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## Review

# The clinical trail of TRAIL

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## ABSTRACT

The naturally occurring tumour necrosis factor related apoptosis-inducing ligand (TRAIL) induces apoptosis through two death receptors, death receptor 4 (DR4) and death receptor 5 (DR5), that are expressed on the cell membrane. Binding of the ligand to the death receptors leads to activation of the extrinsic apoptosis pathway. Chemotherapy on the other hand stimulates the intrinsic apoptosis pathway via activation of p53 in response to cellular damage. Many cancer cells have mutations in p53 causing resistance to chemotherapy-induced apoptosis. Concomitant signalling through the extrinsic pathway may overcome this resistance. Moreover, enthusiasm for TRAIL as an anticancer agent is based on the demonstration of rhTRAIL-induced selective cell death in tumour cells and not in normal cells. In this review, we provide an overview of the TRAIL pathway, the physiological role of TRAIL and the factors regulating TRAIL sensitivity. We also discuss the clinical development of novel agents, i.e. rhTRAIL and agonistic antibodies, that activate the death receptors.

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## 1. Introduction

In recent years, the development of more selective, tumour-biology driven therapies that possess anti-tumour activity and might prevent or overcome resistance to chemo- and radiotherapy has become a main field of interest within cancer research. Recombinant members of the tumour necrosis factor (TNF) family, including Fas-ligand, TNF and TNF related apoptosis-inducing ligand (TRAIL), can induce apoptosis in preclinical models. Administration of Fas-ligand however, is hampered by induced severe liver toxicity in preclinical studies.<sup>1</sup> TNF is currently administered only for limb salvage by regional limb perfusion in soft tissue sarcoma treatment,

since systemic use induces a sepsis-like syndrome.<sup>2,3</sup> TRAIL is still considered a promising anticancer agent. In preclinical models, it induces apoptosis in a wide range of tumour cells and xenografts, without causing toxicity to normal cells.<sup>4</sup> In this review, we will give an overview of current knowledge on TRAIL biology and the translation of this knowledge into clinical therapies based on TRAIL signalling.

## 2. TRAIL signalling pathway

TRAIL was identified in 1995 based on its sequence homology to FasL/APO1L and TNF.<sup>5</sup> TRAIL is a type II membrane protein, which can be cleaved from the cell surface to form a soluble

