Abstract

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors are promising targets for the selective eradication of tumor cells while sparing normal cells. Currently, both recombinant TRAIL proteins and TRAIL receptor agonistic antibodies are being tested in the clinic, showing encouraging antitumor activities and mild side effects. Unfortunately, resistance to TRAIL therapy is frequently encountered requiring combined treatments with sensitizing agents. Standard chemotherapeutics can enhance TRAIL sensitivity; however, more specific and less toxic agents are needed to exploit the full antitumor potential of TRAIL. Here, a brief overview of the TRAIL signaling pathway is given together with a short description of early results obtained with TRAIL therapy in the clinic. Mechanisms of TRAIL resistance and ways to overcome these by targeted agents that either neutralize apoptotic blockades or suppress prosurvival signals also triggered by TRAIL are highlighted, such as inhibitors of IAPs, Bcl-2 family members, HDACi, and modulators of NF-κB, Raf and EGFR signaling.

Keywords: TRAIL; Cancer; Apoptosis; Kinases; Therapy

1. TRAIL and its receptors

More than a decade ago, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, Apo2L, TNFSF10) was identified as a powerful activator of programmed cell death or apoptosis in tumor cells while sparing normal cells [1,2]. This feature has greatly spurred research to explore the potential of TRAIL as an anticancer therapy and to the mechanisms underlying its antitumor properties.

TRAIL belongs to the tumor necrosis factor (TNF) superfamily that includes cytokines such as TNF and FasL (CD95L) and is inserted in the cell membrane with its C-terminal domain exposed, indicative of type II membrane proteins. Cells of the immune system such as natural killer cells, T cells, macrophages and dendritic cells express TRAIL. TRAIL (33 kDa) can be processed by cysteine proteases to yield soluble TRAIL (20 kDa) [3] and both forms function as trimers that are able to trigger apoptosis via interaction with TRAIL receptors present in target cells. The TRAIL receptors are predominantly type I membrane proteins having their N-terminal TRAIL-binding domains exposed. Five receptors that bind TRAIL have been identi-
TRAILR1 (DR4, TNFRSF10a) and TRAILR2 (DR5, TNFRSF10b) contain two cysteine-rich extracellular ligand-binding domains and a cytoplasmic region, called the death domain (DD), which is required for activation of the extrinsic apoptotic pathway after TRAIL binding. TRAILR3 (DcR1, TNFRSF10c), TRAILR4 (DcR2, TNFRSF10d) and circulating osteoprotegerin (OPG, TNFRSF11b) lack functional DDs, and are thought to be mainly involved in negatively regulating apoptosis by sequestering TRAIL or stimulating prosurvival signals. TRAIL receptors have different binding affinities for the ligand, TRAIL-R2 showing the highest and secretory OPG the lowest affinity.

The TRAIL receptors have been mapped as a gene cluster to chromosome 8p22-21 [6]. Examination of mRNA expression of the receptors in human tissues revealed a widespread tissue expression for TRAIL-R1,-2, and -4, whereas TRAIL-R3 expression was more restricted with transcripts detectable only in peripheral blood and spleen [6,7]. The precise role and function of the different TRAIL receptors remains to be defined. For example, TRAIL-R1 and TRAIL-R2 initially were thought to be interchangeable for their apoptosis-inducing potential, however, more recently it was found that either one of these receptors can be dominant in death signaling in a cell-dependent fashion. For instance, TRAIL-R1 appears to be dominant in apoptosis activation in B chronic lymphocytic leukemia cells in spite of TRAIL-R2 being more abundantly expressed [8]. Moreover, evidence is mounting that TRAIL receptors, like other TNF receptor family members, are also involved in non-apoptotic functions [9].

The physiological function of TRAIL is largely unclear, its best-characterized activity being immune surveillance against tumors. TRAIL-deficient knockout mice were viable and displayed no developmental defects, but were more susceptible to tumor initiation following carcinogen treatment [10] and demonstrated an accelerated development of hematological malignancies [11]. In addition, also roles for TRAIL were found in normal hematopoiesis and regulation of innate immunity reviewed in [12].

