release of cytochrome c from the mitochondria. Thus, Bid provides a connection between extrinsic and intrinsic pathways (so called mitochondrial amplification loop). The reasons that determine whether tumor cells rely on type I or II signaling are not well understood but resistance has been attributed to dysfunction of different steps in the TRAIL-induced apoptosis pathway and/or elevation of survival signals (Zhang and Fang, 2005). In particular, it has been proposed that the levels of c-FLIP and XIAP relative to caspase-8 and SMAC/DIABLO might be important determinants (Kim et al., 2000).

Bcl-2 family proteins are involved in the regulation of apoptosis by controlling mitochondrial membrane permeability. Several studies have demonstrated that these proteins can interact with each other and these interactions can neutralize their pro- or anti-apoptotic functions. The balance between anti- and pro-apoptotic members dictates the fate of cell sur-

vival or death. Pro-apoptotic Bcl-2 members can be divided into 2 groups according to their function and the number of BH domains that they possess. Proteins containing BH domains 1-3 are known as multidomain pro-apoptotic proteins such as Bax, Bak and Bok (Youle and Strasser, 2008). BH-3-only pro-apoptotic proteins such as Bik, Bid, Bad, Bim, Bmf, Noxa, Puma and others can form heterodimers with the multidomain proteins Bax and Bak leading to the activation of the mitochondria. Anti-apoptotic proteins such as Bcl-2, Bcl-XL and Mcl-1 can also form hetero-dimeric interactions with Bax and Bak, thereby neutralizing their pro-apoptotic activity. Anti-apoptotic proteins can form hetero-dimers with BH-3-only proteins and this interaction neutralizes the pro-survival function of anti-apoptotic proteins.

4. TRAIL and its receptors

TRAIL is a member of the TNF ligand superfamily of cytokines and is a type II transmembrane protein, which is anchored to the plasma membrane and presented to the cell surface. The extracellular domain of TRAIL can be shed from the cell surface by cysteine proteases to produce soluble TRAIL. Both the soluble and the membrane-bounded TRAIL can trigger apoptosis by interacting with its cognate death receptors expressed by target cells. Of the five human TRAIL receptors that have been identified, both TRAIL R1 (DR4) and TRAIL R2 (DR5) contain a functional death domain in their intracellular portion, unlike decoy receptors TRAIL R3 (DcR1) and TRAIL R4 (DcR2), which lack a functional death domain and are thus incapable of transmitting an apoptotic signal (Pan et al., 1997a; Pan et al., 1997b; Sheridan et al., 1997; Marsters et al., 1997). Soluble TRAIL also binds with low affinity to soluble osteoprotegerin (OPG), which is a decoy receptor for RANKL that blocks the RANK-RANKL interaction (Hofbauer et al., 2000). OPG negatively regulates osteoclastogenesis and soluble OPG can act as a scavenger for soluble TRAIL and therefore inhibits TRAIL-induced apoptosis (Vitovski et al., 2007).

5. Expression of apoptosis-related proteins in ovarian cancer

Because the susceptibility of tumor cells to apoptosis appears to be determined, at least in part, by the ratio between pro- and anti-apoptotic proteins, the expression pattern of anti-apoptotic proteins, Bcl-2, Bcl-X_L and Mcl-1 has been assessed in OC tissues. For example, higher Bcl-2 expression has been generally associated with a favorable outcome in OC (Henriksen et al., 1995; Herod et al., 1996; Marx et al., 1997; Marone et al., 1998). This apparent paradox may be explained by the observation that high Bcl-2 expression delays cell cycle progression by promoting accumulation of cells in S phases without affecting the rate of apoptosis in OC cells (Bélanger et al., 2005). Bcl-X_L expression is generally higher in OC tissues when compared to normal tissues (Marone et al., 1998) but has not been consistently associated with worse outcome (Shigemasa et al., 2002; Williams et al., 2005). This could be related to the observation that the ability of Bcl-X_L to attenuate apoptosis appears to be cell context-dependent in OC (Dodier and Piché, 2006). In at least one study, increased Mcl-1 ex-

pression has been correlated with poor prognostic for patients with OC (Shigemasa et al., 2002). Elevated expression of c-FLIP_L has been reported in a substantial percentage of OC tissues from patients with advanced diseases (Mezzanzanica et al., 2004; Horak et al., 2005a) and has been associated with adverse outcome in some studies (Ouellet et al., 2007; Bagnoli et al., 2009) whereas others have found no such association (Duiker et al., 2010).