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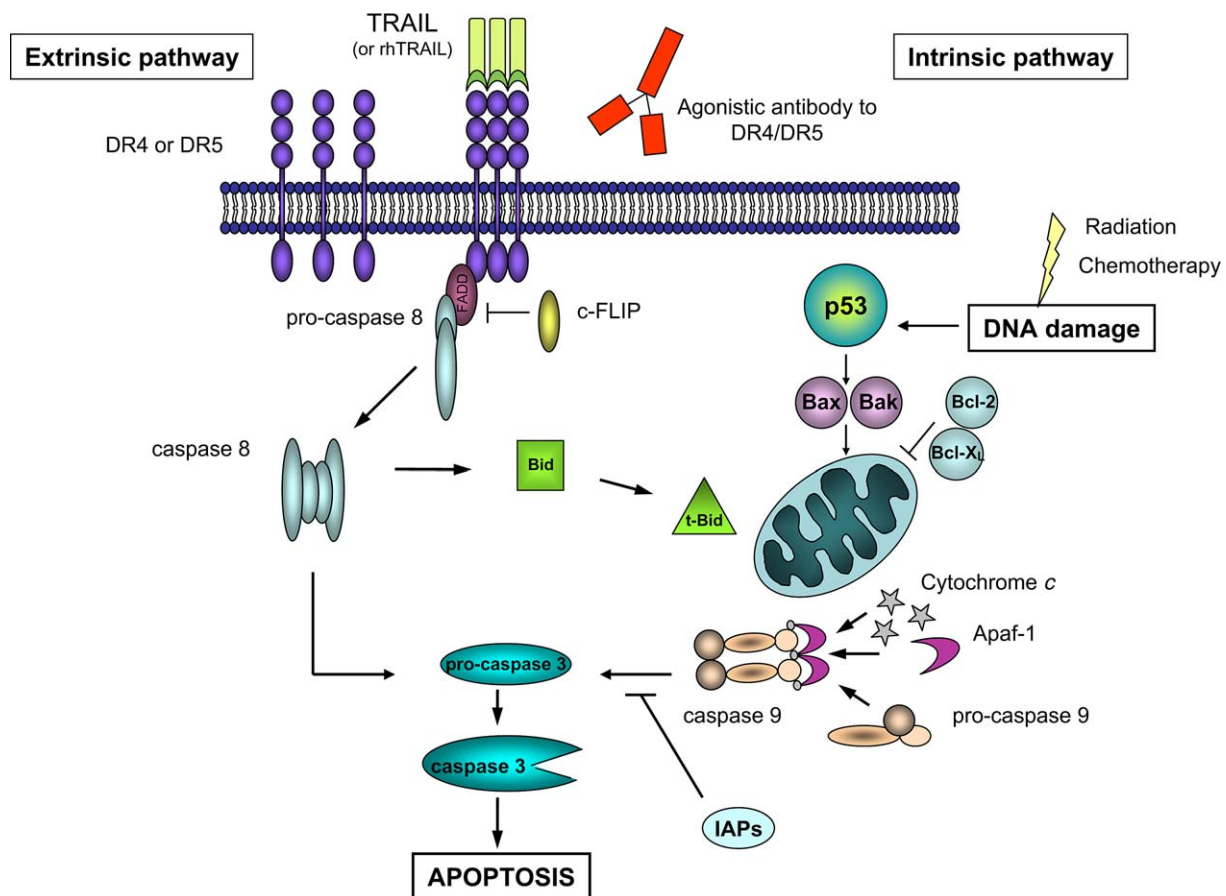
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ligand. Both full-length membrane expressed TRAIL and the soluble ligand can rapidly induce apoptosis in a wide variety of human cancer cell lines.<sup>5</sup> TRAIL can bind to five different receptors: four membrane-bound and one soluble receptor.<sup>6,7</sup> Two of these membrane receptors, death receptor 4 (DR4) and death receptor 5 (DR5), act as agonistic receptors, containing a cytoplasmic death domain through which TRAIL can transmit an apoptotic signal. Two other membrane receptors, decoy receptor 1 (DcR1) and decoy receptor 2 (DcR2), can also bind TRAIL, but may act as antagonistic receptors, lacking an intact death domain. In addition to these four receptors, a fifth soluble antagonistic receptor, osteoprotegerin (OPG) exists. The ability of OPG to act as an antagonistic receptor for TRAIL is disputed, because of its low affinity for TRAIL at 37 °C.<sup>8</sup> The existence of decoy receptors and their widespread expression on normal cells was initially seen as the explanation for protection of normal cells against TRAIL-induced apoptosis. Decoy receptor expression is, however, also present in cancer

cells, without predicting sensitivity or resistance to recombinant human (rh) TRAIL.<sup>9</sup> TRAIL binds as a homotrimer to DR4 and DR5, which results in trimerisation of the receptors (see Fig. 1). This leads to the assembly of a death-inducing signalling complex (DISC). At the DISC, the adaptor protein Fas associated death domain (FADD) acts as a bridge between the death receptor complex and the pro-domain of the initiator caspase 8. Dimerisation of caspase 8 molecules at the DISC leads to the formation of mature caspase 8 that is capable of activating downstream effector caspases such as caspases 3, 6 and 7, which execute apoptosis.<sup>10</sup> This death receptor initiated apoptosis pathway is referred to as the extrinsic apoptosis pathway. Crosstalk exists between the extrinsic pathway and the intrinsic or mitochondria-initiated apoptosis pathway through Bid, a BH3-only protein member of the Bcl-2 gene superfamily, which can be activated by active caspase 8 to trigger mitochondrial perturbation. The intrinsic pathway triggers apoptosis after DNA damage by