2. TRAIL in apoptosis signaling

The mechanisms underlying apoptosis activation by TRAIL have been studied in most detail. After binding of TRAIL to TRAIL-R1 or -R2 the trimerized receptors recruit several cytosolic proteins that form the death-inducing signaling complex (DISC) (see also Fig. 1). FADD (Fas-associated protein with death domain) is an adaptor protein that binds directly to the intracellular death domain of the receptors where it simultaneously binds the inactive pro-form of caspase-8 or caspase-10 via a shared death effector domain (DED) leading to their activation. Then two different routes causing irreversible cell death can be triggered, one whereby caspase-8 directly activates the effector caspases (caspases-3,-6,-7) leading to the disassembly of the cell, and the other involving the caspase-8-dependent cleavage of the BH3-only protein Bid thus engaging the mitochondrial or intrinsic death pathway. Caspase-10 is dispensable for apoptosis activation and may be involved in the proliferative effect of the death receptors. Cleaved Bid, called truncated Bid (tBid), translocates to the mitochondria where it interacts with other proapoptotic members of the Bcl-2 family, Bax and Bak, that form pores in the outer-mitochondrial membrane causing the release of proapoptotic factors such as cytochrome c and Smac/DIABLO. Cytochrome c together with dATP initiates the assembly of a functional apoptosome consisting of Apaf-1 and procaspase-9 leading to caspase-9 activation and subsequent activation of the effector caspases [13,14]. Smac/Diablo is able to neutralize the caspase-inhibiting activity of inhibitor of apoptosis proteins (IAPs), in particular X-linked IAP (XIAP), thus facilitating the apoptotic process.

Depending on the mode of apoptosis activation cells have been classified into type I and II cells. In type I cells, activation of the extrinsic pathway is sufficient for the execution of apoptosis, whereas type II cells require the death receptor dependent activation of the mitochondrial pathway for full induction of apoptosis [15]. Also TRAIL sensitive cells have been classified into type I and II cells and regulation of Bid cleavage has been proposed as a defining factor in these cells [16].

3. TRAIL in non-apoptotic signaling

TNF receptor family members can apart from apoptosis activation induce to varying extents signaling pathways for inflammation, differentiation and cell survival [17]. Although TRAIL has strong apoptosis activation properties in most susceptible cells, evidence is accumulating for its ability to acti-
vate prosurvival and proliferation signaling mediated by NF-κB, JNK and p38 mitogen-activated protein kinase (MAPK) pathways (for review see Ref. [5]). Molecular determinants of kinase activation by TRAIL have been studied by co-immunoprecipitation experiments showing the involvement of a secondary signaling complex subsequent to the assembly of the primary DISC [18]. This secondary complex contains receptor interacting protein 1 (RIP1), TNF receptor/associated factor 2 (TRAF2), and NEMO/IκKγ in addition to FADD/TNF receptor-associated death domain (TRADD) and active caspase-8, which are also being part of the DISC (see also Fig. 1). RIP1 and TRAF2 trigger JNK activation thus inducing the expression of cell proliferation genes via the transcription factor c-Jun (AP1), whereas NEMO recruits IκKα/β to the signaling complex causing the phosphorylation of the inhibitor of κB (IκB) that subsequently releases NF-κB. Nuclear translocated NF-κB is known to regulate the transcription of several anti-apoptotic genes, including c-FLIP, BCL-XL, Mcl-1 and cIAPs. TRAIL-R4 has a small cytosolic portion containing a truncated DD and has been reported also to activate NF-κB in overexpression experiments in monkey kidney epithelial CVI-EBNA cells, thereby conferring resistance to TRAIL [7]. However, other studies failed to demonstrate NF-κB activating properties of TRAIL-R4 in colon cancer cell lines and instead a p53-dependent enhancement of TRAIL-R4 expression was proposed to inhibit apoptosis induced by TRAIL-R2, whereby TRAIL and the intracellular portion of TRAIL-R4 were dispensable [19]. Thus, the more precise way by which activated TRAIL receptors trigger prosurvival signal transduction cascades remains an important field of study for optimizing TRAIL cancer therapy.

