The TRAIL apoptotic pathway in cancer onset, progression and therapy

Ricky W. Johnstone, Ailsa J. Frew and Mark J. Smyth

Abstract | Triggering of tumour cell apoptosis is the foundation of many cancer therapies. Death receptors of the tumour necrosis factor (TNF) superfamily have been largely characterized, as have the signals that are generated when these receptors are activated. TNF-related apoptosis-inducing ligand (TRAIL) receptors (TRAILR1 and TRAILR2) are promising targets for cancer therapy. Herein we review what is known about the molecular control of TRAIL-mediated apoptosis, the role of TRAIL in carcinogenesis and the potential therapeutic utility of recombinant TRAIL and agonistic antibodies against TRAILR1 and TRAILR2.

A multicellular organism maintains cellular homeostasis in normal tissue compartments and eliminates disordered cells by a controlled cellular mechanism known as apoptosis[1,2]. Apoptosis can be induced by various stimuli, and radiation or chemicals in particular have been used in cancer therapy[3,4]. There are two major signalling pathways that lead to apoptosis in mammalian cells: the intrinsic pathway and the extrinsic pathway. The intrinsic pathway is controlled by pro- and anti-apoptotic Bcl2 family proteins at the mitochondria and has a substantial role in chemotherapy- and radiation-induced cell death. By contrast, the extrinsic death pathway is initiated through apoptotic signal transduction cascades mediated by members of the tumour necrosis factor (TNF) receptor superfamily[3]. In cancer cells, apoptosis induced by the extrinsic pathway complements that induced by the intrinsic pathway, so targeting death receptors is considered a useful new therapeutic approach. The pathway involving TNF-related apoptosis-inducing ligand (TRAIL, also known as APO2L and TNFSF10) and TRAIL receptors (TRAILRs) is most promising, as preclinical models suggest that apoptosis of tumour cells is achievable *in vivo* without lethal toxicities[5–7].

Cancer Immunology Program, Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia. Correspondence to R.W.J and M.J.S.
e-mails: ricky.johnstone@petermac.org; mark.smyth@petermac.org
doi:10.1038/nrc2465

TRAIL and its receptors

TRAIL was originally identified and cloned on the basis of its sequence homology to the extracellular domain of CD95 ligand (CD95L, also known as FASLG) and TNF[8,9]. Like other TNF superfamily members, TRAIL forms homotrimers that crosslink receptor molecules on the cell surface. TRAIL ligates two types of receptors: death receptors triggering TRAIL-induced apoptosis and decoy receptors that possibly inhibit this pathway (BOX 1). TRAIL can also bind to OPG (also known as TNFRSF11B, a soluble inhibitor of RANK ligand) at low affinity[5]. To date, four human receptors specific for TRAIL have been identified: the death receptors TRAILR1 (also known as DR4 and TNFRSF10A) and TRAILR2 (also known as DR5, KILLER and TNFRSF10B), and the putative decoy receptors TRAILR3 (also known as DCR1, TRID and TNFRSF10C) and TRAILR4 (also known as DCR2 and TNFRSF10D)[5]. Only one death-inducing receptor has been identified in mice (DR5 or TRAILR2), and this shares sequence homology with human TRAILR1 and TRAILR2 (REF. 10). Two decoy receptors (DCTRAILR1 or DCR1, and DCTRAILR2 or DCR2) have also been characterized in mice[11]. Human TRAILR3 and mouse DCR1 are glycosylphosphatidylinositol-anchored membrane proteins. Human TRAILR4 contains a truncated, non-functional death domain, and mouse DCR2 can be expressed as two alternatively spliced variants, a secreted form (DCR2S) and a transmembrane form (DCR2L).

TRAIL signalling pathways and sensitivity

Apoptotic signalling. Binding of TRAIL or agonistic monoclonal antibodies (mAbs) to TRAILR1 or TRAILR2 results in receptor oligomerization on the cell membrane and initiation of apoptosis (see REF. 12 and references therein) through the recruitment of the FAS-associated protein with death domain (FADD) to death domain motifs in the carboxyl terminus of the receptors. FADD then recruits membrane-proximal caspases (caspase 8 or caspase 10) through its death effector domain (DED). The formation of this multi-protein complex, designated the death-inducing signalling complex (DISC), allows autoactivation of the recruited caspases (FIG. 1). The canonical view of apoptotic signalling downstream of activated caspases 8 and 10 is that caspase 3 is then targeted for

At a glance

- Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a potent stimulator of apoptosis, and tumour cells are significantly more sensitive to TRAIL-induced apoptosis than normal cells. Although the molecular basis for the tumour-selective activity of TRAIL remains to be fully defined, the TRAIL pathway is an attractive therapeutic target for the treatment of cancer.
- In addition to triggering a pro-apoptotic signal through activation of caspases, TRAIL can activate diverse intracellular signalling pathways involving NFκB, phosphoinositoide 3-kinase (PI3K) and mitogen activated protein kinase (MAPK) family proteins that can stimulate cell survival and proliferation.
- TRAIL is an important immune effector molecule in the surveillance and elimination of developing tumours. Moreover, genetic lesions in various components of the TRAIL pathway have been found in human tumour samples, suggesting that inactivation of the TRAIL pathway and/or escape from TRAIL-mediated immunosurveillance might have an important role in tumour onset and progression.
- In preclinical trials, recombinant forms of TRAIL and agonistic anti-TRAIL receptor antibodies can have single-agent activity against TRAIL-sensitive tumour cells *in vitro* and *in vivo*. These agents can synergize with chemotherapeutic drugs and novel molecular therapeutic agents to more effectively kill TRAIL-sensitive tumour cells and TRAIL-resistant tumours.
- Early-phase clinical trials using recombinant TRAIL and agonistic anti-TRAIL receptor antibodies indicate that these agents can be delivered safely and are generally well-tolerated. Although some objective anti-tumour responses have been reported with these agents as monotherapies, they probably hold greater promise for further clinical development when used in combination with other cancer treatments.

proteolytic cleavage, and activated caspase 3 in turn cleaves numerous cellular proteins, resulting in the biochemical and morphological hallmarks of apoptosis (see REFS 12,13 and references therein). However, Bcl2 homology domain 3-interacting domain death agonist (BID) is also a target for active caspase 8. Cleaved BID (tBID) activates the intrinsic apoptotic pathway by binding to BAX, Bcl2-homologous antagonist/killer (BAK) or pro-survival Bcl2 family proteins[14] and serves to amplify the death receptor apoptotic signal (FIG. 1). Depending on the cell type, cleavage of BID may function as a primary mechanism of TRAIL-induced apoptosis or may serve to amplify the apoptotic response by mediating the simultaneous activation of the extrinsic and intrinsic apoptotic pathway[15–21].

Alternative signal transduction pathways mediated through TRAIL receptors. Depending on the cell type, the relative strength and duration of the ligand signal, and the presence, absence or activation state of intracellular proteins that signal downstream of TRAIL receptors, treatment with TRAIL or agonistic mAbs may stimulate apoptosis or, more rarely, cell proliferation[22–24]. Indeed it is conceivable that TRAIL may simultaneously induce multiple intracellular signal transduction pathways that involve proteins such as nuclear factor κB (NFκB) (BOX 2), mitogen activated protein kinases (MAPKs, including extracellular signal-regulated kinases (ERKs), JUN N-terminal kinases (JNKs) and p38), phosphoinositide 3-kinase (PI3K) and Akt (see REF. 12 and references therein, and BOX 3). Although there is considerable debate as to the make-up of the protein complexes that are necessary for TRAIL-mediated activation of these 'alternative' signalling pathways, it appears likely that various combinations of FADD, TNF receptor type 1-associated DEATH domain protein (TRADD), CASP8 and FADD-like apoptosis regulator (CFLAR, also known as c-FLIP), caspases 8 and 10, TNF receptor-associated factor 2 (TRAF2), NFκB essential modulator (NEMO) and RPA-interacting protein (RIP) are involved, possibly in a cell type-dependent manner (FIG. 2). Engagement of one or more of these pathways in a manner that dominates over the pro-apoptotic signal may have dramatic effects on the physiological or therapeutic activities of TRAIL or agonistic anti-TRAILR mAbs.

TRAIL receptor modification and localization. In trying to identify the molecular events that underpin sensitivity of a given cell to TRAIL-mediated apoptosis, most studies have focused on mechanisms involving decreased stimulation of TRAILR1 and/or TRAILR2 by decoy receptors (BOX 1), or suppression of the intracellular apoptotic signalling cascade (BOXES 2,3). However, a recent exciting report indicates that post-translational modification of TRAILR1 and TRAILR2 may have an important role in determining TRAIL sensitivity. Wagner and colleagues used microarray-based gene expression profiling of over 100 human tumour cell lines to identify gene signatures that correlate with TRAIL sensitivity and resistance. This whole-genome screen indicated that genes encoding enzymes that initiate and carry out *O*-glycosylation, such as *GALNT14*, *GALNT3*, *FUT6* and *FUT3*, were significantly over-represented in TRAIL-sensitive cell lines[25]. There is convincing evidence that TRAILR1 and TRAILR2 are *O*-glycosylated, and inhibition of this post-translational modification by pharmacological and genetic (using small interfering RNA (siRNA)-mediated knockdown of *GALNT14* or *FUT6*) methods suppressed TRAIL-mediated apoptosis[25]. *O*-Glycosylation of TRAILR1 and TRAILR2 did not affect the expression of the death receptors but did enhance ligand-mediated receptor clustering and subsequent DISC formation and caspase 8 activation. This landmark study provides novel insight into the molecular processes that might regulate TRAIL activity and raises the possibility that the expression of *GALNT14, GALNT3, FUT6* and *FUT3* and the glycosylation status of TRAILR1 and TRAILR2 could serve as biomarkers for sensitivity to TRAIL-mediated apoptosis.

There is evidence that signal transduction mediated by death receptors such as CD95 (also known as TNFRSF6) and TNFR1 (also known as TNFRSF1A) can be regulated by the localization of these proteins to cholesterol- and sphingolipid-rich lipid rafts within the plasma membrane[26,27]. Recently, it was shown that ligation of TRAILR1 or TRAILR2 localized to lipid rafts induces a pro-apoptotic signal mediated by caspase 8 activation following DISC formation, but TRAIL receptors not associated with lipid rafts mediate the activation of NFκB, ERK1 and ERK2 (REF. 28). Moreover, knockdown of CFLAR by siRNA resulted in the re-localization of the TRAIL DISC to lipid rafts from non-lipid-raft

Box 1 | **Decoy receptors**

TRAILR3 (tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor 3)[130,160,161] and TRAILR4 (REF. 162) can bind TRAIL but are deficient in signalling to caspase 8 and were initially thought to act as decoys to inhibit the apoptotic signal through TRAILR1 and TRAILR2. Indeed, initial observations that *TRAILR3* and *TRAILR4* mRNA appeared to be preferentially expressed in normal cells compared with tumour cells, and that overexpressed TRAILR3 or TRAILR4 inhibited TRAIL-induced apoptosis[130,160,162], indicated that the relative expression of pro-apoptotic and decoy TRAILRs could regulate the sensitivity of cells to the ligand. However, subsequent studies using monoclonal antibodies (mAbs) specific for the different receptors found no correlation between the levels of TRAILR3 or TRAILR4 and relative sensitivity of cells to TRAIL[25,163]. Recent data indicate that the initial model for the regulation of TRAIL-mediated apoptosis, based merely on the relative expression of TRAILR1 and TRAILR2 versus TRAILR3 and TRAILR4, which compete for binding to TRAIL, may have been too simplistic. Indeed, it has been shown that rather than competing for binding to TRAIL, TRAILR4 can suppress TRAILR2-mediated apoptosis by forming a ligand-independent protein complex. The subsequent TRAILR2–TRAILR4 heterocomplex is deficient in mediating a robust death signal[164]. Another study similarly demonstrated that formation of a TRAILR2–TRAILR4 heterocomplex correlates with reduced TRAIL-mediated apoptosis, but in this instance heterocomplex formation was ligand-dependent[165]. It is therefore still not clear whether TRAILR3 and/or TRAILR4 truly regulate the activity of TRAIL in a physiological or pathological situation and, if they do, how this occurs.

membrane concomitantly with increased cleavage and activation of caspase 8 and subsequent apoptosis. Exactly how CFLAR directs ligated TRAILRs to non-lipid-raft domains is unclear, and whether this occurs *in vivo* has not been determined.

The role of TRAIL in regulating tumorigenesis

The TRAIL–TRAILR pathway has been proposed to regulate different physiological processes such as haematopoiesis[29] and T-cell activation and survival[30], as well as a number of pathophysiological conditions including asthma[31], autoimmune diseases[32,33], diabetes[34], inflammation[35] and excessive host immune responses in bacterial meningitis[36]. Although TRAIL-mediated suppression of inflammation might correlate with suppression of tumour development[37], this link remains to be proved. By contrast, there is strong experimental evidence that the TRAIL pathway has a direct role in the regulation of tumour onset and development. As detailed below, TRAIL may be a key effector in mediating host immune surveillance against tumours as they develop. Moreover, loss of function of TRAILRs through mutation or decreased expression, or through changes in key downstream signalling components, may confer intrinsic resistance to TRAIL-induced apoptosis.

TRAIL as a tumour suppressor in mouse experimental tumour models. Cancer immune surveillance is mediated by both components of cellular immunity: innate immunity and adaptive immunity. TRAIL, along with perforin 1 and CD95L, partly mediates spontaneous and activated natural killer (NK) cell-induced cytotoxicity against TRAIL-sensitive lines *in vitro*[38,39]. In mice, the anti-metastatic function of NK cells against TRAIL-sensitive tumour cells was also partly dependent on TRAIL expression and, in particular, basal TRAIL expression and anti-metastatic activity was restricted to liver NK cells in several different tumour models[38,40]. No such TRAIL phenotype was observed when TRAIL-resistant tumour cell lines were examined *in vivo*. Interferon-γ (IFNγ)-mediated TRAIL induction on NK cells was also shown to have a significant role in the anti-tumour efficacy of IFNγ-dependent immunotherapies[40,41]. These findings provided the first evidence for the physiological function of TRAIL as a tumour suppressor.