**Fig. 1 – Apoptotic pathway.** The death receptor initiated apoptosis pathway is referred to as the extrinsic apoptosis pathway. TRAIL binds as a homotrimer to DR4 and DR5, which results in trimerisation of the receptors and subsequent assembly of a death-inducing signalling complex (DISC). At the DISC, the adaptor protein FADD acts as a bridge between the death receptor complex and the initiator caspase 8. Upon recruitment by FADD, caspase 8 will be activated and then activates downstream effector caspases such as caspases 3, 6 and 7. When the intrinsic apoptotic pathway is activated, pro-apoptotic members of the Bcl-2-gene family translocate to the mitochondria, causing subsequent release of cytochrome c and other mitochondrial factors into the cytosol. In the cytosol, cytochrome c binds the adaptor protein Apaf-1 and pro-caspase 9 in the presence of dATP. It hereby forms the apoptosome signalling complex, in which caspase 9 is activated and can activate subsequently the effector caspases 3, 6 and 7. Crosstalk exists between the extrinsic pathway and the intrinsic or mitochondria-initiated apoptosis pathway through Bid. Activated caspase 8 will cleave Bid, which then translocates to the mitochondria to induce cytochrome c release.

chemo- and radiotherapy, hypoxia, starvation and other kinds of severe cellular stress. When the intrinsic pathway is activated, pro-apoptotic members of the Bcl-2-gene family, such as Bax and Bak, translocate to the mitochondria, causing release of cytochrome c and other mitochondrial factors into the cytosol. In the cytosol, cytochrome c binds the adaptor proteins Apaf-1 and pro-caspase 9 in the presence of dATP. This leads to the formation of the apoptosome signalling complex, in which caspase 9 is activated and can subsequently activate the effector caspases 3, 6 and 7.<sup>7</sup>

### 3. The physiological role of TRAIL

Although mice only express one receptor resembling DR4 and DR5,<sup>11</sup> mouse models have provided important information regarding the expression and physiological role of TRAIL *in vivo*. These models include TRAIL knockout mice, the use of neutralizing anti-mouse TRAIL monoclonal antibodies and soluble recombinant human DR5.<sup>12</sup> TRAIL<sup>−/−</sup> mice are viable and display no apparent haematological or reproductive defects.<sup>13</sup> Expression patterns of TRAIL might reveal an indication to its natural function. While TRAIL mRNA and protein expression is found in a variety of cells and tissues,<sup>5,14</sup> studies in mice and humans show that TRAIL is not expressed at the surface of freshly isolated T-cell, B-cells, monocytes, dendritic cells, natural killer (NK) cells or NKT-cells. Only a subset of mouse NK-cells expresses TRAIL at their surface. After stimulation with interferons most NK-cells, monocytes, peripheral T-cells, and dendritic cells express TRAIL at their surface, suggesting an important role of TRAIL in innate immune responses.<sup>12</sup> In mice in which TRAIL is blocked with neutralizing antibodies and in TRAIL<sup>−/−</sup> mice, an essential role of liver NK-cells in prevention against liver metastases was shown.<sup>13,15</sup> Furthermore, TRAIL contributes to host immunosurveillance against primary tumour development. Neutralisation of TRAIL-promoted tumour development in mice inoculated with the carcinogen methylcholanthrene (MCA).<sup>15</sup> This increased tumour promoting effect of MCA was also observed in TRAIL<sup>−/−</sup> mice.<sup>13</sup> Moreover, the preferential emergence of TRAIL-sensitive fibrosarcoma cells in TRAIL<sup>−/−</sup> mice and IFN $\gamma$ -deficient mice implies an immune selection pressure against TRAIL-sensitive cells during tumour development.<sup>15</sup>

In addition to TRAILs proposed role in tumour immune surveillance, various roles of TRAIL are described in autoimmunity. Most studies describe an inhibitory function of TRAIL on the development of experimentally induced autoimmune diseases in mouse models, such as rheumatoid arthritis, diabetes and experimental autoimmune encephalomyelitis. However, TRAIL may also be involved in the acceleration of autoimmune diseases.<sup>16</sup> Thus, TRAIL may exert dual functions depending on time, extent and location of TRAIL expression. A major task is to translate these results to the human situation and find therapeutic strategies involving TRAIL for the prevention or treatment of autoimmune diseases.