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**Fig. 1.** TRAIL activates apoptosis and pro-survival signaling. Activation of TRAIL-R1 and/ or TRAIL-R2 in the cell membrane induces trimerization and formation of a primary complex (DISC) in lipid rafts leading to apoptosis signaling. DISC-induced caspase-8 activation either directly causes activation of effector caspases 3, 6 and 7 and subsequent apoptosis (type I cells) or triggers apoptosis indirectly via tBid cleavage and activation of the mitochondrial pathway (type II cells). A secondary signaling complex is thought to be formed outside lipid raft structures leading to activation of the transcription factors NF-κB or AP1 and increased expression of anti-apoptotic or proliferation stimulatory proteins. The balance between pro- and anti-apoptotic signaling varies between tumor cell types and TRAIL therapy can be optimized by combined use with agents that prime apoptotic – or suppress pro-survival mechanisms (see text for more details).
It should be noted that ongoing research is revealing additional mechanisms of TRAIL signaling such as the involvement of a lysosomal death pathway [20]. However, the more general involvement of these mechanisms in TRAIL antitumor activity remains to be demonstrated and they are, therefore, not discussed in this short review.

4. Regulation of TRAIL signaling

TRAIL signaling is regulated at different levels throughout the pathway, from receptors to downstream caspases. At the receptor level the decoy receptors have been proposed to act as competitors for TRAIL binding to DR4 and DR5 [4,21], although DcR1 and DcR2 act in different ways. DcR1 acts as a simple competitor, whereas DcR2-mediated inhibition involves the formation of heterologous complexes with DR5, acting more like a “regulatory” than “decoy” receptor [22]. The function of soluble OPG as a TRAIL neutralizing receptor has been questioned under physiological conditions due to its low affinity to TRAIL [23]. However, in cell culture models paracrine or autocrine secretion of OPG was found to promote the survival of cancer cells warranting further research to establish the function of OPG in the tumor microenvironment [12]. Posttranslational modification of TRAIL receptors was recently reported to play a role in TRAIL sensitivity. Death receptor O-glycosylation by the peptidyl O-glycosyltransferase GALNT14 promoted the ligand-induced clustering of DR4 and DR5 and subsequent caspase-8 activation [24]. Furthermore, the expression of GALNT14 mRNA in pancreatic, melanoma and NSCLC cells appeared to correlate with TRAIL sensitivity thus further establishing a relationship between glycosylation and TRAIL signaling.

C-FLIP is the major inhibitor of TRAIL signaling at the DISC level. Being a homolog of caspase-8 c-FLIP is also recruited to the DISC where it competes with procaspase-8 for association with FADD thereby inhibiting apoptosis activation [25]. Interestingly, in recent work c-FLIP recruitment has been linked with prosurvival signaling [26]. It was shown that TRAIL sensitive non-small cell lung cancer (NSCLC) cells assemble DISCs in lipid rafts of the plasma membrane that are specific membrane structures enriched in cholesterol and sphingolipids that in general facilitate receptor-dependent signal transduction. DISCs in the lipid rafts caused the activation of caspase-8, whereas on the other hand non-raft DISC assembly involved the recruitment of c-FLIP and RIP leading to the activation of prosurvival signaling i.e. NF-κB and ERK1/2 activation.