In patients with OC, high TRAIL expression in either tumor or stromal cells is a predictor of overall survival (Lancaster et al., 2003; Horak et al., 2005a). Interestingly in Horak's study, almost 50% of the tumor analyzed expressed elevated level of c-FLIP_L and about 80% of tumors displayed low expression of TRAIL R1 and/or TRAIL R2, which could contribute to protect OC cells from TRAIL-induced apoptosis. Loss of TRAIL expression has been associated with worse outcome (Duiker et al., 2010). Furthermore, this group reported that epigenetic silencing of TRAIL R1 occurred in 8% to 27% OC tumor samples (Horak et al., 2005b). Higher expression of TRAIL receptors in OC cells has been associated with a worse outcome (Ouellet et al., 2007; Dong et al., 2008) but other studies have found no correlation between TRAIL R1 or TRAIL R2 expression and survival (Duiker et al., 2010).

6. Resistance in OC cells

The mechanisms of resistance to TRAIL can be divided into three categories based on their mode of acquisition: intrinsic resistance, acquired resistance and environment-mediated resistance (Goncharenko-Khaider et al., 2012). Each of them will be discussed separately.

6.1. Intrinsic resistance

Intrinsic resistance is observed when tumor cells are resistant to a specific drug without previous exposure to this drug. The incidence of intrinsic resistance to TRAIL among patients presenting with OC is not known but intrinsic TRAIL resistance among OC cell lines and primary OC cells is roughly 50% (Cuello et al., 2001a; Vignati et al., 2002; Siervo-Sassi et al., 2003; Lane et al., 2004). Multiple mechanisms have been described for intrinsic TRAIL resistance in OC cells because the susceptibility to TRAIL-induced apoptosis can be regulated at multiple levels in the apoptotic signaling cascade. The loss of TRAIL R1 expression by epigenetic silencing correlated with resistance to TRAIL-induced apoptosis in OC cells (Horak et al., 2005b). Aberrant methylation of TRAIL receptors has been reported in up to 40% of OC tumors (Shivapurkar et al., 2004). Despite these observations in OC tissues, the levels of TRAIL receptors or decoy receptors do not usually correlate with sensitivity or resistance to TRAIL in OC cell lines (Cuello et al., 2001a; Vignati et al., 2002; Lane et al., 2004). However, the modulation of TRAIL receptors expression may sensitize tumor cells to TRAIL. For example, celestrol-induced upregulation of TRAIL R1 and TRAIL R2 enhances TRAIL-induced apoptosis (Zhu et al., 2010).

As mentioned earlier, c-FLIP is an important modulator of TRAIL sensitivity. Therefore, it is not surprising that c-FLIP overexpression has been associated with intrinsic TRAIL resistance in OC cells. A number of studies have demonstrated that the down-regulation of c-

FLIP_L (through different means) enhances TRAIL-induced apoptosis in resistant OC cells (Lane et al., 2004; Clarke et al., 2007; Syed et al. 2007; Park et al., 2009). In addition, the knockdown of c-FLIP_L inhibited human OC cell lines migratory phenotype in a TRAIL-dependent manner *in vitro* and inhibited the invasion of tumor cells into the peritoneal cavity (El-Gazzar et al., 2010a).

Activation of the PI3K/Akt promotes cell survival and resistance to chemotherapy in OC cells (Fraser et al., 2008; Abedini et al., 2010). The constitutive activation of Akt in OC cell lines and primary tumor cells also promotes resistance to TRAIL (Goncharenko-Khaider et al., 2010). There is a close correlation between the activation of Akt in OC cells and the degree of resistance to TRAIL (Goncharenko-Khaider et al., 2010; Lane et al., 2010). The inhibition of Akt phosphorylation reversed cellular resistance to TRAIL whereas the transfection of Akt in tumor cells with low Akt basal activity enhanced TRAIL resistance (Goncharenko-Khaider et al., 2010). Akt confers resistance, in part, by modulating TRAIL-induced Bid cleavage (Goncharenko-Khaider et al., 2010). The role of Akt in TRAIL resistance among OC cells is also supported by the observation that the inhibition of Akt activation by trastuzumab (Cuello et al., 2001b), an ErbB2 receptor inhibitor, or by a small molecule that inhibits hPEBP4 (Qiu et al., 2010), enhanced TRAIL-induced apoptosis.