Besides studies performed in mice, several studies report increased serum levels *in vivo* of soluble TRAIL (sTRAIL) in patients with neoplastic, autoimmune and infectious diseases. In a human endotoxemia model, sTRAIL levels increased 10-

fold after the administration of endotoxin.<sup>17</sup> Patients with systemic lupus erythematosus display raised sTRAIL concentrations<sup>18</sup> and in human multiple sclerosis patients sTRAIL levels might serve as a potential response marker for IFN- $\beta$  treatment.<sup>16</sup> These results confirm a physiological role of TRAIL in various immune reactions in the human situation.

### 4. Regulation of TRAIL sensitivity

Sensitivity to TRAIL-induced apoptosis can be modulated at several levels in the apoptosis signalling pathway. Numerous mechanisms to escape apoptosis induction have been described. These mechanisms account for intrinsic or acquired resistance to TRAIL, and since crosstalk exists between the extrinsic and intrinsic pathway some of them may be in part responsible for resistance to conventional therapies as well. Although it is highly likely that these mechanisms play a major role in the difference in sensitivity of tumour cells and normal cells to TRAIL, a uniform mechanism that can explain why normal cells are resistant to TRAIL-induced apoptosis has not yet been found.

Because of the physiological role of TRAIL in tumour surveillance, downregulation or loss of agonistic TRAIL receptors might contribute to a malignant phenotype. Loss of heterozygosity (LOH) of region 8p, which is the region where the TRAIL receptors are mapped, is a frequent event in many cancers.<sup>19</sup> This LOH may facilitate mutations or deletions in the TRAIL receptors, leading to TRAIL resistance. In spite of this, deletions or mutations were found only in small numbers of non-small cell lung cancers, head and neck cancers, gastric cancers, non-Hodgkin lymphomas and breast cancers.<sup>10</sup> Epigenetic changes such as promoter hypermethylation have been described to be responsible for loss of TRAIL receptor expression in neuroblastoma,<sup>20</sup> small cell lung cancer<sup>21</sup> and ovarian cancer,<sup>22</sup> but are not a common phenomenon.

Downregulation of the initiator caspase 8 may be responsible for resistance to apoptotic signalling. Silencing of caspase 8 expression by DNA methylation as seen in neuroblastomas, primary neuroectodermal brain tumours, small cell lung cancer cell lines and retinoblastoma correlated with resistance to rhTRAIL.<sup>23</sup> Suppression of caspase 8 expression was shown to occur during the development of neuroblastoma metastases *in vivo*, and reconstitution of caspase 8 expression in deficient neuroblastoma cells suppressed metastases formation.<sup>24</sup> This confirms the importance of TRAIL in preventing metastases.

Cellular FLICE-like inhibitory protein (c-FLIP) acts as an important intracellular inhibitor of TRAIL sensitivity. c-FLIP is structurally related to caspase 8 and can bind to FADD, but lacks enzymatic activity. It thus prevents apoptosis by blocking association of caspase 8 with the DISC. Downregulation of c-FLIP renders cells sensitive to rhTRAIL. Besides acting as a specific inhibitor of apoptosis, c-FLIP is also involved in mediating growth signals through activation of the NF $\kappa$ B and ERK signalling pathways.<sup>25</sup> Nonetheless, although the importance of c-FLIP as an anti-apoptotic protein is clear, a consistent correlation between c-FLIP expression and rhTRAIL resistance has not been established in cell line models.

Mitochondrial outer membrane permeabilisation (MOMP) and subsequent release of cytochrome c and other proteins play an essential role in the intrinsic apoptotic pathway. Although regulation and execution of MOMP cannot be exclusively assigned to members of the Bcl-2 family, they act as key proteins in the control of MOMP.<sup>26,27</sup> The Bcl-2 family consists of anti-apoptotic members, such as Bcl-2 or Bcl-X<sub>L</sub>, which can block MOMP, and pro-apoptotic members. The latter can be divided into those that directly induce MOMP, such as Bax and Bak, and those that facilitate activation of Bax and Bak, such as Bim, Bad and PUMA.<sup>28</sup> Altered expression of Bcl-2 family members occurs in several tumour types and can be associated with prognosis or treatment response.<sup>29</sup> Overexpression of Bcl-2 or Bcl-X<sub>L</sub> inhibits rhTRAIL-induced apoptosis in, *e.g.* lung, prostate and pancreatic cancer cells, but was not able to block apoptosis in murine embryonic fibroblasts and Jurkat cells.<sup>10</sup> Inactivation of either Bax or Bak does not seem to impair apoptosis greatly, but inactivation of both can dramatically impair apoptosis.<sup>30–32</sup> In mismatch repair deficient colorectal tumours inactivation of Bax only was sufficient to cause resistance to rhTRAIL.<sup>33</sup> These results show that in rhTRAIL-induced apoptosis, the importance of crosstalk between the extrinsic and intrinsic pathway varies between cell types.