Obvious candidates for playing an important role in TRAIL signaling particularly in type II cells are the Bcl-2 family members. Indeed, several members are known to modulate TRAIL-induced apoptosis. Bax was demonstrated to mediate TRAIL sensitivity, since Bax deficient colon cancer cells were resistant to TRAIL [27]. Overexpression of Bcl-2 inhibits TRAIL-induced apoptosis [28], although it has been proposed that Bcl-2 provides only a partial protection at lower doses of TRAIL and not at higher concentrations [29]. In cholangiocarcinoma cells the selective silencing of various Bcl-2 members by small interfering RNAs identified Mcl-1 as a key sensitizer for TRAIL [30]. Mcl-1 can interact with tBid thereby preventing tBid–Bax/Bak interactions that trigger mitochondrial cell death [31]. Thus, both pro- and antiapoptotic Bcl-2 homologs can facilitate or block TRAIL-induced apoptosis in a cell-dependent manner.

The Myc oncopgenic pathway appears to be an important regulator of TRAIL sensitivity. Initially, Myc was reported to act as a transcriptional repressor of c-FLIP, thus stimulating the assembly of caspase-8 activating DISCs [32]. Then Myc was found to also enhance the expression of DR5 and coupled caspase-8 activation [33], and recently it was shown that Myc interferes with the TRAIL-dependent NF-κB-mediated transcriptional activation of prosurvival genes such as Mcl-1 and cIAP2 [34]. In the latter study, Myc appeared to sensitize cells for TRAIL-induced apoptosis by acting as a transcriptional suppressor of the Mcl-1 and cIAP2 genes by somehow blocking their NF-κB-dependent activation, and Mcl-1 suppression was identified as the key event in sensitization.

5. TRAIL receptor targeting agents

The selective killing of tumor cells by TRAIL has made TRAIL receptors attractive targets for cancer treatment. The cellular and molecular basis for this selectivity is not completely understood. It could be related to the higher expression of the TRAIL receptors in tumor cells, or to the relative high level of decoy receptors in normal cells [35], but may also involve non-functionality of the pathway at more downstream levels. In this context, it could be pro-
posed that oncogenic activation resulting in forced cell cycle progression is connected with the sensitization for death receptor-dependent apoptosis, as was recently shown for Myc and TRAIL sensitivity [34] as discussed above. In line with this, RAS transformation of normal human cells was also reported to convert TRAIL resistance into sensitivity involving the MAPK-dependent up-regulation of TRAIL-R2 and enhanced recruitment and activation of caspase-8 [36].

In preclinical models, recombinant soluble TRAIL has demonstrated impressive anticancer activity. TRAIL potently induced apoptosis in a broad spectrum of human tumor cell lines derived from leukemia, multiple myeloma, and neuroblastoma, as well as lung, colon, breast, prostate, pancreas, kidney and thyroid carcinoma. Importantly, no systemic toxicity was observed in xenograft transplants of breast cancer cells in mice and dose-dependent suppression of tumor growth was observed [37]. However, the formulation of recombinant TRAIL appears to be important for selectivity and antitumor properties. Recombinant TRAIL variants with different tags such as polyhistidine, Flag and leucine zippers, and non-tagged versions have been generated and tested for efficacy and selectivity in preclinical models. Non-tagged versions appeared to have highest tumor selectivity and the addition of zinc was found to further increase TRAIL activity by stabilizing its homotrimeric structure [38]. On the other hand, the different TRAIL-tagged versions inflicted varying levels of cytotoxicity to normal cells, such as primary hepatocytes, astrocytes and keratinocytes of which hepatotoxicity appeared to be the most life-threatening feature, initially raising concerns on the toxicity of TRAIL therapy. However, further studies revealed that hepatotoxicity was only associated with the use of highly aggregated forms of TRAIL (e.g. His-TRAIL and FLAG-TRAIL for review see [39]). The use of TRAIL-R1 or -R2 fully human agonistic monoclonal antibodies (mAbs) is another promising approach. To this date, the fully humanized mAbs HGS-ETR1 (mapatumumab), HGS-ETR2 (lexatumumab) and HGS-TR2J (all three from Human Genome Sciences, Rockville, MD) that target TRAIL-R1 and -R2, respectively, have been most extensively studied. In cell lines and mice models, these antibodies potently induced apoptosis [40,41]. For example, HGS-ETR1 administered intravenously repressed the growth of lung, colon, and renal tumors in athymic mice and combined treatment with 5-fluorouracil or topotecan further enhanced antitumor activity [40]. In primary lymphoma cells, HGS-ETR1 and HGS-ETR2 were able to trigger cell death in approximately 70% of the samples that could be enhanced by chemotherapy [42]. The antitumor activity HGS-ETR1 and HGS-ETR2 in colon cancer cell lines and in xenograft transplantation models could be significantly enhanced by irradiation [43]. An advantage of mAbs compared to soluble TRAIL is that they have high affinity for their targets, thus limiting non-specific binding to decoy receptors or OPG. Furthermore, pharmacokinetic studies in primates and humans showed that the mAbs have a much longer half-life (around 15 days) than recombinant TRAIL (30 min) making them easier to dose and administer [44]. Moreover, apart from activating TRAIL receptor-induced apoptosis the bound mAbs can also recruit immune cells to the tumor site thus providing an additional means for antitumor activity. This was demonstrated in mice in which an agonistic mouse DR5-specific mAb was examined for antitumor effects against syngeneic tumors [45]. Fc-receptor expressing macrophages, natural killer – and dendritic cells were shown to be recruited by the Fc portion of the mAb thus suggesting that activation of innate immune cells may contribute to antitumor activity on top of the direct killing activity of the agonistic antibody.