TRAIL triggers changes in mitochondrial membrane permeability which results in the release of pro-apoptotic proteins such as cytochrome *c* and SMAC/DIABLO from the mitochondria. In a cohort of 75 patients, Mao et al. demonstrated decreased expression of SMAC/DIABLO and increased expression of XIAP in OC compared to normal ovarian tissues (Mao et al., 2007). However, they observed no difference in SMAC/DIABLO and XIAP expression between TRAIL sensitive and resistant cell lines. To assess the biological relevance of these observations, they stably transfected TRAIL resistant OC cell lines with a SMAC/DIABLO expression vector and showed enhanced TRAIL-induced apoptosis in transfected cells. Similarly, the treatment of TRAIL resistant OC cells with a small molecule SMAC/DIABLO mimic enhanced TRAIL- and TRAIL R1 or R2 agonist-induced apoptosis (Petrucci et al., 2007). Others have found a lack of correlation between XIAP protein expression and TRAIL sensitivity (Goncharenko-Khaider et al., 2010). Furthermore, down-regulation of XIAP in TRAIL resistant OC cells failed to enhance TRAIL-induced apoptosis (Goncharenko-Khaider et al., 2010) suggesting that XIAP is not a major factor contributing to TRAIL resistance in OC.

In summary, intrinsic TRAIL resistance appears to be multi-factorial and can be influenced by the activation of survival pathways such as Akt. In this context, the identification of informative and validated biomarkers of TRAIL resistance will be important for selecting patients and predicting the clinical outcome.

6.2. Acquired resistance

Acquired resistance is a mechanism by which tumor cells that were initially sensitive to a drug adapted to survive to prolonged exposure to this drug. Acquired drug resistance constitutes a major problem in the management of OC. This type of resistance is believed to be caused by sequential genetic alterations in tumor cells often associated with sub-lethal exposure to apoptosis-inducing drugs that eventually result in a therapy-resistant phenotype.

For example, in an OC cell line model, resistance to the anti-TRAIL-R2 antibody TRA-8 was induced by repeated exposure to non-apoptosis-inducing doses of the antibody (Li et al., 2006). Interestingly, the apoptotic responses induced by TRAIL, a TRAIL-R1 agonist antibody (2E12), and other apoptotic stimuli were not impaired. Lane et al. demonstrated that TRAIL acquired resistance was due to a rapid degradation of active caspase-3 subunits by the proteasome in the TRAIL resistant variant OC cells OVCAR3 (Lane et al., 2006). Interestingly, TRAIL resistant OVCAR3 cells remained sensitive to chemotherapeutic drugs.

One reassuring finding of these studies in OC and other in different tumor types is the fact that acquired TRAIL resistance does not confer cross-resistance to chemotherapeutic drugs such as cisplatin. In fact, combining standard chemotherapy with TRAIL treatment appears to be beneficial because treatment with platinum compounds upregulates the expression of TRAIL death receptors regardless of the p53 status which leads to increase apoptosis in OC cells (El-Gazzar et al., 2010b).

6.3. Environment-mediated resistance

Environment-mediated drug resistance (*de novo* resistance) is a form of resistance by which tumor cells are transiently protected from drug-induced apoptosis via the induction of survival signaling pathways (Meads et al., 2009). Soluble factors in the tumor environment may engage cell surface receptor to activate survival pathways. Evidence is accumulating that the tumor environment affects both tumor progression and response to chemotherapy in OC. The accumulation of peritoneal fluid that develops during OC progression, which contains a large mass of the tumor cells, represents a unique form of tumor environment. The floating malignant cells are capable of surviving and proliferating despite lacking immediate proximity to blood vessels presumably due to the permissive attributes of this environment. There are several indirect evidences to suggest that ascites alter drug resistance in tumor cells. Proteomic profiling of tumor cells from ascites before and after chemotherapy showed an increase in the activation of survival pathways such as Akt pathway (Davidson et al., 2006). Moreover, OC ascites attenuate TRAIL and drug-induced apoptosis *in vitro* (Lane et al., 2007; Lane et al., 2010a; Lane et al., 2010b). OC ascites contains significant levels of bioactive lipids such as lysophosphatidic acid (LPA), which exceed levels required to activate LPA receptors (Yamada et al., 2004; Lane et al., 2010a). LPA, one of the ligands of G-protein coupled receptors, has been shown to induce cell survival signaling pathways through different mechanisms including PI3K/Akt activation and regulation of DR4 and c-FLIP (Tanyi et al., 2003; Kang et al., 2004; Ishdorj et al., 2008). Furthermore, LPA inhibits cisplatin-induced apoptosis (Tanyi et al., 2003). The role of LPA, as a component of ascites, in modulating drug resistance in OC cells remains however uncertain. For example, the blockade of LPA cascade did not altered TRAIL-induced apoptosis in OC cells (Lane et al., 2010a) and incubation of OC cells with LPA did not protect them from TRAIL-induced apoptosis (Lane et al., 2010).