Although activated T cells exert TRAIL-mediated apoptosis[42], the first indication that TRAIL has a role in T-cell-mediated immune defence against tumours was shown in an allogeneic graft-versus-tumour (GVT) setting[43] where TRAIL expression was shown to be required for optimal GVT activity by donor T cells[43]. By contrast, TRAIL had little or no role in the graft-versus-host disease activity of donor T cells. The idea of using exogenous TRAIL in conjunction with allogeneic bone marrow transplant therapy has been tempered by the knowledge that many leukaemia samples taken from patients with acute lymphocytic leukaemia or acute myeloid leukaemia, for example, were not sensitive to TRAIL *in vitro*[44]. Therefore, combination therapies using activators of the TRAIL pathway and other pro-apoptotic stimuli that sensitize inherently resistant cells to TRAIL-mediated apoptosis may be more efficacious in the clinic. Most primary leukaemia cells are highly resistant to TRAIL and CD95L, suggesting that resistance to death-inducing ligands, particularly to TRAIL, could be one of the mechanisms for a rapid clonal expansion, and a poor sensitivity to the graft-versus-leukaemia effect in infant leukaemias with *MLL* (myeloid/lymphoid or mixed-lineage leukaemia) rearrangement[45]. Encouragingly, in mouse models, GVT activity against subcutaneous colon tumours is efficiently induced by pre-conditioning with irradiation and allogeneic donor lymphocyte infusion, and here TRAIL and IFNγ produced by T cells act cooperatively in the anti-tumour effect[46]. In addition to NK and T cells, other cell types such as IFN-activated myeloid cells and neutrophils have a role in immune and anti-tumoural responses and can store and release biologically active TRAIL[47,48]. The therapeutic potential of harnessing TRAIL-induced apoptosis of tumour cells mediated by host immune cells in a cancer setting was demonstrated in mice transplanted with donor haematopoietic cells retrovirally transduced to overexpress mouse TRAIL. Transplanted syngeneic mammary carcinoma cells grew more slowly in these mice and there was evidence that the tumour cells were undergoing TRAIL-mediated apoptosis without damage to normal tissue[49].

Innate immunity
The innate immune system provides immediate non-specific defence against pathogens. The innate leukocytes include NK cells, mast cells, eosinophils, basophils and the phagocytic cells, including macrophages, neutrophils and dendritic cells. These cells identify and eliminate pathogens, and virus-infected and neoplastic cells.

Adaptive immunity
The adaptive immune system comprises specialized, systemic cells that can recognize and remember specific 'non-self' antigens, responding more vigorously each time this antigen is encountered. The cellular components involved include B lymphocytes, T lymphocytes and antigen-presenting cells (dendritic cells, B cells and macrophages).

Graft versus tumour
(GVT). A beneficial T-cell-mediated immune response to host tumour cells by immune cells present in a donor's transplanted tissue.

Graft-versus-host disease
(GVHD). The pathological consequence of a response initiated by transplanted immunocompetent T lymphocytes into an allogeneic, immunocompromised host. The host is unable to reject the grafted T cells and becomes their target.

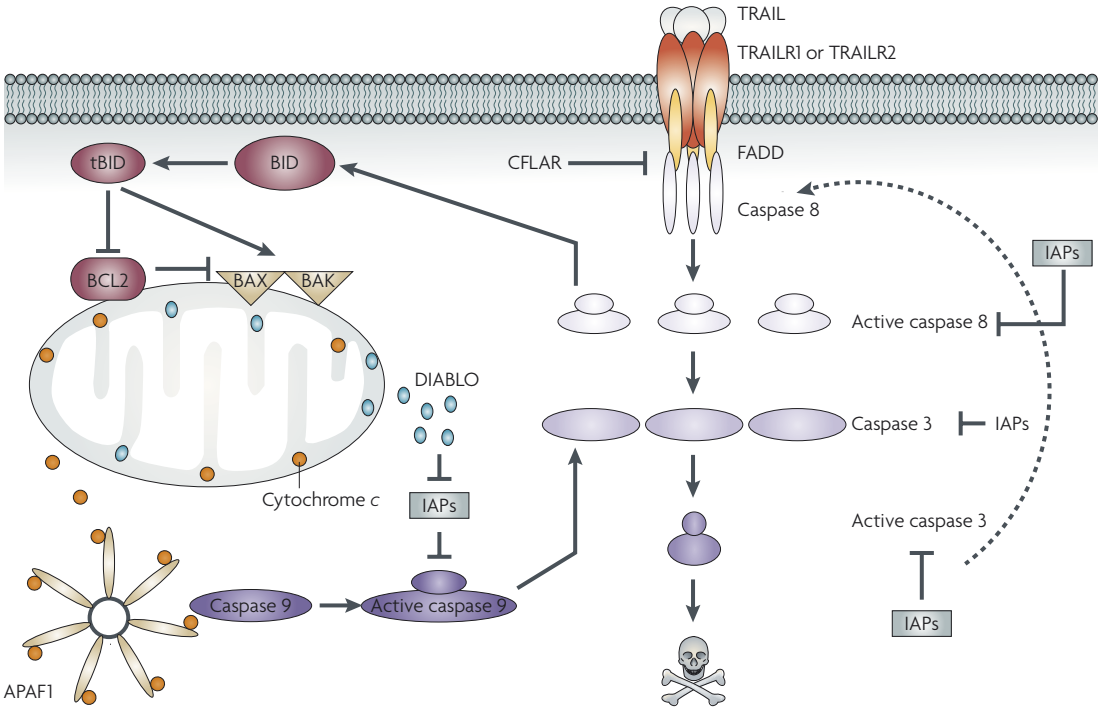

Figure 1 | **Apoptotic signalling through the tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) pathway.** Binding of TRAIL to TRAIL receptor 1 (TRAILR1) or TRAILR2 results in receptor oligomerization and recruitment of FAS-associated protein with death domain (FADD) and caspase 8 to form a functional death-inducing signalling complex (DISC). Upon DISC formation, caspase 8 is cleaved and activated, which in turn can cleave and activate caspase 3 and the BH3-only protein BID. Active, cleaved BID (tBID) can bind to pro-apoptotic BAX and BAK, resulting in mitochondrial membrane permeabilization and release of mitochondrial proteins cytochrome *c* and DIABLO. Cytochrome *c*, apoptotic protease-activating factor 1 (APAF1) and caspase 9 combine with ATP to form a functional apoptosome that results in cleavage and activation of caspase 9, which can then cleave caspase 3. DIABLO suppresses the caspase-inhibitory activities of IAPs. Caspase 3 can cleave a large number of intracellular targets resulting in the morphological and biochemical hallmarks of apoptosis. Caspase 3 can also cleave and activate caspase 8, thereby amplifying the apoptotic signal.

TRAIL as a tumour suppressor in mouse spontaneous tumour models. A role for TRAIL as a tumour suppressor has also been supported by considerable evidence derived in spontaneous or carcinogen-induced tumours in mice. Neutralization of TRAIL promoted tumour development in mice inoculated with the carcinogen methylcholanthrene (MCA)[41,50] and there was preferential emergence of TRAIL-sensitive fibrosarcoma cells in TRAIL-deficient and IFNγ-deficient mice compared with wild-type mice, strongly suggesting immunoediting of TRAIL-sensitive cells during tumour development. The effect of TRAIL in this model is consistent with a role for NK and NKT cells in host immune protection from MCA-induced sarcoma[51,52]. A substantial contribution of TRAIL to immune surveillance against spontaneous tumour development initiated by the loss of one *Trp53* allele was also demonstrated[50,53], but the loss of two p53 alleles negated this effect[54]. Further validation of the importance of TRAIL signalling in tumorigenesis was supported by the report that loss of just one allele of *Trailr2* was sufficient to significantly reduce median lymphoma-free survival on the lymphoma-prone *Eμ–Myc* genetic background[37]. Strangely, TRAILR2-deficient lymphomas developed with equal frequency irrespectively of monoallelic or biallelic loss of *Trailr2*. This finding is inconsistent with other reports[55] and with data in this same paper (survival after sublethal irradiation) that suggest that the gene dosage of *Trailr2* does not regulate the outcome in terms of the response of cells to radiation. These lymphomas also had increased metastatic potential and showed apoptotic defects compared with lymphomas from wild-type littermates. The same group reported that TRAILR2 also suppressed diethylnitrosamine-induced hepatocarcinogenesis, with an increased number of large tumours with apoptotic defects developing in the livers of *Trailr2*-deficient mice. Furthermore, TRAILR2 has been shown to act as a metastasis suppressor in the mouse multistage model of squamous cell carcinoma[55]. DMBA–TPA-treated TRAILR2-deficient mice did not show an increase in primary benign papilloma number or growth rate or progression to squamous cell carcinoma; however, metastasis to lymph nodes was significantly increased. In concert, adherent skin carcinoma cells were TRAIL-resistant *in vitro* but were sensitized to TRAIL on detachment by inactivation of the ERK signalling pathway (BOX 3).

Immunoediting
Describes the complex relationship between a developing tumour under constant pressure from the host immune system. Cancer immunoediting consists of three phases: elimination (that is, cancer immunosurveillance), equilibrium and escape. The immune system not only protects the host against development of primary cancers but also sculpts tumour immunogenicity.

Box 2 | **NFκB and TRAIL**

Signalling through TRAILR1 (tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor 1) or TRAILR2 results in activation of nuclear factor κB (NFκB), probably through RPA-interacting protein (RIP)[12,166]. Consistent with the proposed role of NFκB in regulating TRAIL-induced apoptosis, inhibition of NFκB activity *in vitro* sensitized tumour cells to TRAIL-mediated apoptosis[167–171] and mediated TRAIL-dependent tumour regression *in vivo*[172]. However, although inhibition of NFκB activity can sensitize tumour cells to TRAIL-induced apoptosis *in vitro*, this is not a universal effect on all cell types[173]. A simplistic view is that, when apoptosis is suppressed, TRAIL-mediated activation of the NFκB pathway switches the TRAIL signal from induction of apoptosis to stimulating cell survival and/or proliferation. Indeed, this has been demonstrated in tumour cells that are resistant to TRAIL-mediated apoptosis owing to defective death receptor signalling. These cells showed NFκB-dependent enhanced proliferation[24] or metastasis and invasion[174] *in vitro* following stimulation with TRAIL. However NFκB is a multi-protein complex and, depending on its composition, activation of the pathway may result in entirely different biological outcomes. For example, the RELA subunit can enhance TRAIL-induced expression of TRAILR1, TRAILR2 and BCL-X_s (also known as BCL2L10) and repress expression of inhibitor of apoptosis protein 1 (IAP1), IAP2 and survivin, and so is largely a pro-apoptotic signal. By contrast, REL inhibits expression of caspase 8, TRAILR1 and TRAILR2 and enhances TRAIL-mediated expression of IAP1 and IAP2, a largely anti-apoptotic signal[175]. Thus, even if the canonical death receptor signalling pathway is blocked, stimulation of NFκB activity by TRAIL may still induce tumour cell death, depending on whether RELA or REL is the dominant NFκB transcriptional component (FIG. 2).

Perhaps the simplest demonstration of a role for the TRAIL pathway in carcinogenesis is the low, but significant, incidence of spontaneous tumours of haematopoietic origin in aged C57BL/6 TRAIL-deficient mice[53]. However, the TRAIL pathway does not seem to be critical in all mouse models of tumorigenesis. The loss of host *Trailr2* and *Trail* did not influence intestinal tumour development in adenomatous polyposis coli mutant mice[54] and rat *Erbb2* oncogene-driven mammary carcinoma[53], respectively, both models in which the immune effector control of tumour development is weak or absent. It will be important to validate all of these findings in both TRAILR2-deficient and TRAIL-deficient mice, and in additional models of tumorigenesis. It must also be said that in most of these mouse models it is not appreciated at what stage of tumour development TRAIL may be acting as an extrinsic tumour suppressor.

Genetic lesions in the TRAIL pathway associated with human tumour onset and progression. Overall, at this stage, the case for mutations in the TRAIL–TRAILR pathways predisposing humans to cancer is weak. *TRAILR1* (*DR4*) and *TRAILR2* (*DR5*) map to human chromosome 8p21-22, a site of frequent allelic loss in tumours, and thus some human tumours may have somatic mutations in TRAILRs. Mutations in *DR5* have been identified in a proportion (up to 10–20%) of various human tumours, including cancers of the breast, lung and head and neck, and non-Hodgkin lymphoma (NHL)[56–59]. Most mutations map to the intracellular domain of *DR5* (the region that binds FADD[56,59]), with many tumours retaining wild-type *DR4*. Some mutations have been found repeatedly in different tumour types and in different patients with specific types of cancer, suggesting that TRAIL receptor mutations are selected for during tumorigenesis and may have important functional effects in tumour cells. However, it is poorly understood how the mutations affect signalling. It was recently found that some point mutations that were identified in human tumours resulted in TRAILR2 losing its ability to form a functional DISC and induce apoptosis[60]. As TRAIL can signal through either TRAILR1 or TRAILR2, a simple loss of function mutation in one of the receptors that does not affect the other might not be expected to prevent signalling in response to TRAIL. However, the mutant *DR5* also appeared to have a 'dominant-negative' effect whereby it inhibited the ability of TRAIL to induce apoptosis through functional TRAILR1s. This study provides a molecular basis for the use of specific therapeutic agonists of TRAILRs in patients whose tumours harbour somatic *DR5* mutations. It may also be feasible to avoid the inhibitory effect of mutant TRAILRs using modified versions of TRAIL that target only one of the receptors[61,62]. At this stage a relatively small number of patients have been studied and uncertainties remain about how common these mutations are in different populations of cancer patients.

Evidence that *DR4* has a role in cancer predisposition is variable. A rare allele of *DR4* (A683C) was found to be more frequent in chronic lymphocytic leukaemia, chronic myeloid leukaemia, prostate and bladder cancer, and head and neck squamous cell carcinoma (HNSCC)[63]. The A683C polymorphism did not co-segregate with other known *DR4* polymorphisms. Haplotype analysis revealed a 2.4-fold increased risk for carriers of the rare 626C-683C haplotype (1% prevalence in the general population), suggesting that *DR4* 626C-683C may affect colorectal cancer predisposition[64]. An analysis of known *DR4* polymorphisms, namely G442A, C626G and A1322G, in germline DNA of 97 patients with ovarian cancer and controls did not detect any significant difference between patients and controls[65]. In addition, a case–control study of eight selected polymorphisms in a large sample (1,008 cases and 768 controls) of Spanish women with breast cancer, no differences in genotype or haplotype distribution were found for two *DR4* polymorphisms between cases and controls[66]. Interestingly, however, one allele (2699G) of the decoy receptor *DCR2* appeared to associate with reduced breast cancer risk ($P = 0.05$). Given that it is located in the 3′ untranslated region, its effect might be related to *DCR2* mRNA instability, or linkage disequilibrium with a functional variant residing in either *DCR2* or neighbouring genes. A decreased efficiency of DCR2 to work as a decoy receptor for TRAIL might facilitate the apoptotic pathway in cells at risk. Another study indicated that TRAILR1 expression positively correlated with the tumour grade in breast cancer patients with invasive ductal carcinoma[67].

Downstream regulators of TRAIL signalling, such as caspase 8, caspase 10 and CFLAR, might also be associated with cancer risk. The reproducible, dose-dependent association of a caspase 8 single-nucleotide polymorphism (CASP8 D302H) with breast cancer indicates the potential importance of inherited variation in the apoptosis pathway in breast cancer susceptibility,

Box 3 | **TRAIL, MAPKs and PI3K**

Activation of the phosphoinositide 3-kinase (PI3K)–Akt pathway is thought to be an important oncogenic event stimulating growth and survival of tumour cells[176]. TRAIL (tumour necrosis factor (TNF)-related apoptosis-inducing ligand) can rapidly induce the phosphorylation and activation of PI3K and Akt, and this occurs in cells that ultimately die in response to TRAIL and those that proliferate and survive[177,178]. The potential importance of the PI3K–Akt pathway in mediating sensitivity to TRAIL-induced apoptosis has been demonstrated by genetic studies showing that tumour cells containing an activating somatic mutation in PI3K are relatively resistant to TRAIL-induced apoptosis[179]. In cells that are resistant to TRAIL-induced apoptosis, such as fibroblast-like synoviocytes (commonly found in rheumatoid arthritis), signalling through TRAIL receptors and activation of the PI3K–Akt pathway results in cell proliferation[22].

The mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK), JUN N-terminal kinase (JNK) and p38 can all be activated in response to TRAIL stimulation (see REF. 12 and references therein). There is little consensus on the signalling events leading to activation of these kinases by TRAIL, with differing recent reports indicating that MAPK activation may in fact occur downstream of caspase activation[180,181]. Moreover, the effect that activation of MAPKs may have on TRAIL-mediated apoptosis is similarly confusing at present, with contrasting reports indicating that activated ERK, JNK or p38 either suppress[20,182,183] or enhance[184,185] the apoptotic effects of TRAIL. Whether the different effects of MAPKs on TRAIL-induced biological outcomes reflect variations in molecular programmes within specific cell types or merely variations in *in vitro* experimental systems remains unclear. At present there is a lack of strong genetic or *in vivo* evidence validating important roles of ERK, JNK or p38 in mediating the physiological and therapeutic activities of the TRAIL pathway.

but does not yet pin down the TRAIL receptor pathway as key in this disease setting[68]. In neuroblastoma, inactivation of *CASP8* by hypermethylation has become a hallmark of defective apoptosis in advanced disease, suggesting that *CASP8* may act as a tumour suppressor gene in this cancer[69,70]. To this end, the methylation status of *CASP8* has been linked to *MYCN* amplification in some studies[69] but not in others[71], and thus the prognostic effect of *CASP8* in neuroblastoma has remained controversial. No correlation was observed between caspase 8 expression and *MYCN* amplification in one recent report and, more importantly, loss of caspase 8 protein had no effect on event-free or overall survival in the overall study population or in distinct subgroups of patients[72]. Epigenetic aberrations have also been shown to have an important role in the pathogenesis of most cancers. In primary neuroblastoma tumours, high-risk disease and poor outcome were associated with methylation of *DCR2* and *CASP8* individually, suggesting that clinically aggressive neuroblastoma tumours have aberrant methylation of genes in the TRAIL pathway and providing a rationale for exploring treatment strategies that include demethylating agents[73]. Finally, overexpression of CFLAR, but not TRAILR1 or TRAILR2, provided stage-independent poorer prognosis in patients with colorectal cancer[74]. Therefore, the evidence that TRAIL receptors have a role in human cancer onset and progression remains relatively weak and, although polymorphisms in downstream mediators such as caspase 8 appear prognostic in some instances, these molecules are used by several upstream death receptor complexes and a distinct role for TRAIL remains to be clarified.

Antibody-dependent cell-mediated cytotoxicity (ADCC). Cell death that occurs when the Fc fragment of a mAb, bound to a target cell, interacts with the Fc receptor on monocytes, macrophages or NK cells. These cells in turn kill the target cell or secrete cytokines. ADCC is part of the adaptive immune response owing to its dependence on a prior antibody response.

Complement-dependent cytotoxicity (CDC). The effect of a mAb bound to a target cell initiating the complement cascade, leading to the assembly of the membrane attack complex. This disrupts the target cell membrane, resulting in cell lysis.

Targeting the TRAIL pathway: preclinical studies

Targeting TRAILRs as a monotherapy. Recombinant TRAIL (rTRAIL) is an attractive anticancer agent and preclinical studies in mice and non-human primates have shown that soluble forms of rTRAIL suppressed the growth of TRAIL-sensitive human tumour xenografts, with no apparent systemic toxicity[75,76]. Agonistic anti-human TRAILR1 or TRAILR2 mAbs exhibit potent tumoricidal activities against human tumour xenografts in nude or severe combined immunodeficient mice without apparent toxicity[77–82]. Both mAbs possessing intrinsic agonistic activity and proto-agonistic types of mAb (requiring crosslinking of their Fc domains for agonistic activity) display tumoricidal activity *in vivo*. It is important to recognize, however, that not all tumour cells express both TRAILR1 and TRAILR2 and that, even when both receptors are expressed, only one may be functionally competent. For example, in colon and breast carcinoma cell lines that express both TRAILR1 and TRAILR2, the use of modified rTRAIL specific for either receptor indicated that TRAILR2 could mediate a pro-apoptotic signal whereas ligation of TRAILR1 had little or no effect[83]. By contrast, chronic lymphocytic leukaemia cells were selectively sensitive to ligation of TRAILR1 even though both TRAILR1 and TRAILR2 were expressed on the cell surface[84]. Accordingly, the anti-tumour efficacy of recombinant ligands or mAbs specific for either TRAILR1 or TRAILR2 may be based on the activating potential of either receptor on particular tumour cells rather than on mere expression. Currently, most anti-TRAIL receptor antibodies developed for clinical use target TRAILR2 rather than TRAILR1 (TABLE 1). The reason for this may not be based on more detailed functional studies such as those listed above, but rather on initial studies indicating that TRAILR2 is more highly expressed on tumour cells than TRAILR1 (REF. 5) or that apoptotic signalling through TRAILR2 may be more potent than through TRAILR1 (REF. 83).

The anti-tumour activity of anti-human TRAILR1 mAbs in mice bearing human tumour xenografts was markedly influenced by their isotype, suggesting a crucial contribution of Fc receptors for some effector functions *in vivo*[77]. We have demonstrated that an agonistic hamster anti-mouse TRAILR2-specific mAb (MD5-1) has anti-tumour effects against syngeneic tumours in mice[85]. The MD5-1-induced anti-tumour effect was demonstrated to mainly depend on direct apoptosis induction, rather than antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity, as TRAILR2-expresssing and CFLAR-transfected apoptosis-resistant tumour cell variants were completely resistant to MD5-1 *in vitro* and *in vivo*. A rapid recruitment of FcR-expressing innate immune cells, macrophages and dendritic cells (DCs) into the tumour site was observed after MD5-1 treatment and, intriguingly, small tumour burdens could be eradicated and mice concurrently developed tumour-specific cytotoxic T lymphocytes (CTLs). Subsequent tumour challenge revealed that TRAIL-resistant tumour variants were eliminated by such effector cells. Thus, activating Fc-receptor-mediated immune activation can be a great advantage of antibody-based therapy of tumours[86,87].

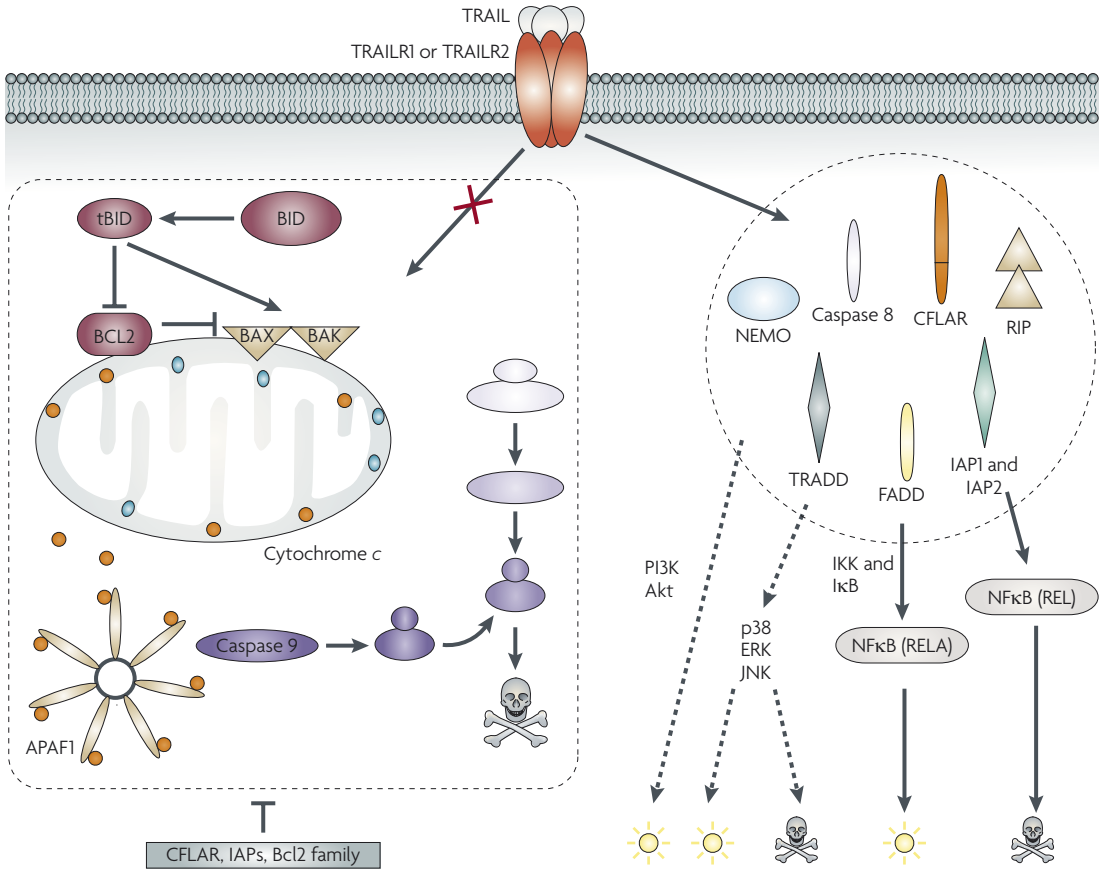

Figure 2 | **Additional signal transduction pathways activated by tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL).** Ligation of TRAIL can result in the simultaneous activation of the pro-apoptotic caspase cascade and additional signalling pathways such as the phosphoinositide 3-kinase (PI3K)–Akt, nuclear factor κB (NFκB) and mitogen-activated protein kinase (MAPK, including p38, extracellular signal-regulated kinase (ERK) and JUN N-terminal kinase (JNK)) pathways. The signalling events downstream of receptor ligation have not been fully dissected but proteins such as FAS-associated protein with death domain (FADD), TNF receptor type 1-associated DEATH domain protein (TRADD), caspase 8, RPA-interacting protein (RIP), CASP8 and FADD-like apoptosis regulator (CFLAR), inhibitor of apoptosis protein 1 (IAP1), IAP2 and possibly NEMO have roles. In general, activation of these additional pathways in the absence of a functional apoptotic cascade has a pro-survival, proliferative effect. However, engagement of REL and, in certain circumstances, MAPK pathway proteins can also result in apoptosis. APAF1, apoptotic protease-activating factor 1; tBID, cleaved BID.

Use of TRAIL and agonistic anti-TRAILR antibodies in combination with other anticancer agents. Although rTRAIL and agonistic anti-TRAILR mAbs may have some clinical activity as monotherapies, perhaps the most attractive use of these agents will be as one arm of combination therapies in conjunction with other anticancer modalities. Indeed, there are many publications demonstrating that combining compounds or antibodies that activate the TRAIL pathway with a range of different pharmaceutical, biological and cellular anticancer agents results in additive or synergistic tumour cell death. Herein, we have chosen to focus on those reports providing one or more of the following: a strong molecular rationale for combining a particular agent(s) with TRAIL; detailed analysis on the molecular events that underpin the combination therapy; or robust *in vivo* data demonstrating that the combination approach provides therapeutic efficacy that is superior to single-agent therapy.

Combining TRAIL with standard anticancer therapies. There have been numerous publications demonstrating that diverse chemotherapeutic drugs or radiotherapy can induce synergistic tumour cell apoptosis when combined with rTRAIL or agonistic anti-TRAILR mAbs (TABLE 2). There is little consensus on how different chemotherapeutic drugs synergize with TRAIL signalling to mediate enhanced tumour cell death, with various studies showing upregulated TRAILR, downregulated CFLAR and enhanced localization of TRAILRs to lipid rafts as being important for the combination effect (TABLE 2). As *DR5* is a transcriptional target of p53 (REF. 88), a simplistic model would be that activation of the p53 pathway in response to cytotoxic agents underpins the synergistic effects seen using chemotherapeutics and stimulators of the TRAIL pathway. However, in tumour cells lacking wild-type p53, chemotherapeutic agents can both upregulate TRAILRs and synergize with TRAIL[89], indicating that other molecular interactions are sufficient to

Table 1 | **Combination studies using recombinant TRAIL or agonsitic anti-TRAILR mAbs and other anticancer agents (part 1)**

Compound	Tumour model	Primary mechanism	Secondary mechanism	Efficacy tested *in vivo*?	Refs
Standard anticancer agents					
Cisplatin	Thoracic cancer cells	DNA crosslinking	Activation of caspases	Yes, H513 xenograft. Strong tumoricidal effect; 3.7-fold increase in survival	197
	Malignant pleural mesothelioma		Enhanced caspase 8, caspase 3 and BID activation. Increase in BAX. Downregulation of survivin and MCL1	No	198
Doxorubicin	Prostate cancer	DNA intercalation & topisomerase II inhibition	Downregulation of c-FLIP$_L$ and c-FLIP$_S$	Yes, PC3 xenograft. Significantly delayed tumour growth	199
	Leukaemic B cells		Clustering of TRAILR2 into ceramide-enriched membrane platforms	No	200
Etoposide	Mesothelioma	DNA damage	Lower threshold for BID cleavage, enhanced caspase 8 cleavage	No	201
Ionizing radiation	Prostate cancer cells	DNA damage	Upregulation of TRAILR1, TRAILR2, BAX and BAK and induction of caspase activation. Downregulation of BCL2	Yes, PC3 xenograft. Irradiation then TRAIL led to tumour irradication & 100% survival	202
Irinotecan (CPT-11)	Prostate cancer	Inhibits topoisomerase I	*In vitro*: upregulation of BAX, downregulation of BCL-X$_L$. *In vivo*: upregulation of BAK and BCL-X$_S$, downregulation of BCL-W and BCL-X$_L$	Yes C4-2 xenograft. Complete tumour elimination in 1/3 mice	203
Paclitaxel, vincristine, vinblastine, etoposide, doxorubicin, campothecin	Prostate and bladder cancer	Topisomerase I & II & microtubule inhibition, DNA damage, DNA intercalation	Upregulation of TRAILR1, TRAILR2, BAX & BAK, & slight downregulation of XIAP, IAP1, IAP2 & survivin	Yes, PC3 xenografts. Inhibited tumour growth, enhanced survival & reduced angiogenesis	204
NFκB pathway inhibitors					
17AAG	Lung cancer	Inhibited HSP90	Decreased expression of RIP & IKKβ & phosphorylation & degradation of IκBα blocked. Disabling the NFκB survival signal led to synergistic apoptosis	No	205
AS602868	Myeloma	Inhibited IKK	Downregulation of c-FLIP$_L$, IAP1, IAP2 BCL-X$_L$ & MYC, enhanced cleavage of XIAP & MCL1 & decreased paracrine IL6 production	No	167
Aspirin	Colon adenocarcinoma & breast cancer	Inhibits activation of IKK complex (IKKβ)	Downregulation of BCL-X$_L$	No	168
	Prostate adenocarcinoma & colorectal carcinoma		Downregulation of BCL2	No	206
BMS-3455541	Mantle cell lymphoma	Inhibited IKK	Downregulation of CFLAR	No	169
Bortezomib & geldanamycin	Pancreatic cancer cell lines	Synergistically block NFκB activation	Downregulation of BCL-X$_L$, BCL2, IAP1 & cyclin D1	No	207
Bortezomib or PS-1145	Pancreatic cancer cell lines	Inhibited IKK	Downregulation of BCL-X$_L$ & XIAP	Yes, Panc-1 xenograft. Reversed TRAIL resistance & synergistically inhibited tumour growth	170
Curcumin	Prostate cancer cells	Blocks phosphorylation of IκBα	Increased TRAILR1 but not TRAILR2 expression; TRAILR3 upregulated but TRAILR4 unchanged	No	208
Nitrosylcobalamin	Melanoma	Inhibits IKK activation, decreases IκBα phosphorylation & inhibits NFκB DNA binding	Inhibition of XIAP	Yes A375 xenograft. Synergistic anti-tumour activity	209

15d-PGJ$_2$, 15-deoxy-Δ-prostaglandin J$_2$; BAK, Bcl2-homologous antagonist/killer; BID, Bcl2 homology domain 3-interacting domain death agonist; c-FLIP$_L$, CFLAR isoform 1; c-FLIP$_S$, CFLAR isoform 2; CLL, chronic lymphocytic leukaemia; DISC, death-inducing signalling complex; EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; HSP90, heat shock protein 90; IAP, inhibitor of apoptosis protein; IκB, NFκB inhibitor; IL6, interleukin 6; IKK, IκB kinase; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MCL1, myeloid leukaemia cell 1; mTOR, mammalian target of rapamycin; NFκB, nuclear factor κB; PI3K, phosphoinositide 3-kinase; RIP, RPA-interacting protein; SAHA, suberoylanilide hydroxamic acid; SBHA, suberic bishydroxamate; Ser, serine; TRAIL, tumour necrosis factor (TNF)-related apoptosis-inducing ligand; TRAILR, TRAIL receptor; XAntag, small molecule antagonist of XIAP.