The IAP-family of genes has an evolutionary conserved role in apoptosis regulation in animals ranging from insects to humans.<sup>34,35</sup> IAPs are characterised by the presence of one to three baculovirus IAP repeat (BIR) domains, through which they can bind and inactivate caspases. Many IAPs also possess a C-terminal RING domain that enables them to ubiquitylate themselves and other interacting proteins.<sup>36</sup> XIAP is the most potent human IAP member, which can bind and inactivate caspases 3, 7 and 9.<sup>34</sup> cIAP-1 and cIAP-2 have been shown to bind caspases 7 and 9, but this does not seem to lead to inactivation of these caspases.<sup>37</sup> Overexpression of members of the IAP family correlates with survival in many tumour types.<sup>38</sup> Targeting IAPs *in vitro* sensitises many tumour cells to rhTRAIL.

TRAIL sensitivity can also be regulated by activation of pro-apoptotic pathways. The transcription factor NFκB can exert anti-apoptotic effects most likely through upregulation of anti-apoptotic genes, such as c-FLIP, XIAP, cIAP-1, cIAP-2 and Bcl-X<sub>L</sub>. It is known that TNF regulates its own action by activation of NFκB. TRAIL is able to activate NFκB as well. This activation can be mediated through the agonistic receptors DR4 and DR5, as well as the antagonistic receptor DcR2. Interestingly, TRAIL seems to activate NFκB only when its apoptosis inducing capacity is blocked,<sup>39</sup> suggesting another role for NFκB in TRAIL signalling than for TNF. Constitutively active NFκB can prevent rhTRAIL-induced apoptosis in some cases and blocking or interfering with NFκB can sensitise cells to rhTRAIL.<sup>10</sup> Several newly developed drugs, such as proteasome inhibitors and 17-allylamino-17-demethoxygeldanamycin (17-AAG),<sup>40</sup> can augment rhTRAIL-induced apoptosis by inhibition of NFκB.

The PI3K-AKT pathway is one of the key pathways controlling survival, proliferation and growth. It can promote tumour cell survival through direct interference with apoptosis through inactivation of Bax, Bad and caspase 9, upregulation of c-FLIP<sup>41</sup> and stabilisation of XIAP.<sup>42</sup> Furthermore, it can

activate NFκB and increase p53 degradation. Several components of the AKT pathway are deregulated in a wide range of cancers<sup>43</sup> and contribute to resistance to chemotherapy and radiation. Numerous reports describe an inhibitory role of AKT in TRAIL signalling. Downregulation of constitutively active AKT reversed resistance to rhTRAIL.<sup>7</sup>

## 5. RhTRAIL and agonistic antibodies targeting the TRAIL pathway

The unravelling of the TRAIL pathway has resulted in many preclinical studies that have confirmed the potential of rhTRAIL for the treatment of cancer. RhTRAIL induces marked anti-tumour effects in a broad range of tumour cell lines.<sup>44</sup> Substantial anti-tumour activity without systemic toxicity, is demonstrated in mouse xenograft models treated with rhTRAIL as a single agent.<sup>44,45</sup> However, the identification of key factors involved in the regulation of sensitivity to TRAIL has shown that this regulation is highly complex and that tumour cells may present with intrinsic or acquired resistance to rhTRAIL. Fortunately, numerous studies have shown that combinations of rhTRAIL and chemotherapy or radiotherapy show synergistic effects in several human tumour types and can overcome resistance to either of the agents.<sup>4</sup> These data have provided a solid ground for the exploration of the clinical applicability of agents targeting the TRAIL pathway.