A wide variety of cytokines can be measured in OC ascites and interleukin-6 (IL-6) and interleukin-8 (IL-8) are among the most abundant (Giuntoli et al., 2009; Lane et al., 2011; Matte et al., 2012). A number of studies have reported an association between serum lev-

els of IL-6 and prognosis, where elevated levels correlated with a poor relapse-free and overall survival (Plante et al., 1994; Scambia et al., 1995; Tempfer et al., 1997). Interestingly, it was recently shown that elevated ascites levels of IL-6, but not IL-8, were an independent predictor of shorter progression-free survival (Lane et al., 2011). Whether IL-6 is a critical soluble factor in ascites-mediated TRAIL resistance is unclear but a recent study suggests that IL-6 may indeed be an important component of the tumor environment that support tumor growth (Kulbe et al., 2012). Recently, high levels of IL-10, OPG and leptin in ascites were found to correlate with shorter PFS (Matte et al., 2012). Furthermore, in this study, IL-10 neutralizing antibodies attenuated the protective effect of ascites against TRAIL-induced apoptosis suggesting that IL-10 is one of the factors in ascites that promote ascites-induced TRAIL resistance.

The role of integrins in mediating cell proliferation, migration and survival in ovarian cancer is well established (Carreiras et al., 1999; Cruet-Hennequart et al., 2003; Lane et al., 2008). Integrins transmit signals directly through ligation-dependent recruitment of non-receptor tyrosine kinases from the focal adhesion kinase (FAK) leading to the activation of several cell signaling pathways including the PI3K/Akt pathway (Stupack and Cheresch, 2002). Recently, it has been shown that the PI3K/Akt cascade is activated by OC ascites (Lane et al., 2010a). The ability of different ascites to induce Akt phosphorylation in tumor cells strongly correlates with their ability to inhibit TRAIL-induced apoptosis. The PI3K/Akt pathway most likely couples signals from ascites-activated cell surface receptors which regulate the expression and/or phosphorylation of apoptosis-regulating targets. Ascites-induced activation of $\alpha v \beta 5$ integrins leads to focal adhesion kinase (FAK) phosphorylation and FAK induces the activation of Akt (Lane et al., 2010a). This leads to Akt-mediated up-regulation of c-FLIPs expression in ovarian cancer cells (Lane et al., 2007).

Collectively, these data support the role of ascites to promote resistance to TRAIL-induced apoptosis, at least *in vitro*. Whether this is relevant *in vivo* remains unclear for the moment. However, the prosurvival activity of ascites against TRAIL-induced apoptosis has been associated with shorter PFS in women with OC suggesting that ascites-mediated resistance might be clinically relevant (Lane et al., 2010b).

7. TRAIL targeting agents

Different strategies have been used to activate the TRAIL signaling pathway in cancer therapy. A variety of recombinant forms of soluble TRAIL have been developed and fused with different tags (Pitti et al., 1996; Schneider et al., 2000; Ganten et al., 2006). Major limitations however of recombinant soluble TRAIL (rsTRAIL) include the short half-life *in vivo* and relative lack of specificity as rsTRAIL can also bind decoy receptors TRAIL R3 and TRAIL R4. Despite these potential limitations, rsTRAIL (dulcanermin) has entered phase I and phase II clinical trials. Alternatively, various humanized TRAIL receptor agonist antibodies have been developed which target TRAIL R1 (Mapatumumab) or TRAIL R2 (Apomab, Conatumumab, Lexatumumab, Tigatuzumab and LBY-135), and are currently being evaluated clin-

ically (Table 1). These antibodies have a significantly increased half-life and consequently their bioavailability is increased at the tumor site.