Table 1 | **Combination studies using recombinant TRAIL or agonsitic anti-TRAILR mAbs and other anticancer agents (part 2)**

Compound	Tumour model	Primary mechanism	Secondary mechanism	Efficacy tested *in vivo*?	Refs
SN50	Multiple myeloma	Blocks NFκB nuclear translocation & inhibited transcription	Augments TRAIL sensitivity or reversed TRAIL resistance	No	210
Sorafenib	Colon adenocarcinoma	Decreased NFκB DNA binding ability	Downregulation of IAP2 and MCL1	Yes, HT29 xenograft. TRAIL-resistant tumours regressed	211
HDAC inhibitors					
Depsipeptide	Prostate cancer	Inhibition of HDAC	Increased TRAILR1 & TRAILR2 in membrane lipid rafts	No	97
LAQ824	Jurkat, B lymphoblast, primary myeloid leukaemia blast samples	Inhibition of HDAC	Upregulated TRAILR1 and TRAILR2. Downregulated CFLAR, BCL2, BCL-X_L, XIAP & survivin. Increased TRAIL-induced DISC assembly	No	93
Sodium butyrate, SAHA	Leukaemia cell lines	Inhibition of HDAC	Increased BID activation, BAX translocation, & caspase, p21 & BCL2 cleavage; downregulation of XIAP, cyclin D1	No	94
SBHA	Melanoma	Inhibition of HDAC	Upregulated pro-apoptotic caspase 8 & 3, BID, BAK, BAX & BIM; downregulated anti-apoptotic BCL-X_L, MCL1 & XIAP	No	95
Valproic acid	CLL	Inhibition of HDAC	Downregulation of CFLAR	No	212
PI3K pathway inhibitors					
15d-PGJ_2	Leukaemic HL-60 cells	Downregulation of Akt expression & phosphorylation	Downregulation of XIAP, BCL2 & CFLAR; activation of intrinsic & extrinsic apoptotic pathways	No	213
1L-6-hydroxy-methyl-chiro-inositol 2(*R*)-2-*O*-methyl-3-*O*-octodecylcarbonate	Leukaemic HL-60 cells	Akt inhibitor	Downregulation of anti-apoptotic proteins IAP1, IAP2 & CFLAR, & of BAD phosphorylation at Ser136	No	100
Fusion protein of TRAIL & EGFR blocking antibody (scFv425:sTRAIL)	$EGFR^+$ A431, Jurkat	EGFR inhibition coupled to apoptosis induced by crosslinked TRAIL on cell surface	Downregulation of PI3K and MAPK signaling. CFLAR downregulation and BAD dephosphorylation	No	101
Gefitinib (Iressa)	Bladder cancer cell lines	EGFR inhibition	Downregulation of active Akt and XIAP	Yes, preliminary toxicity studies show combination is well-tolerated	214
Rapamycin	Glioma	mTOR inhibitor, p70 S6 kinase 1 pathway inhibition	Downregulation of *c-FLIP*$_S$ but not *c-FLIP*$_L$ mRNA. Suppresses polyribosomal accumulation of *c-FLIP*$_S$ mRNA and c-$FLIP_S$ protein expression	No	105
Wortmannin, LY294002	Prostate cancer	PI3K inhibitor, downregulates Akt	Allows cleavage of Bid, enables apoptosis through mitochondria	No	215
Wortmannin, LY294002	Leukaemia cell line HL-60	PI3K inhibitor, downregulates Akt	Downregulated CFLAR levels through NFκB-dependent mechanism	No	102
IAP inhibitors					
Flavopiridol	Leukaemia cell lines	Transcriptional downregulation of XIAP	Enhanced caspase 8 and 3 cleavage, BID activation and BAX translocation	No	216
DIABLO-mimic compound	Breast cancer cell lines	Binds IAPs, relieves their inhibition of caspases 3, 7 & 9	Caspase 3 activation through IAP-dependent mechanism	No	108
Smac-mimic compound 3	Glioblastoma	Binds and eliminates XIAP, IAP1 and IAP2 activities	Enhanced caspase 8 cleavage	No	109
XAntag	Pancreatic cancer cell lines	Inhibition of XIAP	Activation of effector caspases 3 and 7	No	110

15d-PGJ_2, 15-deoxy-Δ-prostaglandin J_2; BAK, Bcl2-homologous antagonist/killer; BID, Bcl2 homology domain 3-interacting domain death agonist; c-$FLIP_L$, CFLAR isoform 1; c-$FLIP_S$, CFLAR isoform 2; CLL, chronic lymphocytic leukaemia; DISC, death-inducing signalling complex; EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; HSP90, heat shock protein 90; IAP, inhibitor of apoptosis protein; IκB, NFκB inhibitor; IL6, interleukin 6; IKK, IκB kinase; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MCL1, myeloid leukaemia cell 1; mTOR, mammalian target of rapamycin; NFκB, nuclear factor κB; PI3K, phosphoinositide 3-kinase; RIP, RPA-interacting protein; SAHA, suberoylanilide hydroxamic acid; SBHA, suberic bishydroxamate; Ser, serine; TRAIL, tumour necrosis factor (TNF)-related apoptosis-inducing ligand; TRAILR, TRAIL receptor; XAntag, small molecule antagonist of XIAP.

confer TRAIL-sensitivity in the absence of a functional p53 pathway. Some of these studies have been broadened to include *in vivo* analyses of the combination approach using xenograft models. These have had encouraging results showing superior activity of the combination over either single agent tested. However, there is evidence that normal human hepatocytes, lymphocytes and osteoblasts are sensitive *in vitro* to TRAIL-induced apoptosis following co-treatment with chemotherapeutic drugs[90,91], indicating that such combinations may have toxicity profiles that limit the use of these therapies in humans. Careful preclinical testing is required to characterize these potentially harmful toxic side effects, especially as such combinations are already being assessed in humans (TABLE 1).

TRAIL and NFκB pathway inhibitors. Constitutive NFκB activity or sustained activation of the NFκB pathway following TRAILR1 or TRAILR2 ligation can suppress TRAIL-mediated apoptosis (BOX 2). Accordingly, agents that directly or indirectly suppress NFκB activity have been shown to strongly synergize with activators of the TRAIL pathway *in vitro* and *in vivo* (TABLE 2). Interestingly, decreased expression of CFLAR, inhibitor of apoptosis proteins (IAPs) and pro-survival Bcl2 family proteins are common downstream effects of inhibiting NFκB activity (TABLE 2), and CFLAR and IAPs in particular have been shown to suppress TRAIL-induced apoptosis (BOX 4).

TRAIL and histone deacetylase inhibitors. Histone deacetylase inhibitors (HDACi) are promising new anticancer agents that can induce tumour cell apoptosis, inhibit cell proliferation by blocking progression through the G1 or G2/M phases of the cell cycle, induce cellular differentiation, suppress angiogenesis and modulate anti-tumour immunity[92]. The molecular rationale for combining HDACi with TRAIL or agonistic anti-TRAILR mAbs is based on findings that HDACi can induce expression of TRAILRs[93–95] (TABLE 2). HDACi can also increase the expression of pro-apoptotic genes, including those in the intrinsic pathway[96] (such as caspases, BAX and BAK) and decrease expression of pro-survival genes (such as CFLAR and XIAP (also known as BIRC4))[92]. Interestingly, the HDACi depsipeptide induced the localization of TRAILRs to lipid rafts, resulting in enhanced TRAIL-mediated apoptosis[97]. These *in vitro* results suggest that a combination of HDACi and TRAIL-stimulatory agents may be therapeutically desirable. Whether the combination of HDACi with TRAILR agonists will prove effective in the clinic remains to be seen, with some results suggesting cause for concern. A recent study has shown that *ex vivo* treatment of healthy liver explants with TRAIL does not result in apoptosis, but that treatment with depsipeptide sensitizes normal hepatocytes to TRAIL-mediated apoptosis[98]. However, we have recently demonstrated that a combination of vorinostat and the agonistic anti-mTRAILR2 mAb MD5-1, but not either agent alone, eradicated established mouse mammary tumours in the absence of significant toxicity[99].

TRAIL and kinase inhibitors. Activation of signalling cascades involving the PI3K–Akt pathway might negatively regulate TRAIL-induced apoptosis, so suppressing the activity of one or more key proteins in this pathway may augment TRAIL-mediated apoptosis (BOX 3). Pharmacological inhibition of PI3K or Akt can be achieved in a number of ways: first, inhibiting the activity of receptor tyrosine kinases that function upstream of PI3K–Akt using agents such as gefitinib (Iressa); second, specifically inhibiting PI3K or Akt by using, for example, LY294002, which inhibits PI3K; or, third, using compounds that inhibit PI3K or Akt in a less specific manner, such as amiloride. All these approaches have been shown to sensitize cells to apoptosis induced by TRAIL[100–104] (TABLE 2). Mammalian target of rapamycin (mTOR, also known as FRAP1) is an important downstream target of PI3K–Akt, and inhibition of mTOR activity by rapamycin also sensitizes tumour cells to TRAIL-mediated cell death[105]. Given the surge in activity to develop new compounds that specifically target the PI3K–Akt–mTOR signalling pathway[106], and the apparent synergy between inhibition of this pathway and stimulators of the TRAIL pathway, this appears to be an attractive area for further preclinical and clinical investigation.

TRAIL and inhibitors of CFLAR and IAPs. Given the potent inhibitory effects of CFLAR and IAPs in mediating resistance to TRAIL-induced apoptosis (BOX 4), directly or indirectly inhibiting the expression and/or function of these proteins is an attractive option for combination studies involving TRAIL or agonistic anti-TRAILR mAbs. At present, small-molecule inhibitors or cell-permeable peptides directly targeting CFLAR have not been produced, although efforts to develop such agents are expanding[107]. By contrast, small-molecule IAP inhibitors have been developed and these can synergize with TRAIL to induce tumour cell apoptosis[108–110]. The simplest mechanistic model to explain the synergistic apoptosis seen using IAP inhibitors and activators of the TRAIL pathway is that suppression of IAP activity allows for more potent caspase activation following TRAILR oligomerization, resulting in enhanced apoptotic signal transduction. However, recently four papers published by different groups using different IAP antagonists indicated that these agents mediate single-agent anti-tumour activity through the induction of TNFα expression, resulting in autocrine activation of TNFR1 (REFS 111–114). A series of elegant genetic studies demonstrated that IAP inhibitors mediate the activation of canonical and non-canonical NFκB pathways with activation of the non-canonical pathway occurring through stabilization of NIK that is targeted for degradation by IAP1 (also known as BIRC3) and IAP2 (also known as BIRC2) (REFS 111,112). Given that activation of canonical NFκB signalling appears to antagonize TRAIL-mediated apoptosis (BOX 2), it would seem counterintuitive that enhanced NFκB signalling may sensitize cells to TRAIL-mediated death. However, it is possible that non-canonical NFκB signalling alters the overall NFκB signal emanating from the TRAILRs in such a way that a more pro-apoptotic environment exists.

Table 2 | **Clinical trials using recombinant human TRAIL or agonistic anti-TRAILR mAbs**

Agent	Phase	Tumour type	Patients (*n*)	Observed responses (number of patients)	Most common adverse events	Refs
Mapatumumab (HGS-ETR1)	1	Solid	49	Stable disease (19)	Fatigue, fever, myalgia	131
Mapatumumab (HGS-ETR1)	I	Solid and NHL	15	None reported	Thrombocytopenia	132
Mapatumumab (HGS-ETR1)	I	Solid	24	Stable disease (8)	Thrombocytopenia, hypertension	133
Mapatumumab (HGS-ETR1) plus paclitaxel and carboplatin	I	Solid	28	Partial response (4)	Neutropenic fever (attributed to chemotherapy), hypersensitivity (attributed to HGS-ETR1), fatigue, myalgia, transaminitis, anorexia, arthralgia	134
Mapatumumab (HGS-ETR1)	II	NSCLC	32	Stable disease (9)	Fatigue, cough, nausea, dyspnea, constipation, vomiting	135
Mapatumumab (HGS-ETR1)	II	NHL	40	Complete response (1) Partial response (2) Stable disease (12)	Shingles, fever	136
Lexatumumab (HGS-ETR2)	I	Solid	37	Stable disease (12)	Constipation, fatigue, mild nausea at 10 mg per kg Sepsis, acute renal failure, transaminitis, hyperbilirubinaemia at 20 mg per kg	137
Lexatumumab (HGS-ETR2)	I	Solid	31	Stable disease (10)	Hyperamylasaemia	138
Lexatumumab (HGS-ETR2) plus gemcitibine, pemetrexed, doxorubicin or FOLFIRI	1b	Solid and haematological	41	Partial response (doxorubicin and FOLFIRI arms)	Anaemia, fatigue and dehydration	139
AMG 655 (anti-DR5)	I	Solid	16	Partial response (1) Stable disease (4)	Pyrexia, fatigue, hypomagnesaemia, increased serum lipase	141
Apomab (anti-DR5)	I	Solid	26	Stable disease (1)	None reported	142
AMG951 (rhAPO2L/TRAIL)	I	Solid, NHL	51	Partial response (1) Stable disease (13)	Fatigue, nausea, vomiting, anaemia, pyrexia, diarrhoea, headache, anorexia	143
AMG951 (rhAPO2L/TRAIL)	1A	Solid and haematological	39	None reported	None reported	144
AMG951 (rhAPO2L/TRAIL)	1A	CRC, sarcoma, NSCLC	31	Partial response (1) Stable disease (5)	None reported	145
AMG951 (rhAPO2L/TRAIL) plus rituximab	IB	NHL	7	Complete response (2) Partial response (1) Stable disease (2)	None reported	146

CRC, colorectal carcinoma; DR5, death receptor 5; FOLFIRI, folinic acid, fluorouracil and irinotecan; mAb, monoclonal antibody; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; TRAIL, tumour necrosis factor (TNF)-related apoptosis-inducing ligand; TRAILR, TRAIL receptor.