The safety profile of rhTRAIL has been evaluated in non-human primates, because of the homology in sequence identity of TRAIL and TRAIL receptors. An 84–99% extracellular protein sequence identity is shared between humans and cynomolgus monkeys and a 97–99% extracellular sequence identity exists between humans and chimpanzees. No toxicity has been observed in cynomolgus monkeys and chimpanzees after administration of rhTRAIL.<sup>44–46</sup> Fas-ligand and earlier recombinant versions of TRAIL could not proceed to the clinic due to liver toxicity. These early variants of TRAIL contained an exogenous sequence tag, for example polyhistidine. Moreover, they were not optimised for zinc content, which is crucial for stability and biologic activity. The tagged TRAIL versions therefore had a tendency to form insoluble aggregates. In preclinical studies they caused apoptosis in normal cells, including hepatocytes, possibly as a result of high-order multimerisation of death receptors. In contrast, administration of the optimised version of rhTRAIL in non-human primates did not induce hepatotoxic effects.<sup>46</sup> Pharmacokinetic studies with rhTRAIL show a half-life of 21–31 minutes in non-human primates. Furthermore, the rhTRAIL clearance is highly correlated to glomerular filtration rate in various species, suggesting that rhTRAIL is primarily eliminated through the kidneys.<sup>45</sup> Based on these results, a phase I study with rhTRAIL (Genentech, San Francisco, CA, USA) has been initiated.

DR4 and DR5 can also be targeted using agonistic antibodies. Upon binding to the receptor these antibodies activate the apoptotic pathway. The potential advantage of this approach is the specific binding to the target receptor: the antibodies bind selectively and with high affinity to their cognate receptor. Furthermore, the half-life of these antibodies is longer



than the half-life of rhTRAIL. Three fully human monoclonal antibodies are under development, one directed at DR4 and two directed at DR5. HGS-ETR1 (mapatumumab; Human Genome Sciences, Rockville, MD, USA) is an agonistic antibody to DR4, whereas HGS-ETR2 and HGS-TR2J (both Human Genome Sciences) are agonistic antibodies to DR5. Compared to HGS-ETR2, HGS-TR2J seems to be a more potent DR5 antibody, because it is more effective at inducing tumour regression in xenograft models. Whereas HGS-ETR2 needs cross-linking reagents for exertion of its apoptotic activity, HGS-TR2J induces apoptosis independently of cross-linking reagents, and is therefore capable of directly activating the apoptotic pathway.<sup>47</sup> Whether the difference between both antibodies will have any implications with regard to efficacy and toxicity profile in humans will become apparent in the clinical studies with these agents.

In preclinical studies, HGS-ETR1 inhibited tumour growth and induced apoptosis in a broad range of tumour cell lines and human tumour mouse xenograft models. Preliminary data from two clinical phase I studies have been reported.<sup>48,49</sup> These dose-escalation studies have been conducted in patients with advanced solid malignancies and non-Hodgkin's lymphoma. They received doses of HGS-ETR1 varying from 0.01 to 20 mg/kg every 28 d. An alternate dose level of 10 mg/kg every 14 d is still being evaluated. HGS-ETR1 has been well tolerated so far and the maximum tolerated dose has not been reached. Stable disease was the best response observed. The mean terminal elimination half-life was approximately 17 d.

Data from three phase II studies with single agent HGS-ETR1 in patients with colorectal cancer, non-small cell lung cancer and non-Hodgkin's lymphoma have recently been presented. In a phase II study in patients with relapsed or refractory non-Hodgkin's lymphoma, the efficacy, safety and tolerability of HGS-ETR1 as single agent were evaluated.<sup>50</sup> Forty patients were enrolled in one of two treatment groups, receiving either 3 mg/kg every 21 d ( $n = 8$ ) or 10 mg/kg every 21 d ( $n = 32$ ). Tumour responses (one complete and two partial responses) were seen in three patients (8%), who were all diagnosed with follicular lymphoma. Stable disease was observed in 12 patients (30%). The antibody was well tolerated, with minimal toxicity observed. Another phase II study was conducted in 32 heavily pre-treated patients with relapsed or refractory non-small cell lung cancer.<sup>51</sup> Patients received 10 mg/kg of HGS-ETR1 every 21 d until disease progression. Stable disease was observed in nine patients (29%) with a median duration of 2.3 months. In a phase II study involving 38 patients with relapsed or refractory colorectal cancer, HGS-ETR1 was administered at a dose of 20 mg/kg every 14 d during cycles 1 and 2, and at 10 mg/kg every 14 d in cycles 3–6.<sup>52</sup> The best response observed was stable disease in 12 patients (32%) for a median of 2.6 months.