Name	Targets	Compagny	Clinical stage development
Apomab/Drozitumab (PRO95780) (human monoclonal antibody agonist)	TRAIL R2	Genetech	Phase II
Conatumumab (AMG 655) (human monoclonal antibody agonist)	TRAIL R2	Amgen	Phase I/II
Dulanermin (rs TRAIL)	TRAIL R1 and TRAIL R2	Amgen/Genetech	Phase I/II
Lexatumumab (HGS-ETR2) (monoclonal antibody agonist)	TRAIL R2	Human Genome Sciences	Phase I
Mapatumumab (TRM-1, HGS-ETR1)	TRAIL R1	Human Genome Sciences	Phase II
Tigatuzumab (CS-1008) (humanized monoclonal antibody agonist)	TRAIL R2	Daiichi Sankyo	Phase I/II
LBY-135 (humanized monoclonal antibody agonist)	TRAIL R2	Novartis	Phase I

Table 1. TRAIL-targeting agents

8. Therapeutic potential of TRAIL agonistic agents in ovarian cancer: Preclinical studies

8.1. Monotherapy

The anti-tumor activity of dulanermin has been extensively evaluated in preclinical models (Ashkenazi et al., 1999; Hylander et al., 2005; Pollack et al., 2001). Furthermore, preclinical *in vitro* studies have demonstrated that OC cell lines displayed variable sensitivity to recombinant human TRAIL (Cuello et al., 2001; Vignati et al., 2002; Siervo-Sassi et al., 2003; Lane et al., 2004). TRAIL-resistant cell lines usually remain sensitive to chemotherapy and conversely, cisplatin-resistant cell lines may be sensitive to TRAIL. Collectively, these results suggest that both platinum-sensitive and platinum-resistant OC are candidates for TRAIL-targeting therapy (Tomek et al., 2004). To increase cancer cell-directed toxicity of TRAIL, fusion proteins of rsTRAIL with target moiety to epidermal growth factor receptor (EGFR) have been developed and were shown to have superior pro-apoptotic activity compared to soluble TRAIL in tumor cells that expressed high levels of EGFR such as the OC cell line OVCAR3 (Bremer et al., 2008).

8.2. Combination therapy

Several studies demonstrated that the combination of TRAIL with cisplatin was more efficient than either molecule alone in various OC cell lines *in vitro* (Cuello et al., 2001; Vignati et al., 2002; Siervo-Sassi et al., 2003; Tomek et al., 2004; Liu et al., 2006). In a mouse model of OC, treatment with rhTRAIL-DR5 or rhTRAIL in combination with cisplatin significantly reduced tumor growth compared to rhTRAIL-DR5 alone (97% and 85% reduction in the combination arms versus 63% reduction in the rhTRAIL-DR5 arm alone) (Duiker et al., 2009). In this study, the beneficial effect of combined treatment was related to the observation that cisplatin strongly enhanced TRAIL R2 surface expression. Similar to cisplatin, proteasome inhibitors and nelfinavir, an HIV protease inhibitor, up-regulate TRAIL R2 and enhance the sensitivity of ovarian cancer cells and tissue explants to an apoptosis-inducing TRAIL receptor antibody (Saulle et al., 2007; Brüning et al., 2008; Brüning et al., 2009; Pasquini et al., 2010). For example, mapatumumab (TRAIL R1 agonist) and lexatumumab (TRAIL R2 agonist) were more efficient than TRAIL to induce apoptosis in primary OC cells and enhanced apoptosis induced by the proteasome inhibitor bortezomid (Pasquini et al., 2010). Using a model of acquired cisplatin resistant cell lines, Duiker et al. showed that cisplatin enhances TRAIL-induced apoptosis in cisplatin-resistant ovarian cancer cells, and induction of caspase-8 protein expression is the key factor of TRAIL sensitization (Duiker et al., 2011). Estes et al. evaluated the cytotoxicity of TRAIL R2 agonist (TRA-8) in nineteen chemotherapy-naïve primary ovarian tumor samples (stage III/IV) (Estes et al., 2007). Using a similar *ex vivo* model, increased cytotoxicity was observed when TRA-8 was used in combination with chemotherapeutic drugs (Frederick et al., 2009). The potential of TRA-8 was further evaluated in a xenograft mouse model of OC (Bevis et al., 2011). When used alone, TRA-8 produced only a modest benefit in terms of tumor growth inhibition. However, animals treated with the combination of carboplatin, docetaxel and TRA-8 demonstrated a better outcome when compared to carboplatin and docetaxel only.

Because TRAIL cytotoxicity in OC cells relies on the activation of both the extrinsic and the intrinsic apoptosis pathways, the combination of TRAIL with pro-apoptotic proteins is of interest. For example, SMAC/DIABLO or LBW242, a SMAC/DIABLO mimic, sensitizes OC cell lines to the antitumor effects of TRAIL and anticancer drugs commonly used in clinic (Mao et al., 2007; Petrucci et al., 2007; Petrucci et al., 2012). These observations suggest that the LBW242 could be of value for the development of experimental strategies for treatment of ovarian cancer. Radicol, an Hsp90 inhibitor, potentiates the apoptotic effect of TRAIL on ovarian carcinoma cell lines by increasing the activation of the caspase-8- and Bid-dependent pathway and the mitochondria-mediated apoptotic pathway, leading to caspase activation (Kim et al., 2012).