Screens for other genes that affect TRAIL-induced apoptosis. Loss-of-function 'synthetic lethal' screens to identify genes that functionally interact with TRAIL using TRAIL-treated cells and kinome-specific siRNA libraries[115,116] or a genome-wide siRNA library[117] identified both sensitizer and inhibitor genes. Members of the glycogen synthase kinase family (*GSK3A* and *GSK3B*) were identified in all three screens as regulating TRAIL-induced apoptosis. In one screen, knockdown of *GSK3A* inhibited TRAIL-induced apoptosis[115], and in other screens knockdown of *GSK3A*[117] and *GSK3B*[116] sensitized cells to TRAIL. The association between loss of function of *GSK3B* and enhanced TRAIL sensitivity is particularly interesting given that GSK3β negatively regulates the expression of the oncogene *MYC*. Enhanced expression of MYC confers sensitivity to TRAIL-mediated apoptosis[118,119], whereas knockdown of *Myc*[115,119] and transcriptional regulators of *Myc* such as TCF4 conferred resistance to TRAIL[115]. Interestingly, *BUB1* (encoding a protein kinase involved in regulating the spindle checkpoint), *PAK1* (encoding a protein kinase activated by CDC42 and RAC1) and various cyclin-dependent kinases were identified in two different screens as TRAIL suppressor genes. It is therefore apparent that unbiased functional genomics screens are a powerful tool to identify molecular pathways that regulate the apoptotic activity of TRAIL and identify proteins that may either be targeted by pharmacological or biological agents to enhance TRAIL-mediated apoptosis or, in the case of overexpression of MYC, may act as biomarkers for TRAIL sensitivity.

Combining TRAIL with immune-based therapies. The anti-mTRAILR2 mAb MD5-1 triggers tumour cell death directly through caspase activation, but also evokes tumour-specific CTL that could also eliminate anti-TRAILR2-resistant variants[85]. Thus, combining primary tumour cell death with T-cell activation may be an attractive approach to augment the therapeutic effect of the anti-mTRAILR2 mAb and other mAb-based tumour targeting[120]. Based on this hypothesis, we have combined MD5-1 with agonistic anti-CD40 mAb

Box 4 | **IAPs and CFLAR**

TRAIL (tumour necrosis factor (TNF)-related apoptosis-inducing ligand)-mediated apoptosis can be inhibited by apoptotic regulatory mechanisms that function downstream of receptor ligation. CASP8 and FADD-like apoptosis regulator (CFLAR) is an inhibitor of death receptor signalling that, owing to the presence of a death effector domain (DED) but lack of cysteine protease activity, interacts with FADD and/or caspase 8 through DED–DED interactions but inhibits TRAIL-mediated caspase 8 autoactivation and subsequent apoptosis[186,187] (FIG. 1). CFLAR is overexpressed in diverse tumour types[188] and this, coupled with its defined role in inhibiting death receptor-mediated apoptosis and ability to promote cell proliferation[189], indicates that it may function as a *bona fide* oncoprotein.

Members of the inhibitor of apoptosis protein (IAP) family such as IAP1, IAP2 and XIAP were initially proposed to block apoptosis by binding to active caspases 3, 7 and 9 and inhibiting subsequent cleavage of downstream substrates. Overexpression of these proteins has been reported in a variety of tumour types and is associated with poor disease prognosis[190]. In addition to the somewhat passive role of blocking the proteolytic activity of caspases, IAPs have E3 ligase activity[191] and may induce caspase ubiquitylation resulting in altered caspase stability or function[192]. IAPs are neutralized by DIABLO (also known as SMAC), a protein released from the mitochondria following membrane permeabilization[193,194]. High levels of IAPs can reportedly suppress TRAIL-mediated apoptosis, and introduction of exogenous DIABLO[195] or knockdown of endogenous *XIAP* by RNA interference[196] sensitizes TRAIL-resistant cells to TRAIL-mediated apoptosis. Targeting IAPs using small-molecule mimetics of DIABLO to sensitize tumour cells to TRAIL-mediated apoptosis is an attractive therapeutic approach currently being tested in preclinical models.

and agonistic anti-CD137 (4-1BB) mAb treatments to enhance activation of T cells, and coined the combination "trimAb". Indeed, trimAb promptly induced tumour-specific T cells in the draining lymph node and resulted in the complete rejection of established TRAIL-sensitive mouse tumours[121]. Moreover, trimAb therapy could induce complete regression of tumour masses containing 90% apoptosis-resistant variants. Importantly, trimAb therapy also induced complete tumour rejection in a large proportion of mice bearing established MCA-induced sarcomas, despite the known heterogeneity of these tumours and their capacity to evade natural immunity[122]. TrimAb-induced tumour rejection was mediated by $CD8^+$ T cells, and a great advantage of trimAb therapy is that tumour antigens do not have to be defined for therapeutic application. We have also reported that IL21 enhanced the induction of tumour-specific CTLs by MD5-1 alone or trimAb in mice[123,124]. Thus, cytokines enhancing CTL expansion, effector function or survival could be favourable reagents to combine with anti-death-receptor therapy. However, taking such a complex experimental combination to the clinic has significant logistical hurdles that need to be overcome.

The proteasome inhibitor bortezomib has direct anti-tumour effects and sensitizes tumour cells to killing through TRAIL. In a tumour-purging assay, in which tumour bone marrow cell mixtures were placed into lethally irradiated mice, only treatment with a combination of NK cells and bortezomib resulted in significant tumour-free survival of the recipients[125]. These results demonstrated that bortezomib treatment can sensitize tumour cells to NK cell-mediated killing by TRAIL.

Recently there have been reports indicating that a combination of anti-CD20 antibodies (rituximab) and rTRAIL or agonistic anti-TRAILR mAbs is efficacious against NHL in experimental mice[126,127]. Moreover, a rituximab-refractory NHL cell line remained sensitive to the combination of rituximab and rTRAIL *in vivo* but was resistant to the monotherapy. Interestingly, depletion of endogenous NK cells or complement reduced the *in vivo* efficacy of the combination, indicating that engagement of the innate immune response has an important therapeutic role[127].

TRAIL and agonistic anti-TRAILR mAbs: clinical use

Early-phase clinical trials have been initiated in cancer patients, testing for safety, pharmacokinetics and preliminary evidence of anti-tumour activity using soluble rTRAIL (co-developed by Genentech and Amgen), and mAbs targeting TRAILR1, such as mapatumumab (HGS-ERT1 developed by Human Genome Sciences (HGS)), and TRAILR2, such as lexatumumab (HGS-ETR2 developed by HGS), AMG 655 (developed by Amgen) and apomab (developed by Genentech) (TABLE 1). Given the possible role of decoy receptor expression in regulating sensitivity to TRAIL (BOX 1), some tumour cells expressing TRAILR1 or TRAILR2 may still be protected from rTRAIL-induced apoptosis. mAbs generally have a longer half-life *in vivo* than recombinant proteins and, thus, specific targeting of death-inducing TRAILR1 and/or TRAILR2 by agonistic mAbs is theoretically a more effective approach than using the rTRAIL ligand. Conversely, although cancer cells express more TRAILR1 and TRAILR2 than normal cells[128], anti-TRAILR1 or anti-TRAILR2 mAbs may be potentially more toxic than rTRAIL, as the decoy receptors might protect TRAILR1- and TRAILR2-expressing normal cells from TRAIL-induced killing[129,130]. It is tempting to speculate that the relative sensitivity of a cell to TRAIL could be predicted based on the expression of TRAILRs that can or cannot directly transmit an apoptotic signal. However, a true understanding is needed of the complex molecular interplay between the functions of the different receptors and the roles of various downstream signal transduction pathways (BOXES 2–4) that are activated by TRAIL.

In three phase I trials, patients with advanced solid malignancies were treated with intravenous doses of humanized mapatumumab ranging from 0.01 to 20 mg per kg[131–133] (TABLE 1). In one trial, the half-life of mapatumumab at 10 mg per kg every 14 days was 18.8 days (±10.1 days). No complete or stable responses were reported, although ~37% of patients reported had stable disease. A study using mapatumumab in combination with gemcitabine and cisplatin has reported no major toxicities to date, and partial responses were reported in 4 out of 28 patients enrolled in the trial[134]. Two phase II studies with mapatumumab have commenced in patients with non-small-cell lung carcinoma[135] and NHL[136] (TABLE 1). Encouragingly, 1 complete response and 2 partial responses were observed in the NHL study with 12 out of the 40 patients in the trial showing stable disease and few adverse events recorded.

Clinical trials using lexatumumab[137,138] have also demonstrated that this agent is well-tolerated when used as a monotherapy at 10 mg per kg; however, dose-limiting toxicities were observed at 20 mg per kg with one patient going into renal failure[137] (TABLE 1). The half-life of lexatumumab at 10 mg per kg averaged 16.4 (±10.9) days. Twelve patients had durable stable disease that lasted a median of 4.5 months, including three patients with sarcoma having prolonged stable disease (≥6.7 months). Combination therapies using lexatumumab and chemotherapeutic drugs were generally well-tolerated[139]. Tumour shrinkage was observed in the FOLFIRI (folinic acid, fluorouracil and irinotecan) and doxorubicin arms. In addition, a second anti-TRAILR2 mAb (HGS-TR2J) developed by HGS in collaboration with Kirin Pharma is in a phase I clinical trial, although few details are currently available[140]. Recently, both Amgen and Genentech have developed their own anti-TRAILR2 mAbs and both have entered phase I clinical testing. A partial response (1 patient) and stable disease (4 patients) have been reported in 16 patients with solid cancers treated with the Amgen agent AMG 655 (REF. 141), and stable disease was observed in 1 out of 26 patients with solid tumours treated with apomab[142] (TABLE 1).

Results from three phase I trials using AMG 951 (rhApo2L/TRAIL) have been reported[143–145] (TABLE 1). Thirty-nine patients enrolled in a phase 1A study had pharmacokinetics assessments at dose levels ranging from 0.5 to 15 mg per kg. AMG 951 clearance appeared proportional to dose, and doses that can be safely administered in humans produced serum concentrations consistent with those demonstrating efficacy in tumour xenograft models. AMG 951 is also being tested as a targeted therapy in combination with rituximab[146], which, as detailed above, shows promising synergistic activities in preclinical models. Six subjects with low-grade NHL (4 with follicular NHL and 2 with small-cell NHL) were enrolled and treated with 4 mg per kg rhApo2L/TRAIL and rituximab, and 1 subject (with follicular NHL) was enrolled and treated with 8 mg per kg rhApo2L/TRAIL and rituximab. There have been no dose-limiting toxicities or serious adverse events or grade 3/4 adverse events reported to date. At this stage, 5 subjects had undergone tumour response assessment: 2 patients with complete response, 1 with partial response and 2 with stable disease. Thus, the combination of rhApo2L/TRAIL at 4 mg per kg per day and rituximab appears safe and shows evidence of activity in subjects with low-grade NHL that has relapsed following previous rituximab-containing therapy. Enrolment is continuing to test rhApo2L/TRAIL at 8 mg per kg plus rituximab for expanded safety data and further dose optimization.

Although these early trials are promising, it is important to recognize that the utility of rTRAIL and agonistic anti-TRAILR mAb therapies is limited to patients with TRAIL-sensitive tumours. It is also obvious that TRAIL sensitivity will vary among individual cancer patients, even if preferential TRAIL-sensitivity and TRAILR1 and TRAILR2 expression in certain cancer cell types were reported. The efficacy of TRAILR-targeting therapies will be dramatically improved when diagnostic methods determining TRAIL sensitivity of clinically detectable human cancers are developed. Along those lines, a phase 1A trial is underway evaluating the safety and tolerability of AMG 951 in patients with advanced tumours, and the aim is to develop and validate high-throughput pharmacodynamic assays to monitor AMG 951 activity in easily accessible patient samples such as serum[145]. Increases in serum caspase 3 and caspase 7 and genomic DNA levels were observed in >50% of the patients with colorectal cancer, lung cancer and sarcoma evaluated. Preliminary analyses showed the percentage increase correlated using both analytes and was dose-dependent. These findings support the use of serum-based pharmacodynamic assays to monitor rhApo2L/TRAIL activity in patients with advanced tumours.

Barriers to clinical application of the TRAIL pathway. It is clear that the potential toxicity of therapy versus the efficacy of tumour treatment will be limiting in patients. Induction of apoptosis in normal human cells, such as hepatocytes and keratinocytes, by some rTRAIL and anti-TRAILR mAbs has been reported *in vitro*[147–150], and two recent studies have shown that the TRAIL-DR5 pathway may contribute to hepatotoxicity and bile duct toxicity in mice either treated with anti-mouse DR5 mAb or intervened by bile duct ligation[151–152]. Thus, administration of rTRAIL or agonistic anti-TRAILR mAbs might induce cytotoxicity in some normal cells in patients. Hepatotoxicity with increased serum alanine aminotransferase, aspartate aminotransferase and bilirubin was reported in a few patients when treated with higher doses (20 mg per kg) of lexatumumab (TABLE 1). As this is an observation in humans rather than a test of toxicity in animals, and it supports the recent mouse studies of Takeda and colleagues showing that the anti-DR5 mAb can induce cholangitis and cholestatic liver injury in mice[152], it needs to be taken seriously. Even if this unfavourable effect were observed only in a dose-limiting manner and using one anti-human anti-TRAILR mAb, the mechanisms underlying hepatotoxicity would need to be delineated, as this will provide novel information for safer treatment with anti-TRAILR2 mAbs in humans in the future. Intriguingly, it was recently reported that TRAIL strongly induced apoptosis in explants from steatotic and hepatitis C virus-infected livers[98]. The authors of this study therefore cautioned care in the use of TRAIL in patients with inflammatory liver diseases. It is possibly too early to know whether hepatotoxicity or bile duct toxicity might limit recombinant TRAIL- and anti-DR5-based therapeutic approaches in humans. Certainly, extreme care needs to be taken when progressing to combination therapies where new sensitivities to the TRAIL–TRAILR pathway may manifest.