As an alternative for selective agonistic antibodies, receptor-selective recombinant TRAIL variants have been generated. Phage-display technology allowed the selection for such variants whereby three to six-ligand amino acid substitutions appeared to mediate selectivity [46]. These variants were further optimized and characterized and used to show predominant apoptosis signaling through TRAIL-R1 in lymphoid cancer cells [47]. Computational design strategies have been recently used to generate a TRAIL-R2 selective variant in which two amino acid substitutions were sufficient to create receptor selectivity [48]. Interestingly, this variant was more potent than wild type TRAIL in triggering apoptosis in TRAIL-R2-dependent ovarian cancer cells. Thus, these selective TRAIL variants appear to provide a valuable addition to the TRAIL arsenal although more work is required to further explore their possible superior antitumor properties in preclinical studies.

Finally, gene therapeutic approaches with TRAIL-expressing adenoviral vectors are also being explored. For example, efficient tumor cell killing by
adenoviral-expressed TRAIL has been demonstrated in several tumor cell lines and mice bearing non-small cell lung cancer xenografts [49,50]. While promising, currently the use of adenoviral vectors for cancer treatment has its limitations, such as problems related to viral delivery and poor cell infection efficiencies.

6. TRAIL resistance and strategies for sensitization

TRAIL resistance has been reported in approximately 50% of tested tumor cell lines thus tempering the expectations of monotherapy in the clinic [4]. In a number of tumors, including breast and lung cancer, resistance to TRAIL has been correlated with mutations in TRAIL-R1 and TRAIL-R2 in approximately 10% of the tumors examined [51,52]. Although these mutations may cause a structural failure in TRAIL signaling in a small percentage of tumors, genetic aberrations were found in only one TRAIL receptor leaving the other receptor functional. Other types of resistance that have been identified usually occur in the TRAIL signaling regulatory circuit and can be reverted by combination treatment with agents that modulate the inhibitory signal and/or priming of proapoptotic signals.

In this respect and as mentioned earlier the antitumor properties of TRAIL can be greatly enhanced when used in combination with standard chemotherapeutics as has been demonstrated in many studies using different tumor cell lines and mice models. For example, the combination of recombinant TRAIL and 5-fluorouracil was superior to either therapy alone in inhibiting the growth of established colon cancer cell-derived tumors in mice [53]. Similarly, TRAIL given in combination with paclitaxel or irradiation demonstrated synergistic activity in lung and breast cancer models, respectively [54,55]. The potentiating effect of these treatments on TRAIL antitumor activity has been related to an increase of TRAIL-R1/-R2 levels, reduction of c-FLIP levels, or an apoptosis-priming effect involving the mitochondrial pathway [56,57]. However, care should be taken when combining TRAIL with other therapeutic agents because of possible toxicity to normal tissues of some combinations as illustrated by the finding that TRAIL/cisplatin combination therapy was toxic towards primary hepatocytes in contrast to TRAIL/5-fluorouracil [58].