The enhanced efficacy of TRAIL in combination with other agents in preclinical models is encouraging and suggests that combination therapies with TRAIL probably represent the best clinical option at this point. Because TRAIL resistance in OC can be induced by various pathways, a combination of molecules that targets critical steps in the TRAIL signaling cascade is likely to be the most efficient approach.

9. Clinical trials with TRAIL targeting agents in OC patients

A large number of phase I/II clinical trials have been undertaken with TRAIL targeting agents either as monotherapy or in combination with chemotherapeutic drugs in a wide range of solid and haematological malignancies (Table 2). For the purpose of this discussion, we have only considered clinical studies with TRAIL targeting agents that included patients with OC.

Name	Status	Clinical stage	Clinical Trials Identifier
Apomab/Drozitumab			
A study of PRO95780 in patients with previously untreated, advanced-stage NSCLC	Completed	Phase II	NCT00480831
A study of PRO95780 in combination with Rituximab in patients with NHL that has progressed following previous Rituximab therapy	Completed	Phase II	NCT00517049
A study of PRO95780 in combination with Cetuximab and Irinotecan chemotherapy or the FOLFIRI regimen with Bevacizumab in patients with previously treated metastatic colorectal cancer	Completed	Phase I	NCT00497497
A study of PRO95780 administered in combination with the FOLFOX regimen and Bevacizumab in patients with previously untreated, locally advanced, recurrent, and metastatic colorectal Cancer	Completed	Phase I	NCT00851136
Conatumumab			
Phase I/II study of Conatumumab and Gemcitabine Hydrochloride followed by Conatumumab, Capecitabine, and 3-dimensional conformal radiotherapy in patients with locally advanced pancreatic cancer	Approved – not yet active	Phase I	NCT01017822
A phase 1b/2 study of AMG 655 in combination with Paclitaxel and Carboplatin for the first-line treatment of advanced NSCLC	Completed	Phase I/II	NCT00534027
Phase 1b/2 study of AMG 655 with mFOLFOX6 and Bevacizumab for first-line metastatic colorectal cancer	Completed	Phase I/II	NCT00625651
Phase 1b/2 study of AMG 655 with Doxorubicin for the first-line treatment of unresectable soft tissue sarcoma	Completed	Phase I/II	NCT00626704

Name	Status	Clinical stage	Clinical Trials Identifier
A study of AMG 655 or AMG 479 in combination with Gemcitabine for treatment of metastatic pancreatic cancer	Completed	Phase I/II	NCT00630552
AMG655/Panitumumab combination in metastatic colorectal cancer study	Completed	Phase I/II	NCT00630786
AMG 655 in combination with AMG 479 in advanced, refractory solid tumors	Completed	Phase I/II	NCT00819169
Phase 2 safety & efficacy of FOLFIRI in combination with AMG 479 or AMG 655 vs FOLFIRI in KRAS-mutant metastatic colorectal carcinoma	Completed	Phase II	NCT00813605
Phase 1b Lymphoma Study of AMG 655 in Combination With Bortezomib or Vorinostat	Completed	Phase I	NCT00791011
Dulanermin			
A study of AMG 951 [rhApo2L/TRAIL] in subjects with previously untreated NSCLC treated with chemotherapy +/- Bevacizumab	Completed	Phase II	NCT00508625
A study of Dulanermin administered in combination with Camptosar®/Eribitux® chemotherapy or FOLFIRI (with or without Bevacizumab) in subjects with previously treated metastatic colorectal cancer	Completed	Phase I	NCT00671372
A study of Dulanermin administered in combination with the FOLFOX regimen and Bevacizumab in patients with previously untreated, locally advanced, recurrent, or metastatic colorectal cancer	Completed	Phase I	NCT00873756
Lexatumumab			
Phase I study of Lexatumumab with or without recombinant interferon gamma in pediatric patients with relapsed or refractory solid tumors or lymphoma	Completed	Phase I	NCT00428272
Mapatumumab Mapatumumab, Cisplatin and radiotherapy for advanced cervical cancer	Active	Phase I/II	NCT01088347
Study of TRM-1 (TRAIL-R1 monoclonal antibody) in subject with relapsed or refractory NSCLC	Completed	Phase II	NCT00092924
Study of TRM-1 (TRAIL-R1 monoclonal antibody) in subjects with relapsed or refractory NHL	Completed	Phase II	NCT00094848