Immature human and mouse DCs are sensitive to TRAIL-mediated apoptosis *in vitro*[153,154], and immature mouse DCs generated *in vitro* were eliminated *in vivo* by NK cells in a TRAIL-dependent manner[153]. Moreover, negative regulatory functions of TRAILR2 on innate immune responses have been shown using TRAILR-deficient mice[155]. These reports suggested a possible immune suppressive effect of rTRAIL or anti-TRAILR mAb treatments that might result in an increased

frequency of infectious diseases during therapy. However, to date, there is no evidence for severe immune suppression by TRAIL or anti-TRAILR mAb treatments in any experimental setting or clinical trial. Even so, it will be important to monitor immune status during TRAIL or anti-TRAILR mAb therapies, when combined with chemotherapy and/or radiotherapy. A combination of antibodies that target different steps within the immune response that collectively induce anti-tumour immunity is one of most rational strategies to eradicate established tumour masses. However, we advise caution in the use of such approaches given the immense power of the immune system; a clear assessment of unfavourable side effects, including autoimmune reactions, must be made. Moreover, it has been noted that overactivation of the immune response can result in hepatotoxicity (for example, concanavalin A-induced hepatitis). Moreover, it has been reported that hepatocytes can have augmented TRAIL sensitivity during viral infections, alcohol intake and cholestasis[98,156–159]. Thus, disorders of hepatic function are the most likely side effects during TRAILR-targeting monotherapy or combination therapy. Regardless, targeting TRAILRs should be safer and more useful than cancer therapies that target other members of the death-inducing TNF receptor superfamily[5].

Conclusion and perspective

Since the initial identification of TRAIL and TRAILRs a little over a decade ago there has been exceptional progress in understanding how TRAIL selectively kills tumour cells, to identify and molecularly decipher the pro- and anti-apoptotic pathways that are activated following TRAILR ligation and to develop therapeutic agents that can engage the pathway. The development of rTRAIL and agonistic anti-TRAILR mAbs has rapidly advanced and encouraging clinical trial results have been reported, but questions still remain regarding the role(s) of the decoy TRAILRs and the cellular and molecular contexts in which activation of NFκB, PI3K–Akt and other signal transduction pathways impinge on the physiological and therapeutic activities of TRAIL. Moreover, there is experimental evidence that ligation of TRAILRs in cells that cannot initiate an apoptotic signal results in activation of pathways that may stimulate cell proliferation, survival and migration, and the physiological effects of such situations need to be fully appreciated. Answers to these questions will undoubtedly provide a clearer scientific rationale for combining agents that activate the TRAIL pathway with small molecules or biologicals that are most likely to be therapeutically advantageous.

1. Danial, N. N. & Korsmeyer, S. J. Cell death: critical control points. *Cell* **116**, 205–219 (2004).
2. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
3. Johnstone, R. W., Ruefli, A. A. & Lowe, S. W. Apoptosis: a link between cancer genetics and chemotherapy. *Cell* **108**, 153–164 (2002).
4. Fesik, S. W. Promoting apoptosis as a strategy for cancer drug discovery. *Nature Rev. Cancer* **5**, 876–885 (2005).
5. Ashkenazi, A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nature Rev. Cancer* **2**, 420–430 (2002).
6. Smyth, M. J. *et al.* Nature's TRAIL — on a path to cancer immunotherapy. *Immunity* **18**, 1–6 (2003).
7. Yagita, H., Takeda, K., Hayakawa, Y., Smyth, M. J. & Okumura, K. TRAIL and its receptors as targets for cancer therapy. *Cancer Sci.* **95**, 777–783 (2004).
8. Pitti, R. M. *et al.* Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J. Biol. Chem.* **271**, 12687–12690 (1996).
9. Wiley, S. R. *et al.* Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* **3**, 673–682 (1995).
References 8 and 9 describe the initial identification and cloning of TRAIL.
10. Wu, G. S., Burns, T. F., Zhan, Y., Alnemri, E. S. & El-Deiry, W. S. Molecular cloning and functional analysis of the mouse homologue of the KILLER/DR5 tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptor. *Cancer Res.* **59**, 2770–2775 (1999).
11. Schneider, P. *et al.* Identification of a new murine tumor necrosis factor receptor locus that contains two novel murine receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *J. Biol. Chem.* **278**, 5444–5454 (2003).
12. Falschlehner, C., Emmerich, C. H., Gerlach, B. & Walczak, H. TRAIL signalling: decisions between life and death. *Int. J. Biochem. Cell Biol.* **39**, 1462–1475 (2007).
References 5 and 12 provide excellent reviews of the molecular and biological activities of the TRAIL pathway.
13. Ashkenazi, A. & Dixit, V. M. Apoptosis control by death and decoy receptors. *Curr. Opin. Cell Biol.* **11**, 255–260 (1999).
14. Willis, S. N. & Adams, J. M. Life in the balance: how BH3-only proteins induce apoptosis. *Curr. Opin. Cell Biol.* **17**, 617–625 (2005).
15. Keogh, S. A., Walczak, H., Bouchier-Hayes, L. & Martin, S. J. Failure of Bcl-2 to block cytochrome *c* redistribution during TRAIL-induced apoptosis. *FEBS Lett.* **471**, 93–98 (2000).
16. Walczak, H., Bouchon, A., Stahl, H. & Krammer, P. H. Tumor necrosis factor-related apoptosis-inducing ligand retains its apoptosis-inducing capacity on Bcl-2- or Bcl-x_L-overexpressing chemotherapy-resistant tumor cells. *Cancer Res.* **60**, 3051–3057 (2000).
17. Belka, C. *et al.* Sensitization of resistant lymphoma cells to irradiation-induced apoptosis by the death ligand TRAIL. *Oncogene* **20**, 2190–2196 (2001).
18. Hinz, S. *et al.* Bcl-X_L protects pancreatic adenocarcinoma cells against CD95- and TRAIL-receptor-mediated apoptosis. *Oncogene* **19**, 5477–5486 (2000).
19. Fulda, S., Meyer, E. & Debatin, K. M. Inhibition of TRAIL-induced apoptosis by Bcl-2 overexpression. *Oncogene* **21**, 2283–2294 (2002).
20. Zhang, X. D., Borrow, J. M., Zhang, X. Y., Nguyen, T. & Hersey, P. Activation of ERK1/2 protects melanoma cells from TRAIL-induced apoptosis by inhibiting Smac/DIABLO release from mitochondria. *Oncogene* **22**, 2869–2881 (2003).
21. Rudner, J. *et al.* Type I and type II reactions in TRAIL-induced apoptosis — results from dose–response studies. *Oncogene* **24**, 130–140 (2005).
22. Morel, J., Audo, R., Hahne, M. & Combe, B. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces rheumatoid arthritis synovial fibroblast proliferation through mitogen-activated protein kinases and phosphatidylinositol 3-kinase/Akt. *J. Biol. Chem.* **280**, 15709–15718 (2005).
23. Baader, E. *et al.* Tumor necrosis factor-related apoptosis-inducing ligand-mediated proliferation of tumor cells with receptor-proximal apoptosis defects. *Cancer Res.* **65**, 7888–7895 (2005).
24. Ehrhardt, H. *et al.* TRAIL induced survival and proliferation in cancer cells resistant towards TRAIL-induced apoptosis mediated by NF-κB. *Oncogene* **22**, 3842–3852 (2003).
25. Wagner, K. W. *et al.* Death-receptor *O*-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. *Nature Med.* **13**, 1070–1077 (2007).
Landmark paper describing how *O*-glycosylation of TRAILRs determines the relative sensitivity of cells to TRAIL-mediated apoptosis.
26. Muppidi, J. R. & Siegel, R. M. Ligand-independent redistribution of Fas (CD95) into lipid rafts mediates clonotypic T cell death. *Nature Immunol.* **5**, 182–189 (2004).
27. Legler, D. F., Micheau, O., Doucey, M. A., Tschopp, J. & Bron, C. Recruitment of TNF receptor 1 to lipid rafts is essential for TNFα-mediated NF-κB activation. *Immunity* **18**, 655–664 (2003).
References 26 and 27 describe how apoptotic signaling through death receptors may be regulated by the recruitment of receptors in lipid rafts.
28. Song, J. H. *et al.* Lipid rafts and nonrafts mediate tumor necrosis factor related apoptosis-inducing ligand induced apoptotic and nonapoptotic signals in non small cell lung carcinoma cells. *Cancer Res.* **67**, 6946–6955 (2007).
29. Secchiero, P. & Zauli, G. Tumor-necrosis-factor-related apoptosis-inducing ligand and the regulation of hematopoiesis. *Curr. Opin. Hematol.* **15**, 42–48 (2008).
30. Janssen, E. M. *et al.* $CD4^+$ T-cell help controls $CD8^+$ T-cell memory via TRAIL-mediated activation-induced cell death. *Nature* **434**, 88–93 (2005).
This key work demonstrated for the first time a role for TRAIL in immunoregulation.
31. Weckmann, M. *et al.* Critical link between TRAIL and CCL20 for the activation of TH2 cells and the expression of allergic airway disease. *Nature Med.* **13**, 1308–1315 (2007).
32. Cretney, E., Shanker, A., Yagita, H., Smyth, M. J. & Sayers, T. J. TNF-related apoptosis-inducing ligand as a therapeutic agent in autoimmunity and cancer. *Immunol. Cell Biol.* **84**, 87–98 (2006).
33. Hilliard, B. *et al.* Roles of TNF-related apoptosis-inducing ligand in experimental autoimmune encephalomyelitis. *J. Immunol.* **166**, 1314–1319 (2001).
34. Lamhamedi-Cherradi, S. E., Zheng, S., Tisch, R. M. & Chen, Y. H. Critical roles of tumor necrosis factor-related apoptosis-inducing ligand in type 1 diabetes. *Diabetes* **52**, 2274–2278 (2003).
35. Zheng, S. J., Wang, P., Tsabary, G. & Chen, Y. H. Critical roles of TRAIL in hepatic cell death and hepatic inflammation. *J. Clin. Invest.* **113**, 58–64 (2004).
36. Hoffmann, O. *et al.* TRAIL limits excessive host immune responses in bacterial meningitis. *J. Clin. Invest.* **117**, 2004–2013 (2007).