Preclinical studies in which HGS-ETR1 was combined with chemotherapy showed an increased cytotoxic effect in human tumour cells and mouse xenograft models. Two phase Ib studies are currently ongoing to evaluate the safety and tolerability of HGS-ETR1 in combination with gemcitabine and cisplatin, and with paclitaxel and carboplatin.<sup>53,54</sup> So far, these combinations have been well tolerated and the maxi-

mum tolerated dose has not yet been reached. Pharmacokinetic analyses show no signs of drug interaction.

HGS-ETR2 showed growth inhibitory effects as single agent in various human tumour xenografts derived from glioma, non-small cell lung cancer, colorectal cancer and breast cancer. This antibody was evaluated in two phase I studies in patients with advanced solid tumours. In the first study, 31 patients have been treated at the dose levels of 0.1–10.0 mg/kg every 14 d.<sup>55</sup> HGS-ETR2 was well tolerated with minimal toxicity. Stable disease was observed in 10 patients (32.3%). In the second study, HGS-ETR2 was administered every 21 d at doses up to 20 mg/kg in 37 patients.<sup>56</sup> At 20 mg/kg, four patients experienced dose-limiting toxicity. One patient in the 20 mg/kg cohort developed acute renal failure, sepsis and elevated AST (grade 4), ALT (grade 4) and bilirubin (grade 3) levels. The renal failure was considered possibly related and the liver function abnormalities were probably related to the study drug. The sepsis was considered not related to HGS-ETR2. The patient died of renal failure. Three additional patients at this dose level developed dose-limiting toxicities, consisting of hyperamylasemia grade 3 ( $n = 2$ ) and grade 4 elevations of AST and ALT. Administration of HGS-ETR2 was safe with minimal toxicity at the maximum tolerated dose of 10 mg/kg. Stable disease was the best response observed in 11 patients (29.7%).

Preclinical studies with HGS-TR2J showed regression or growth inhibition in human cancer cell lines and xenograft models, both as single agent and in combination with several chemotherapeutics. A phase I clinical trial is currently ongoing.

It has to be realised that all these reports on clinical studies are preliminary. Studies on mice and on human immune cells show a physiological role of TRAIL in innate immune responses. However, since many questions regarding the precise role of TRAIL in the human body remain unanswered, administration of external doses of rhTRAIL may lead to unforeseen immunological effects. Thus far, no immunological side-effects have been reported following the administration of agonistic antibodies, but this may not be predictive for the effect of rhTRAIL, since the antibodies are only directed at one of the TRAIL receptors. In addition, because the mechanism of tumour selectivity by rhTRAIL has not been fully explained, possible toxicity to normal tissue needs to be monitored tightly, especially in combinatory regimens of rhTRAIL and other cancer agents.

## 6. Potential of combination therapies

Apart from the use of rhTRAIL and agonistic antibodies as monotherapy, combinations with traditional chemotherapeutics are particularly interesting. Moreover, new compounds that target proteins involved in the TRAIL signalling pathway are under development and they may enhance the effect of rhTRAIL. Belonging to this group of new agents are the histone-deacetylase (HDAC) inhibitors. HDAC inhibitors affect various cellular processes in tumour cells by activating transcription of target genes.<sup>57,58</sup> They induce differentiation, growth arrest and/or apoptosis. HDAC inhibitors can activate both the extrinsic and intrinsic apoptotic pathways. The

combination of HDAC inhibitors and rhTRAIL results in a synergistic apoptotic response in cancer cell lines due to upregulation of DR4, DR5 and pro-apoptotic members of the Bcl-2 family, downregulation of anti-apoptotic Bcl-2 family members, and activation of caspases 3, 9 and 8.<sup>58</sup> Phase I and II studies with several HDAC inhibitors are ongoing.<sup>57</sup>