Name	Status	Clinical stage	Clinical Trials Identifier
Study of Mapatumumab in combination with Bortezomib (Velcade) and Bortezomib alone in subjects with relapsed or refractory multiple myeloma	Completed	Phase II	NCT00315757
A Study of Mapatumumab in combination with Paclitaxel and Carboplatin in Subjects With NSCLC	Completed	Phase II	NCT00583830
Study of Mapatumumab in combination with Sorafenib in subjects with advanced hepatocellular carcinoma	Completed	Phase II	NCT01258608
Tigatuzumab			
An imaging and pharmacodynamic trial of CS-1008 in patients with metastatic colorectal cancer	Active	Phase I	NCT01220999
Open-label study of CS1008 for subjects with untreated and unresectable pancreatic cancer	Completed	Phase II	NCT00521404
Combination chemotherapy with CS-1008 to treat ovarian cancer	Completed	Phase II	NCT00945191
CS-1008 with Carboplatin/Paclitaxel in chemotherapy naive subjects with metastatic or unresectable NSCLC	Completed	Phase II	NCT00991796
CS1008- in combination with Sorafenib compared to Sorafenib alone in subjects with advanced liver cancer	Completed	Phase II	NCT01033240
Abraxane with or without Tigatuzumab in patients with metastatic, triple negative breast cancer	Completed	Phase II	NCT01307891
Study of CS-1008 in patients with advanced solid malignancies and lymphomas (without leukemic component)	Completed	Phase I	NCT00320827
Study of CS-1008 in combination with FOLFIRI in patients who have failed other treatments	Completed	Phase I	NCT01124630
Abbreviations: NHL, non Hodgkin lymphoma; NSCLC, non-small cell lung cancer			

Table 2. Active or completed clinical trials with TRAIL targeting agents

TRAIL-based treatment strategies that entered clinical studies have included dulanermin. In a phase I study involving 71 patients with advanced or metastatic solid tumors or non-Hodgkin lymphoma (NHL), dulanermin appeared safe and well tolerated (Herbst et al., 2010). Partial response and stable disease were observed in 3% and 53% of patients respectively in this study. Additional clinical studies with dulanermin in combination with other drugs have been performed most often in patients with lung cancer (Soria et al., 2010; Soria et al., 2011).

Although there have been several published early-phase trials with antibody targeting TRAIL-R1 or TRAIL-R2, only two have included patients with OC. The feasibility of intravenous mapatumumab administration, as a single-agent, has been examined in a phase I pharmacokinetic and biological correlative study in patients with advanced solid malignancies refractory to standard therapy (Tolcher et al., 2007). Of the 49 patients enrolled in the study, two had advanced OC. Mapatumumab dosing ranged from 0.01 to 10 mg/kg and was administered every 2-4 weeks. Overall, mapatumumab was well tolerated and toxicity was generally limited to grade 1-2 events. No objective response was observed for mapatumumab in this unselected phase I study. Hotte et al. evaluated the safety and tolerability of mapatumumab in a phase I clinical trial involving 41 patients with malignant solid tumors refractory to conventional therapy in which 22% of the patients had OC (Hotte et al., 2008). Mapatumumab was administered intravenously every 4 weeks and patients received a median of 2 cycles (range, 1-33) with mapatumumab doses ranging from 0.01 to 20 mg/kg. The patient that received 33 cycles of mapatumumab had a diagnosis of borderline OC. She experienced no cumulative toxicity. Indeed, mapatumumab was generally well tolerated and common adverse events included fatigue, hypotension, nausea and fever. No objective response was observed. Conatumumab (AMG 655), a TRAIL R2-specific antibody is currently being evaluated in patients with advanced refractory solid tumors that includes ovarian tumors in combination with ganitumab, a fully human monoclonal antibody against insulin-like growth factor receptor 1 (National Cancer Institute (NCI) Clinical Trials Identifier Number : NCT00819169).

Of the two studies published with mapatumumab in combination with chemotherapy, one included a patient with OC (primary peritoneal carcinoma) (Leong et al., 2009). A phase II using tigatuzumab (CS-1008), a humanized TRAIL-R2 antibody, in combination with paclitaxel and carboplatin is underway (NCI Clinical Trials Identifier Number: NCT00945191).