37. Finnberg, N., Klein-Szanto, A. J. & El-Deiry, W. S. TRAIL-R deficiency in mice promotes susceptibility to chronic inflammation and tumorigenesis. *J. Clin. Invest.* **118**, 111–123 (2008).
38. Takeda, K. *et al.* Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nature Med.* **7**, 94–100 (2001).
A landmark paper first describing natural host TRAIL as an extrinsic tumor suppressor.
39. Kayagaki, N. *et al.* Expression and function of TNF-related apoptosis-inducing ligand on murine activated NK cells. *J. Immunol.* **163**, 1906–1913 (1999).
40. Smyth, M. J. *et al.* Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) contributes to interferon γ-dependent natural killer cell protection from tumor metastasis. *J. Exp. Med.* **193**, 661–670 (2001).
41. Cretney, E. *et al.* Increased susceptibility to tumor initiation and metastasis in TNF-related apoptosis-inducing ligand-deficient mice. *J. Immunol.* **168**, 1356–1361 (2002).
42. Kayagaki, N. *et al.* Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs. *J. Exp. Med.* **189**, 1451–1460 (1999).
43. Schmaltz, C. *et al.* T cells require TRAIL for optimal graft-versus-tumor activity. *Nature Med.* **8**, 1433–1437 (2002).
44. Wuchter, C. *et al. In vitro* susceptibility to TRAIL-induced apoptosis of acute leukemia cells in the context of TRAIL receptor gene expression and constitutive NF-κB activity. *Leukemia* **15**, 921–928 (2001).
45. Inukai, T. *et al.* Resistance of infant leukemia with MLL rearrangement to tumor necrosis factor-related apoptosis-inducing ligand: a possible mechanism for poor sensitivity to antitumor immunity. *Leukemia* **20**, 2119–2129 (2006).
46. Tateishi, K. *et al.* TRAIL-induced cell death cooperates with IFN-γ activation in the graft-versus-tumor effect against colon tumors. *Int. J. Cancer* **118**, 2237–2246 (2006).
47. Cassatella, M. A. On the production of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2L) by human neutrophils. *J. Leukoc. Biol.* **79**, 1140–1149 (2006).
48. Simons, M. P., Nauseef, W. M. & Griffith, T. S. Neutrophils and TRAIL: insights into BCG immunotherapy for bladder cancer. *Immunol. Res.* **39**, 79–93 (2007).
49. Song, K., Benhaga, N., Anderson, R. L. & Khosravi-Far, R. Transduction of tumor necrosis factor-related apoptosis-inducing ligand into hematopoietic cells leads to inhibition of syngeneic tumor growth *in vivo*. *Cancer Res.* **66**, 6304–6311 (2006).
50. Takeda, K. *et al.* Critical role for tumor necrosis factor-related apoptosis-inducing ligand in immune surveillance against tumor development. *J. Exp. Med.* **195**, 161–169 (2002).
51. Smyth, M. J. *et al.* Differential tumor surveillance by natural killer (NK) and NKT cells. *J. Exp. Med.* **191**, 661–668 (2000).
52. Smyth, M. J., Crowe, N. Y. & Godfrey, D. I. NK cells and NKT cells collaborate in host protection from methylcholanthrene-induced fibrosarcoma. *Int. Immunol.* **13**, 459–463 (2001).
53. Zerafa, N. *et al.* Cutting edge: TRAIL deficiency accelerates hematological malignancies. *J. Immunol.* **175**, 5586–5590 (2005).
54. Yue, H. H., Diehl, G. E. & Winoto, A. Loss of TRAIL-R. does not affect thymic or intestinal tumor development in p53 and adenomatous polyposis coli mutant mice. *Cell Death Differ.* **12**, 94–97 (2005).
55. Grosse-Wilde, A. *et al.* TRAIL-R deficiency in mice enhances lymph node metastasis without affecting primary tumor development. *J. Clin. Invest.* **118**, 100–110 (2008).
56. Lee, S. H. *et al.* Alterations of the DR5/TRAIL receptor 2 gene in non-small cell lung cancers. *Cancer Res.* **59**, 5683–5686 (1999).
57. Lee, S. H. *et al.* Somatic mutations of TRAIL-receptor 1 and TRAIL-receptor 2 genes in non-Hodgkin's lymphoma. *Oncogene* **20**, 399–403 (2001).
58. Fisher, M. J. *et al.* Nucleotide substitution in the ectodomain of trail receptor DR4 is associated with lung cancer and head and neck cancer. *Clin. Cancer Res.* **7**, 1688–1697 (2001).
59. Shin, M. S. *et al.* Mutations of tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1) and receptor 2 (TRAIL-R2) genes in metastatic breast cancers. *Cancer Res.* **61**, 4942–4946 (2001).
60. Bin, L. *et al.* Tumor-derived mutations in the TRAIL receptor DR5 inhibit TRAIL signaling through the DR4 receptor by competing for ligand binding. *J. Biol. Chem.* **282**, 28189–28194 (2007).
61. MacFarlane, M., Kohlhaas, S. L., Sutcliffe, M. J., Dyer, M. J. & Cohen, G. M. TRAIL receptor-selective mutants signal to apoptosis via TRAIL-R1 in primary lymphoid malignancies. *Cancer Res.* **65**, 11265–11270 (2005).
62. van der Sloot, A. M. *et al.* Designed tumor necrosis factor-related apoptosis-inducing ligand variants initiating apoptosis exclusively via the DR5 receptor. *Proc. Natl Acad. Sci. USA* **103**, 8634–8639 (2006).
63. Wolf, S. *et al.* Ala228 variant of trail receptor 1 affecting the ligand binding site is associated with chronic lymphocytic leukemia, mantle cell lymphoma, prostate cancer, head and neck squamous cell carcinoma and bladder cancer. *Int. J. Cancer* **118**, 1831–1835 (2006).
64. Frank, B. *et al.* Death receptor 4 variants and colorectal cancer risk. *Cancer Epidemiol. Biomarkers Prev.* **15**, 2002–2005 (2006).
65. Horak, P. *et al.* Perturbation of the tumor necrosis factor-related apoptosis-inducing ligand cascade in ovarian cancer: overexpression of FLIPL and deregulation of the functional receptors DR4 and DR5. *Clin. Cancer Res.* **11**, 8585–8591 (2005).
66. Martinez-Ferrandis, J. I. *et al.* Polymorphisms in TRAIL receptor genes and risk of breast cancer in Spanish women. *Cancer Biomarkers* **3**, 89–93 (2007).
67. Sanlioglu, A. D. *et al.* TRAIL death receptor-4 expression positively correlates with the tumor grade in breast cancer patients with invasive ductal carcinoma. *Int. J. Radiat. Oncol. Biol. Phys.* **69**, 716–723 (2007).
68. MacPherson, G. *et al.* Association of a common variant of the CASP8 gene with reduced risk of breast cancer. *J. Natl Cancer Inst.* **96**, 1866–1869 (2004).
69. Teitz, T. *et al.* Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nature Med.* **6**, 529–535 (2000).
70. Hopkins-Donaldson, S. *et al.* Loss of caspase-8 expression in highly malignant human neuroblastoma cells correlates with resistance to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis. *Cancer Res.* **60**, 4315–4319 (2000).
71. Harada, K. *et al.* Aberrant promoter methylation and silencing of the RASSF1A gene in pediatric tumors and cell lines. *Oncogene* **21**, 4345–4349 (2002).
72. Fulda, S. *et al.* Loss of caspase-8 expression does not correlate with MYCN amplification, aggressive disease, or prognosis in neuroblastoma. *Cancer Res.* **66**, 10016–10023 (2006).
73. Yang, Q. *et al.* Methylation of CASP8, DCR2, and HIN-1 in neuroblastoma is associated with poor outcome. *Clin. Cancer Res.* **13**, 3191–3197 (2007).
74. Ullenhag, G. J. *et al.* Overexpression of FLIPL is an independent marker of poor prognosis in colorectal cancer patients. *Clin. Cancer Res.* **13**, 5070–5075 (2007).
75. Ashkenazi, A. *et al.* Safety and antitumor activity of recombinant soluble Apo2 ligand. *J. Clin. Invest.* **104**, 155–162 (1999).
76. Walczak, H. *et al.* Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand *in vivo*. *Nature Med.* **5**, 157–163 (1999).
References 75 and 76 provided the first evidence that rTRAIL had anti-tumour activity *in vivo* and could be safely administered to experimental animals.
77. Chuntharapai, A. *et al.* Isotype-dependent inhibition of tumor growth *in vivo* by monoclonal antibodies to death receptor 4. *J. Immunol.* **166**, 4891–4898 (2001).
78. Griffith, T. S. *et al.* Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL. *J. Exp. Med.* **189**, 1343–1354 (1999).
79. Ichikawa, K. *et al.* Tumoricidal activity of a novel anti-human DR5 monoclonal antibody without hepatocyte cytotoxicity. *Nature Med.* **7**, 954–960 (2001).
Important description of an agonistic anti-DR5 mAb leading to clinical trials.
80. Motoki, K. *et al.* Enhanced apoptosis and tumor regression induced by a direct agonist antibody to tumor necrosis factor-related apoptosis-inducing ligand receptor 2. *Clin. Cancer Res.* **11**, 3126–3135 (2005).
81. Pukac, L. *et al.* HGS-ETR1, a fully human TRAIL-receptor 1 monoclonal antibody, induces cell death in multiple tumour types *in vitro* and *in vivo*. *Br. J. Cancer* **92**, 1430–1441 (2005).
82. Zeng, Y. *et al.* Monoclonal antibody to tumor necrosis factor-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) induces apoptosis in primary renal cell carcinoma cells *in vitro* and inhibits tumor growth *in vivo*. *Int. J. Oncol.* **28**, 421–430 (2006).
83. Kelley, R. F. *et al.* Receptor-selective mutants of apoptosis-inducing ligand 2/tumor necrosis factor-related apoptosis-inducing ligand reveal a greater contribution of death receptor (DR) 5 than DR4 to apoptosis signaling. *J. Biol. Chem.* **280**, 2205–2212 (2005).
84. MacFarlane, M. *et al.* Chronic lymphocytic leukemic cells exhibit apoptotic signaling via TRAIL-R1. *Cell Death Differ.* **12**, 773–782 (2005).
85. Takeda, K. *et al.* Induction of tumor-specific T cell immunity by anti-DR5 antibody therapy. *J. Exp. Med.* **199**, 437–448 (2004).
86. Clynes, R., Takechi, Y., Moroi, Y., Houghton, A. & Ravetch, J. V. Fc receptors are required in passive and active immunity to melanoma. *Proc. Natl Acad. Sci. USA* **95**, 652–656 (1998).
87. Clynes, R. A., Towers, T. L., Presta, L. G. & Ravetch, J. V. Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nature Med.* **6**, 443–446 (2000).
Key paper demonstrating the importance of FcR in the anti-tumour activity of therapeutic antibodies.
88. Wu, G. S. *et al.* KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. *Nature Genet.* **17**, 141–143 (1997).
89. Wen, J. *et al.* Antileukemic drugs increase death receptor 5 levels and enhance Apo-2L-induced apoptosis of human acute leukemia cells. *Blood* **96**, 3900–3906 (2000).
90. Meurette, O. *et al.* Cytotoxicity of TRAIL/anticancer drug combinations in human normal cells. *Ann. N. Y. Acad. Sci.* **1090**, 209–216 (2006).
91. Koschny, R., Walczak, H. & Ganten, T. M. The promise of TRAIL — potential and risks of a novel anticancer therapy. *J. Mol. Med.* **85**, 923–935 (2007).
92. Bolden, J. E., Peart, M. J. & Johnstone, R. W. Anticancer activities of histone deacetylase inhibitors. *Nature Rev. Drug Discov.* **5**, 769–784 (2006).
93. Guo, F. *et al.* Cotreatment with histone deacetylase inhibitor LAQ824 enhances Apo-2L/tumor necrosis factor-related apoptosis inducing ligand-induced death inducing signaling complex activity and apoptosis of human acute leukemia cells. *Cancer Res.* **64**, 2580–2589 (2004).
94. Rosato, R. R., Almenara, J. A., Dai, Y. & Grant, S. Simultaneous activation of the intrinsic and extrinsic pathways by histone deacetylase (HDAC) inhibitors and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically induces mitochondrial damage and apoptosis in human leukemia cells. *Mol. Cancer Ther.* **2**, 1273–1284 (2003).
95. Zhang, X. D., Gillespie, S. K., Borrow, J. M. & Hersey, P. The histone deacetylase inhibitor suberic bishydroxamate: a potential sensitizer of melanoma to TNF-related apoptosis-inducing ligand (TRAIL) induced apoptosis. *Biochem. Pharmacol.* **66**, 1537–1545 (2003).
96. Lindemann, R. K. *et al.* Analysis of the apoptotic and therapeutic activities of histone deacetylase inhibitors by using a mouse model of B cell lymphoma. *Proc. Natl Acad. Sci. USA* **104**, 8071–8076 (2007).
97. Vanoosten, R. L., Moore, J. M., Ludwig, A. T. & Griffith, T. S. Depsipeptide (FR901228) enhances the cytotoxic activity of TRAIL by redistributing TRAIL receptor to membrane lipid rafts. *Mol. Ther.* **11**, 542–552 (2005).
98. Volkmann, X. *et al.* Increased hepatotoxicity of tumor necrosis factor-related apoptosis-inducing ligand in diseased human liver. *Hepatology* **46**, 1498–1508 (2007).
99. Frew, A. J. *et al.* Combination therapy of established cancer using a histone deacetylase inhibitor and a TRAIL receptor agonist. *Proc. Natl Acad. Sci. USA* **105**, 11317–11322 (2008).

100. Martelli, A. M. *et al.* A new selective AKT pharmacological inhibitor reduces resistance to chemotherapeutic drugs, TRAIL, all-*trans*-retinoic acid, and ionizing radiation of human leukemia cells. *Leukemia* **17**, 1794–1805 (2003).
101. Bremer, E. *et al.* Simultaneous inhibition of epidermal growth factor receptor (EGFR) signaling and enhanced activation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor-mediated apoptosis induction by an scFv:sTRAIL fusion protein with specificity for human EGFR. *J. Biol. Chem.* **280**, 10025–10033 (2005).
102. Bortul, R. *et al.* Constitutively active Akt1 protects HL60 leukemia cells from TRAIL-induced apoptosis through a mechanism involving NF-κB activation and $cFLIP_L$ up-regulation. *Leukemia* **17**, 379–389 (2003).
103. Thakkar, H. *et al.* Pro-survival function of Akt/protein kinase B in prostate cancer cells. Relationship with TRAIL resistance. *J. Biol. Chem.* **276**, 38361–38369 (2001).
104. Kim, K. M. & Lee, Y. J. Amiloride augments TRAIL-induced apoptotic death by inhibiting phosphorylation of kinases and phosphatases associated with the P13K–Akt pathway. *Oncogene* **24**, 355–366 (2005).
105. Panner, A., James, C. D., Berger, M. S. & Pieper, R. O. mTOR controls FLIPS translation and TRAIL sensitivity in glioblastoma multiforme cells. *Mol. Cell Biol.* **25**, 8809–8823 (2005).
106. McCubrey, J. A. *et al.* Targeting survival cascades induced by activation of Ras/Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways for effective leukemia therapy. *Leukemia* **22**, 708–722 (2008).
107. Safa, A. R., Day, T. W. & Wu, C. H. Cellular FLICE-like inhibitory protein (C-FLIP): a novel target for cancer therapy. *Curr. Cancer Drug Targets.* **8**, 37–46 (2008).
108. Bockbrader, K. M., Tan, M. & Sun, Y. A small molecule Smac-mimic compound induces apoptosis and sensitizes TRAIL- and etoposide-induced apoptosis in breast cancer cells. *Oncogene* **24**, 7381–7388 (2005).
109. Li, L. *et al.* A small molecule Smac mimic potentiates TRAIL- and TNFα-mediated cell death. *Science* **305**, 1471–1474 (2004).
110. Karikari, C. A. *et al.* Targeting the apoptotic machinery in pancreatic cancers using small-molecule antagonists of the X-linked inhibitor of apoptosis protein. *Mol. Cancer Ther.* **6**, 957–966 (2007).
111. Vince, J. E. *et al.* IAP antagonists target cIAP1 to induce TNFα-dependent apoptosis. *Cell* **131**, 682–693 (2007).
112. Varfolomeev, E. *et al.* IAP antagonists induce autoubiquitination of c-IAPs, NF-κB activation, and TNFα-dependent apoptosis. *Cell* **131**, 669–681 (2007).
113. Petersen, S. L. *et al.* Autocrine TNFα signaling renders human cancer cells susceptible to Smac-mimetic-induced apoptosis. *Cancer Cell* **12**, 445–456 (2007).
114. Gaither, A. *et al.* A Smac mimetic rescue screen reveals roles for inhibitor of apoptosis proteins in tumor necrosis factor-α signaling. *Cancer Res.* **67**, 11493–11498 (2007).
115. Aza-Blanc, P. *et al.* Identification of modulators of TRAIL-induced apoptosis via RNAi-based phenotypic screening. *Mol. Cell* **12**, 627–637 (2003).
116. Rottmann, S., Wang, Y., Nasoff, M., Deveraux, Q. L. & Quon, K. C. A TRAIL receptor-dependent synthetic lethal relationship between MYC activation and GSK3β/FBW7 loss of function. *Proc. Natl Acad. Sci. USA* **102**, 15195–15200 (2005).
117. Ovcharenko, D., Kelnar, K., Johnson, C., Leng, N. & Brown, D. Genome-scale microRNA and small interfering RNA screens identify small RNA modulators of TRAIL-induced apoptosis pathway. *Cancer Res.* **67**, 10782–10788 (2007).
118. Wang, Y. *et al.* Synthetic lethal targeting of MYC by activation of the DR5 death receptor pathway. *Cancer Cell* **5**, 501–512 (2004).
References 115–118 describe synthetic lethal screens to identify regulators of TRAIL-mediated apoptosis. Three of the screens used siRNA libraries.
119. Ricci, M. S. *et al.* Direct repression of FLIP expression by c-myc is a major determinant of TRAIL sensitivity. *Mol. Cell Biol.* **24**, 8541–8555 (2004).
120. Pardoll, D. & Allison, J. Cancer immunotherapy: breaking the barriers to harvest the crop. *Nature Med.* **10**, 887–892 (2004).
121. Uno, T. *et al.* Eradication of established tumors in mice by a combination antibody-based therapy. *Nature Med.* **12**, 693–698 (2006).
122. Dunn, G. P., Old, L. J. & Schreiber, R. D. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* **21**, 137–148 (2004).
123. Smyth, M. J. *et al.* IL-21 enhances tumor-specific CTL induction by anti-DR5 antibody therapy. *J. Immunol.* **176**, 6347–6355 (2006).
124. Smyth, M. J. *et al.* IL-21 enhances antibody-mediated tumor rejection. *Cancer Res.* **68**, 1–7 (2008).
125. Hallett, W. H. *et al.* Sensitization of tumor cells to NK cell-mediated killing by proteasome inhibition. *J. Immunol.* **180**, 163–170 (2008).
126. Maddipatla, S., Hernandez-Ilizaliturri, F. J., Knight, J. & Czuczman, M. S. Augmented antitumor activity against B-cell lymphoma by a combination of monoclonal antibodies targeting TRAIL-R1 and CD20. *Clin. Cancer Res.* **13**, 4556–4564 (2007).
127. Daniel, D. *et al.* Cooperation of the proapoptotic receptor agonist rhApo2L/TRAIL with the CD20 antibody rituximab against non-Hodgkin lymphoma xenografts. *Blood* **110**, 4037–4046 (2007).
128. Daniels, R. A. *et al.* Expression of TRAIL and TRAIL receptors in normal and malignant tissues. *Cell Res.* **15**, 430–438 (2005).
129. Degli-Esposti, M. To die or not to die — the quest of the TRAIL receptors. *J. Leukoc. Biol.* **65**, 535–542 (1999).
130. Sheridan, J. P. *et al.* Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science* **277**, 818–821 (1997).
131. Tolcher, A. W. *et al.* Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor-related apoptosis-inducing ligand receptor-1. *J. Clin. Oncol.* **25**, 1390–1395 (2007).
132. Le, L. H. *et al.* Phase I study of a fully human monoclonal antibody to the tumor necrosis factor-related apoptosis-inducing ligand death receptor 4 (TRAIL-R1) in subjects with advanced solid malignancies or non-Hodgkin's lymphoma (NHL). *J. Clin. Oncol.* **22**, 2533 (2004).
133. Hotte, S. J. *et al.* HGS-ETR1, a fully human monoclonal antibody to the tumor necrosis factor-related apoptosis-inducing ligand death receptor 1 (TRAIL-R1) in patients with advanced solid cancer: Results of a phase 1 trial. *J. Clin. Oncol.* **23**, 3052 (2004).
134. Chow, L. Q. *et al.* HGS-ETR1, an antibody targeting TRAIL-R1, in combination with paclitaxel and carboplatin in patients with advanced solid malignancies: Results of a phase 1 and PK study. *J. Clin. Oncol.***24**, 2515 (2006).
135. Greco, F. A. *et al.* Phase 2 study of mapatumumab, a fully human agonistic monoclonal antibody which targets and activates the TRAIL receptor-1, in patients with advanced non-small cell lung cancer. *Lung Cancer* (2008).
136. Younes, A. *et al.* Results of a phase 2 trial of HGS-ETR1 (agonistic human monoclonal antibody to TRAIL receptor 1) in subjects with relapsed/refractory non-Hodgkin's lymphoma (NHL). *Blood* **106**, Abstract 489 (2005).
137. Plummer, R. *et al.* Phase 1 and pharmacokinetic study of lexatumumab in patients with advanced cancers. *Clin. Cancer Res.* **13**, 6187–6194 (2007).
References 131 and 137 are the first published results of early-phase clinical trials using anti-TRAILR2 (lexatumumab) and -TRAILR1 (mapatumumab) mAbs respectively.
138. Patnaik, A. *et al.* HGS-ETR2 — A fully human monoclonal antibody to TRAIL-R2: results of a phase I trial in patients with advanced solid tumors. *J. Clin. Oncol.* **24**, 3012 (2006).
139. Sikic, B. I. *et al.* A phase Ib study to assess the safety of lexatumumab, a human monoclonal antibody that activates TRAIL-R2, in combination with gemcitabine, pemetrexed, doxorubicin or FOLFIRI. *J. Clin. Oncol.* **25**, 14006 (2007).
140. Rowinsky, E. K. Targeted induction of apoptosis in cancer management: the emerging role of tumor necrosis factor-related apoptosis-inducing ligand receptor activating agents. *J. Clin. Oncol.* **23**, 9394–9407 (2005).
141. LoRusso, P. *et al.* First-in-human study of AMG 655, a pro-apoptotic TRAIL receptor-2 agonist, in adult patients with advanced solid tumors. *J. Clin. Oncol.* **25**, 3534 (2007).
142. Camidge, D. *et al.* A phase I safety and pharmacokinetic study of apomab, a human DR5 agonist antibody, in patients with advanced cancer. *J. Clin. Oncol.* **25**, 3582 (2007).
143. Herbst, R. S. *et al.* A phase I safety and pharmacokinetic (PK) study of recombinant Apo2L/TRAIL, an apoptosis-inducing protein in patients with advanced cancer. *J. Clin. Oncol.* **24**, 3013 (2006).
144. Ling, J. *et al.* Apo2L/TRAIL pharmacokinetics in a phase 1a trial in advanced cancer and lymphoma. *J. Clin. Oncol.* **24**, 3047 (2006).
145. Pan, Y. *et al.* Application of pharmacodynamic assays in a phase Ia trial of Apo2L/TRAIL in patients with advanced tumors. *J. Clin. Oncol.* **25**, 3535 (2007).
146. Yee, L. *et al.* A phase IB safety and pharmacokinetic (PK) study of recombinant human Apo2L/TRAIL in combination with rituximab in patients with low-grade non-Hodgkin lymphoma. *J. Clin. Oncol.* **25**, 8078 (2007).
147. Jo, M. *et al.* Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. *Nature Med.* **6**, 564–567 (2000).
148. Lawrence, D. *et al.* Differential hepatocyte toxicity of recombinant Apo2L/TRAIL versions. *Nature Med.* **7**, 383–385 (2001).
149. Mori, E. *et al.* Human normal hepatocytes are susceptible to apoptosis signal mediated by both TRAIL-R1 and TRAIL-R2. *Cell Death Differ.* **11**, 203–207 (2004).
150. Qin, J., Chaturvedi, V., Bonish, B. & Nickoloff, B. J. Avoiding premature apoptosis of normal epidermal cells. *Nature Med.* **7**, 385–386 (2001).
References 147–150 describe apoptosis of normal cells induced by TRAIL that is dependent on the method used for production of the recombinant soluble protein. Non-tagged, zinc-containing rTRAIL was not toxic against cultured primary hepatocytes or keratinocytes.
151. Kahraman, A. *et al.* TRAIL mediates liver injury by the innate immune system in the bile duct-ligated mouse. *Hepatology* **47**, 1317–1330 (2008).
152. Takeda, K. *et al.* Death receptor 5 mediated-apoptosis contributes to cholestatic liver disease. *Proc. Natl Acad. Sci. USA* **105**, 10895–10900 (2008).
153. Hayakawa, Y. *et al.* NK cell TRAIL eliminates immature dendritic cells *in vivo* and limits dendritic cell vaccination efficacy. *J. Immunol.* **172**, 123–129 (2004).
154. Leverkus, M. *et al.* Maturation of dendritic cells leads to up-regulation of cellular FLICE-inhibitory protein and concomitant down-regulation of death ligand-mediated apoptosis. *Blood* **96**, 2628–2631 (2000).
155. Diehl, G. E. *et al.* TRAIL-R. as a negative regulator of innate immune cell responses. *Immunity* **21**, 877–889 (2004).
156. Higuchi, H., Bronk, S. F., Taniai, M., Canbay, A. & Gores, G. J. Cholestasis increases tumor necrosis factor-related apoptotis-inducing ligand (TRAIL)-R2/DR5 expression and sensitizes the liver to TRAIL-mediated cytotoxicity. *J. Pharmacol. Exp. Ther.* **303**, 461–467 (2002).
157. Janssen, H. L., Higuchi, H., Abdulkarim, A. & Gores, G. J. Hepatitis B virus enhances tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) cytotoxicity by increasing TRAIL-R1/death receptor 4 expression. *J. Hepatol* **39**, 414–420 (2003).
158. Mundt, B. *et al.* Involvement of TRAIL and its receptors in viral hepatitis. *FASEB J.* **17**, 94–96 (2003).
159. Mundt, B. *et al.* Tumour necrosis factor related apoptosis inducing ligand (TRAIL) induces hepatic steatosis in viral hepatitis and after alcohol intake. *Gut* **54**, 1590–1596 (2005).
160. Pan, G. *et al.* An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* **277**, 815–818 (1997).
161. Degli-Esposti, M. A. *et al.* Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *J. Exp. Med.* **186**, 1165–1170 (1997).