Interestingly, subtoxic concentrations of chemotherapeutics were found to enhance TRAIL-induced apoptosis. For example, in renal cell carcinoma cells low-dose doxorubicin could enhance apoptosis induced by mapatumumab (HGS-ETR1) by increasing the cell surface expression of DR4 and subsequent caspase activation [59]. Hence, non-toxic chemotherapeutic regimens may be combined with TRAIL-based therapy to enhance its efficacy.

Non-toxic compounds found in natural food products have also been demonstrated to augment the activity of TRAIL. For example, curcumin a polyphenol found in turmeric enhanced TRAIL-induced apoptosis in prostate cancer cells by stimulating both the intrinsic and extrinsic pathways [60]. Resveratrol present in grapes was demonstrated to enhance TRAIL antitumor activity in a panel of cancer cell lines [61] and different mechanisms underlying this enhancement have been reported, including down-regulation of the cell cycle regulator/IAP Survivin, redistribution of death receptors in lipid rafts [62] and reactive oxygen species-dependent activation of the mitochondrial pathway as well as stimulation of caspase-8 activation [63].

7. TRAIL in combination with other targeted agents

The increasing knowledge of the TRAIL signaling pathway has lead to the rationalized use of specific agents directed against potential apoptotic blockades in the pathway or agents that suppress the prosurvival signaling abilities of TRAIL. Below several examples are given for these TRAIL sensitizing strategies. Targeting of antiapoptotic Bcl-2 family members in combination with TRAIL-based therapy has been tested in different tumor cell types. The small molecule Bcl-2 inhibitor HA14-1 [64] was able to restore TRAIL-induced apoptosis in Bcl-2 overexpressing colon cancer cells [65]. The cottonseed-derived phytochemical gossypol, having BH3-mimicking properties and acting as an inhibitor Bcl-2/Bcl-XL, was demonstrated to sensitize for TRAIL-mediated cell death in thoracic cancer cells [66]. As a means to bypass mitochondrial blockades Smac mimicking peptides and small molecules that inhibit XIAP were tested in combination with TRAIL. Overexpression of Smac could sensitize different tumor cell types with high expression levels of Bcl-2 for TRAIL-induced apoptosis, and moreover, Smac peptides could potentiate the antitumor activity of TRAIL in a glioblastoma xenograft model [67]. A small molecule Smac mimic was able to enhance TRAIL-induced cell killing in glioblastoma cells [68].
The proteasome inhibitor bortezomib (PS341, Velcade) has been tested in a considerable number of studies for its TRAIL sensitizing abilities in hematological and solid tumors. The ability of bortezomib to prevent NF-κB activation by inhibiting the proteasome-dependent degradation of IκB was the main rational for combined use with TRAIL. However, it turns out that bortezomib in more than one way enhances TRAIL-induced cell killing, involving stimulation of both the extrinsic and intrinsic death pathway. For example, in NSCLC cells bortezomib enhanced the surface expression of TRAIL-R2, reduced c-FLIP expression, increased the expression of several BH3-only proteins (Noxa, Bim, Bik) as well as Mcl-1 resulting in a netto mitochondria-priming effect, and suppressed NF-κB-dependent prosurvival signaling [69]. Although all these events contribute to sensitization, the bortezomib-dependent effects on TRAIL receptor levels and DISC formation are essential for enhancing apoptosis since overexpression of the caspase-8 inhibitor CrmA and siRNA mediated down-regulation, the bortezomib-dependent effects on TRAIL-R2, blocked the enhancing effect of bortezomib [69,70]. Nonetheless, specific inhibitors of NF-κB signaling including the IKK inhibitor PS-1145 (Millennium Pharmaceuticals, Cambridge, MA) and IκB-kinase 2 inhibitor AS602868 (Merck Serono International SA, Geneva, Switzerland) were main sensitizers for TRAIL-induced apoptosis in pancreatic cancer models [71] and myeloma models [72], respectively, indicating that blocking NF-κB activation in these tumor types was sufficient for TRAIL sensitization. In pancreatic cancer cells PS-1145 and bortezomib had similar sensitizing effects, suggesting that in these cells NF-κB is the primary cause for TRAIL resistance. The TRAIL-induced apoptosis suppressing ability of NF-κB may thus vary in a tumor type-dependent way.