10. Conclusions and future directions

The inherent properties of TRAIL or its agonists offer a new targeted therapy for OC. Pre-clinical studies using TRAIL or its agonists have demonstrated the therapeutic potential of these molecules and formed the basis of ongoing phase I/II clinical trials. Although these treatments appear to be clinically well tolerated so far, intrinsic, acquired and environment-mediated resistance may limit the effectiveness of these approaches. However, the development of combination treatments appears to be capable of overcoming, at least in part, some of these limitations. As the search for more effective treatment for OC continues, the morbidity and mortality will hopefully improve. TRAIL treatment strategies have been used so far in the context of salvage treatment and the optimal patient population that will mostly benefit from these treatments remains to be defined. Although significant progress has been made in our understanding of the molecular basis of TRAIL resistance in OC, efforts should continue to further improve this knowledge as this will likely lead to the development of specific biomarkers of resistance and more efficient targeted therapies.

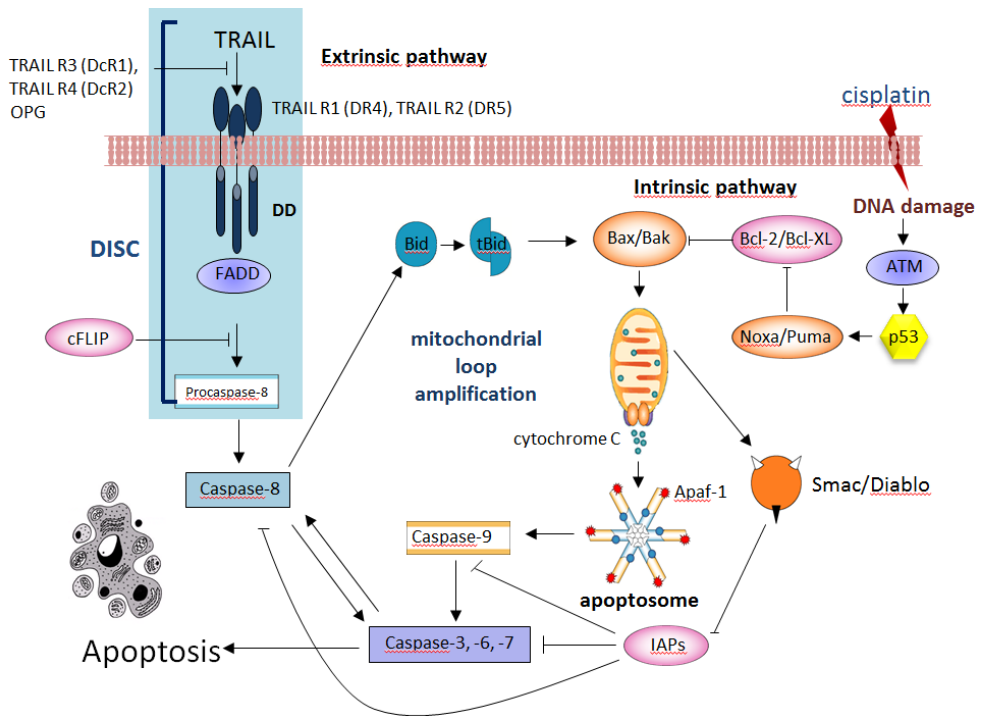


Figure 1. Apoptotic pathways. Binding of TRAIL to death receptors (TRAIL R1, TRAIL R2) leads to the recruitment of the adaptor molecule, FADD. Pro-caspase-8 binds to FADD leading to DISC formation and resulting in its activation. Activated caspase-8 directly activates executioner caspases (caspase-3, -6, and -7) (type I cells) or cleaves Bid (type II cells). Translocation of the truncated Bid (tBid) to the mitochondria promotes the assembly of Bax-Bak oligomers and mitochondria outer membrane permeability changes. Cytochrome c is released into cytosol resulting in apoptosome assembly. Active caspase-9 then propagates a proteolytic cascade of effector caspases activation that leads to morphological hallmarks of apoptosis. Further cleavage of pro-caspase-8 by effector caspases generates a mitochondrial amplification loop that further enhances apoptosis. When FLIP levels are elevated in cells, caspase-8 preferentially recruits FLIP to form a caspase-8-FLIP heterodimer which does not trigger apoptosis. Chemotherapeutic drugs such as cisplatin cause DNA damage which is sensed by the ataxia telangiectasia mutated homolog (ATM) leading to the activation of p53 dependent activation of genes such as PUMA and Noxa which can bind to anti-apoptotic proteins Bcl-2/Bcl-XL thereby opposing their effect. This leads to mitochondrial permeabilization and activation.

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