Other novel drugs that enhance apoptosis when combined with rhTRAIL in human tumour cells are proteasome inhibitors.<sup>59</sup> The proteasome is responsible for the degradation of proteins. Proteasome inhibitors affect multiple processes leading to inhibition of tumour growth and apoptosis. The proteasome inhibitor bortezomib, that is registered for refractory multiple myeloma, inhibits NF $\kappa$ B activation, decreases levels of the anti-apoptotic protein c-FLIP, and induces cell surface expression of DR4 and DR5 in various cells. This may account for the sensitisation of tumour cells to rhTRAIL through proteasome inhibition.<sup>59</sup>

Targeting of anti-apoptotic proteins that are involved in the TRAIL signalling pathway, such as Bcl-2 and the IAPs, is yet another approach to increase sensitivity for rhTRAIL.<sup>60</sup> Bcl-2 can be downregulated by anti-sense therapy and this strategy is under clinical development. An anti-sense oligonucleotide targeting XIAP, the predominant inhibitor of caspases 3, 7 and 9, is currently under investigation in a phase I clinical trial. Also, small molecule inhibitors directed at anti-apoptotic proteins are evaluated in preclinical studies.<sup>61</sup>

The combination of rhTRAIL with therapeutics targeting other pathways may also be of interest. Inhibition of pro-survival routes, including the PI3K/AKT pathway, can be especially interesting. Mammalian target of rapamycin (mTOR) is a kinase that acts downstream of AKT, and is important for the regulation of cell growth and proliferation. Inhibition of mTOR by rapamycin or its analogues results in cell cycle arrest by prevention of progression from G1 to S phase in dividing cells. In phase I and II studies, responses have been observed with a rapamycin analogue in several solid tumours. Preclinical data show an increased sensitivity to rhTRAIL-induced apoptosis when rhTRAIL is combined with rapamycin.<sup>62</sup>

Other promising therapeutic targets are the heat shock proteins (HSP). These chaperone proteins are essential for the proper folding and assembly of proteins, their intracellular transportation and the proteolytic turnover of many of the key regulators of cell growth and survival. HSP90 plays a crucial role in these processes and protects cancer cells from apoptosis through stabilisation of AKT and NF $\kappa$ B. Inhibition of HSP90 leads to cell cycle arrest and apoptosis. The HSP90 inhibitor 17-AAG is currently under clinical investigation in phase I trials. In the preclinical setting, combinations of HSP90 inhibitors with rhTRAIL or with HGS-ETR1 and -ETR2 show synergistic effects on apoptosis.<sup>63,64</sup>

EGFR and HER2, members of the epidermal growth factor receptor family, are involved in proliferation, angiogenesis, invasion and survival of cancer cells. Trastuzumab, the antagonistic HER2 antibody, is used for breast cancer treatment. In preclinical studies, the combination of rhTRAIL and trastuzumab enhances apoptosis in HER2 overexpressing cancer cell lines.<sup>65</sup> Trastuzumab downregulates the HER2 receptor, resulting in decreased activation of the pro-survival AKT pathway, and increased sensitivity for rhTRAIL. RhTRAIL-induced apoptosis is also increased by inhibition of EGFR.<sup>66</sup>

However, because the effect of rhTRAIL on hepatocytes when administered in humans is yet unclear, caution should be exerted when combining rhTRAIL with drugs that may affect the liver.

## 7. Conclusion

Over a decade of TRAIL research, we have acquired considerable knowledge of the TRAIL signalling pathway and of crucial factors involved in its regulation. RhTRAIL has been shown to induce apoptosis in tumour cells and xenografts without inducing toxicity in normal cells. Furthermore, in the preclinical setting numerous combinations of classical chemotherapeutics and targeted drugs with rhTRAIL have shown even more potent anti-tumour effects. Phase I and II studies with rhTRAIL and agonistic antibodies to the TRAIL death receptors are ongoing. Besides providing insights in the efficacy and the possible adverse effects, clinical studies may also lead to an even more profound apprehension of the TRAIL-pathway and may give rise to true tumour-tailored therapy by combining knowledge on the TRAIL signalling pathway with clinical response data and tumour characteristics.

## Conflict of interest statement

E.G.E de Vries is study coordinator of a study with HGS-ETR1. The University Medical Centre Groningen receives the study drug and financial support to perform this study.

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