The multikinase inhibitor sorafenib was recently demonstrated to enhance TRAIL-induced apoptosis by inhibiting Raf causing the down-regulation of Mcl-1 [34,73,74]. Two mechanisms for Mcl-1 reduction have been proposed, one in which sorafenib-dependent inhibition of Raf blocks the MEK/ERK1/2 induced activation of NF-κB, and a second where Raf inhibition prevents the translation of the Mcl-1 message via diminished eIF4E phosphorylation, which also has been linked to an observed decrease of cFLIP expression [74]. Tumor xenograft experiments in mice using TRAIL resistant colon cancer cells revealed that combined TRAIL/sorafenib therapy was highly effective in causing tumor regression, making this an interesting drug combination for further evaluation in clinical studies.

The parallel inhibition of survival/proliferation signaling pathways such as the EGFR pathway has also been explored as a potential TRAIL sensitizing therapy. The EGFR-tyrosine kinase inhibitor gefitinib (ZD1839, Iressa) was demonstrated to enhance TRAIL-induced apoptosis in esophageal squamous cell carcinoma likely through the inhibition of the PI3K/Akt survival pathway leading to down-regulation of Bcl-XL and augmentation of the intrinsic pathway [75]. In bladder cancer cells gefitinib reversed TRAIL resistance by suppressing the apoptosis-inhibiting activity of Akt, in particular down-regulation of XIAP expression [76].

Another class of agents that are potent sensitizers of TRAIL are the histone deacetylase inhibitors (HDACi). HDACs together with histone acetyltransferases regulate the acetylation status of histone tails, a posttranslational modification that controls gene expression whereby acetylation increases the accessibility of transcription factors to DNA. The inhibition of HDACs is a promising anticancer strategy and was shown to depend on activation of different apoptosis mechanisms, including activation of the mitochondrial pathway associated with Bid cleavage [77] and by the death receptor pathway via the up-regulation of both ligands (TRAIL, FasL) and receptors (TRAIL-R2/Fas) [78]. When combined with TRAIL, HDACi were shown to strongly enhance apoptosis in hematological and solid tumors in in vitro and in vivo models [79,80].

Thus, an increasing number of possible rationalized TRAIL combination therapies are being tested to enhance the antitumor properties of TRAIL.

8. TRAIL therapy in the clinic

As illustrated above in preclinical models TRAIL therapy holds great promise for the treatment of cancer. However, the proof of the pudding is in the eating, and results of clinical studies have been eagerly awaited. Recently the first reports have appeared in the public domain.

Apo2/TRAIL (Genentech, South San Francisco, CA; Amgen, Thousand Oaks, CA) is currently evaluated in phase I trials. A preliminary report on this study in which fifty-one patients were enrolled and treated with escalating doses (0.5, 1.5, 4, 8 and 15 mg/kg) of Apo2/TRAIL for five consecutive days every 3 weeks did not show dose-limiting tox-
cities [81]. One patient with chondrosarcoma demonstrated a partial response at 8 mg/kg and enrollment for the study is ongoing.

The results of a phase 1 study with mapatumumab have been published recently [82]. Forty-nine patients with advanced solid malignancies were treated with escalating doses of antibody (0.01–10 mg/kg) intravenously administered over 30–120 min every 28 or 14 days. A dose of 10 mg/kg could be safely administered showing only mild toxicity (fatigue, fever and myalgia) with no clinical significant hematological toxicity. No signs of hepatoxicity were observed. Pharmacokinetic analyses revealed a plasma half-life of approximately 18 days. Immunohistochemical analyses of assessable specimens derived from patients detected TRAIL-R1 expression in 68% of the cases, heterogeneous within and between tumors, suggesting that the relevant target was not present in a large portion of this unselected patient population. No objective responses were observed although 19 patients had stable disease of which two beyond 8 months of treatment. A phase 2 study has been initiated with mapatumumab at two dose levels (3 and 10 mg/kg) every 21 days in patients with relapsed or refractory non-Hodgkin’s Lymphoma [83]. Three of the 14 patients with follicular lymphoma had clinical responses, including one complete response (CR). Recently, a phase 1 study with lexatumumab (HGS-ETR2) has been completed in patients with advanced solid malignancies [84]. In dose escalation studies ranging from 0.1 to 20 mg/kg, intravenous administration of lexatumumab for 2 h every 21 days at 10 mg/kg was identified as the maximal tolerated dose showing good tolerability and no toxicity. Also here no responses were observed, although 12 patients (32%) had prolonged disease stabilization with a median of 4.5 months, including most notably 3 patients with metastatic sarcoma showing stable disease for more than approximately 7 months. Furthermore, immunohistochemical analysis of TRAIL-R2 expression in tumor samples could not establish a relationship between receptor expression levels or proportion of tumor cells with positive staining and beneficial effect of treatment.

These phase 1 single agent safety studies do not allow the drawing of conclusions regarding antitumor activity of the antibodies, although it is anticipated based on the results in preclinical studies, that combinations with other agents will greatly enhance antitumor activity. Therefore, the field is anxiously looking forward to learn the results of ongoing follow up studies in which mapatumumab or lexatumumab are combined with chemotherapeutic agents.

9. Conclusions and future perspectives

TRAIL therapy holds promise as a novel selective antitumor therapy, which is supported by early results obtained in clinical studies in a range of advanced cancers of hematological and solid tumor origin revealing mild toxicities and evidence of beneficial therapeutic effects. Whether recombinant TRAIL or TRAIL receptor agonistic monoclonal antibodies will be most effective remains to be demonstrated in ongoing clinical studies and follow-ups. Both approaches have advantages and disadvantages, with antibodies having favorable pharmacokinetic properties and possibly possess additional antitumor activity by recruiting immune cells to the tumor site via antibody-dependent mechanisms, whereas recombinant TRAIL has no preferential binding and will kill both TRAIL-R1 and -R2-dependent tumor cells. The generation of selective recombinant TRAIL variants however has provided an alternative receptor-specific strategy. In this respect, it remains to be elucidated which molecular mechanisms underlie receptor dependency when both receptors are present on the target cells, and whether there are minimal levels of receptor required to trigger efficient apoptosis. Regardless, it will be important to find effective ways to overcome TRAIL resistance that can occur at different and/or multiple levels in the signaling pathway, from TRAIL receptor expression levels to down-stream mechanisms of either apoptosis or pro-survival signaling. Combined treatment with standard chemotherapeutics can enhance TRAIL-induced apoptosis, including low-dose chemotherapy. On the other hand, combinations with targeted agents may be an option to increase the efficacy of TRAIL therapy. Several examples are described in this review, including the use of Smac mimics and Bcl-2 inhibiting agents to sensitize for TRAIL-induced apoptosis or the use of kinase inhibitors like sorafinib and gefitinib to suppress prosurvival signaling. In this respect, it will be important to preselect patients that will mostly benefit from either one of these combination approaches. This will require pin-pointing of the most critical cause of TRAIL resistance in specimens taken from the tumor and subsequent selection of a (targeted) agent.
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