162. Marsters, S. A. *et al.* A novel receptor for Apo2L/TRAIL contains a truncated death domain. *Curr. Biol.* **7**, 1003–1006 (1997).
163. Griffith, T. S. *et al.* Functional analysis of TRAIL receptors using monoclonal antibodies. *J. Immunol.* **162**, 2597–2605 (1999).
164. Clancy, L. *et al.* Preligand assembly domain-mediated ligand-independent association between TRAIL receptor 4 (TR4) and TR2 regulates TRAIL-induced apoptosis. *Proc. Natl Acad. Sci. USA* **102**, 18099–18104 (2005).
165. Merino, D. *et al.* Differential inhibition of TRAIL-mediated DR5-DISC formation by decoy receptors 1 and 2. *Mol. Cell Biol.* **26**, 7046–7055 (2006).
166. Chaudhary, P. M. *et al.* Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-κB pathway. *Immunity* **7**, 821–830 (1997).
167. Romagnoli, M. *et al.* Canonical nuclear factor κB pathway inhibition blocks myeloma cell growth and induces apoptosis in strong synergy with TRAIL. *Clin. Cancer Res.* **13**, 6010–6018 (2007).
168. Ravi, R. *et al.* Regulation of death receptor expression and TRAIL/Apo2L-induced apoptosis by NF-κB. *Nature Cell Biol.* **3**, 409–416 (2001).
169. Roue, G. *et al.* Selective inhibition of IκB kinase sensitizes mantle cell lymphoma B cells to TRAIL by decreasing cellular FLIP level. *J. Immunol.* **178**, 1923–1930 (2007).
170. Khanbolooki, S. *et al.* Nuclear factor-κB maintains TRAIL resistance in human pancreatic cancer cells. *Mol. Cancer Ther.* **5**, 2251–2260 (2006).
171. Morales, J. C., Ruiz-Magana, M. J. & Ruiz-Ruiz, C. Regulation of the resistance to TRAIL-induced apoptosis in human primary T lymphocytes: role of NF-κB inhibition. *Mol. Immunol.* **44**, 2587–2597 (2007).
172. Luo, J. L., Maeda, S., Hsu, L. C., Yagita, H. & Karin, M. Inhibition of NF-κB in cancer cells converts inflammation- induced tumor growth mediated by TNFα to TRAIL-mediated tumor regression. *Cancer Cell* **6**, 297–305 (2004).
173. Ganten, T. M. *et al.* Proteasome inhibition sensitizes hepatocellular carcinoma cells, but not human hepatocytes, to TRAIL. *Hepatology* **42**, 588–597 (2005).
174. Ishimura, N., Isomoto, H., Bronk, S. F. & Gores, G. J. Trail induces cell migration and invasion in apoptosis-resistant cholangiocarcinoma cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **290**, G129–G136 (2006).
175. Chen, X., Kandasamy, K. & Srivastava, R. K. Differential roles of RelA (p65) and c-Rel subunits of nuclear factor κB in tumor necrosis factor-related apoptosis-inducing ligand signaling. *Cancer Res.* **63**, 1059–1066 (2003).
176. Manning, B. D. & Cantley, L. C. AKT/PKB signaling: navigating downstream. *Cell* **129**, 1261–1274 (2007).
177. Secchiero, P. *et al.* TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. *Circulation* **107**, 2250–2256 (2003).
178. Zauli, G. *et al.* PI-3K/Akt and NF-κB/IκBα pathways are activated in Jurkat T cells in response to TRAIL treatment. *J. Cell Physiol.* **202**, 900–911 (2005).
179. Samuels, Y. *et al.* Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell* **7**, 561–573 (2005).
180. Song, J. J. & Lee, Y. J. Differential cleavage of Mst1 by caspase-7/-3 is responsible for TRAIL-induced activation of the MAPK superfamily. *Cell Signal* **20**, 892–906 (2008).
181. Siegmund, D. *et al.* Role of caspases in CD95L- and TRAIL-induced non-apoptotic signalling in pancreatic tumour cells. *Cell Signal* **19**, 1172–1184 (2007).
182. Sah, N. K. *et al.* Translation inhibitors sensitize prostate cancer cells to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by activating c-Jun N-terminal kinase. *J. Biol. Chem.* **278**, 20593–20602 (2003).
183. Weldon, C. B. *et al.* Sensitization of apoptotically-resistant breast carcinoma cells to TNF and TRAIL by inhibition of p38 mitogen-activated protein kinase signaling. *Int. J. Oncol.* **24**, 1473–1480 (2004).
184. Frese, S. *et al.* PG490-mediated sensitization of lung cancer cells to Apo2L/TRAIL-induced apoptosis requires activation of ERK2. *Oncogene* **22**, 5427–5435 (2003).
185. Ohtsuka, T. *et al.* Synergistic induction of tumor cell apoptosis by death receptor antibody and chemotherapy agent through JNK/p38 and mitochondrial death pathway. *Oncogene* **22**, 2034–2044 (2003).
186. Irmler, M. *et al.* Inhibition of death receptor signals by cellular FLIP. *Nature* **388**, 190–195 (1997).
187. Schneider, P. *et al.* TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF-κB. *Immunity* **7**, 831–836 (1997).
188. Wajant, H. Targeting the FLICE Inhibitory Protein (FLIP) in cancer therapy. *Mol. Interv.* **3**, 124–127 (2003).
189. Mitsiades, C. S. *et al.* Fas signaling in thyroid carcinomas is diverted from apoptosis to proliferation. *Clin. Cancer Res.* **12**, 3705–3712 (2006).
190. Vucic, D. & Fairbrother, W. J. The inhibitor of apoptosis proteins as therapeutic targets in cancer. *Clin. Cancer Res.* **13**, 5995–6000 (2007).
191. Vaux, D. L. & Silke, J. IAPs, RINGs and ubiquitylation. *Nature Rev. Mol. Cell Biol.* **6**, 287–297 (2005).
192. Zhang, H. G., Wang, J., Yang, X., Hsu, H. C. & Mountz, J. D. Regulation of apoptosis proteins in cancer cells by ubiquitin. *Oncogene* **23**, 2009–2015 (2004).
193. Verhagen, A. M. *et al.* Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* **102**, 43–53 (2000).
194. Du, C., Fang, M., Li, Y., Li, L. & Wang, X. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. *Cell* **102**, 33–42 (2000).
195. Ng, C. P. & Bonavida, B. X-linked inhibitor of apoptosis (XIAP) blocks Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of prostate cancer cells in the presence of mitochondrial activation: sensitization by overexpression of second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI (Smac/DIABLO). *Mol. Cancer Ther.* **1**, 1051–1058 (2002).
196. Vogler, M., Durr, K., Jovanovic, M., Debatin, K. M. & Fulda, S. Regulation of TRAIL-induced apoptosis by XIAP in pancreatic carcinoma cells. *Oncogene* **26**, 248–257 (2007).
197. Shamimi-Noori, S. *et al.* Cisplatin enhances the antitumor effect of tumor necrosis factor-related apoptosis-inducing ligand gene therapy via recruitment of the mitochondria-dependent death signaling pathway. *Cancer Gene Ther.* **15**, 356–370 (2008).
198. Belyanskaya, L. L. *et al.* Human agonistic TRAIL receptor antibodies Mapatumumab and Lexatumumab induce apoptosis in malignant mesothelioma and act synergistically with cisplatin. *Mol. Cancer* **6**, 66 (2007).
199. El-Zawahry, A., McKillop, J. & Voelkel-Johnson, C. Doxorubicin increases the effectiveness of Apo2L/TRAIL for tumor growth inhibition of prostate cancer xenografts. *BMC Cancer* **5**, 2 (2005).
200. Dumitru, C. A., Carpinteiro, A., Trarbach, T., Hengge, U. R. & Gulbins, E. Doxorubicin enhances TRAIL-induced cell death via ceramide-enriched membrane platforms. *Apoptosis* **12**, 1533–1541 (2007).
201. Broaddus, V. C. *et al.* Bid mediates apoptotic synergy between tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and DNA damage. *J. Biol. Chem.* **280**, 12486–12493 (2005).
202. Shankar, S., Singh, T. R. & Srivastava, R. K. Ionizing radiation enhances the therapeutic potential of TRAIL in prostate cancer *in vitro* and *in vivo*: Intracellular mechanisms. *Prostate* **61**, 35–49 (2004).
203. Ray, S. & Almasan, A. Apoptosis induction in prostate cancer cells and xenografts by combined treatment with Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand and CPT-11. *Cancer Res.* **63**, 4713–4723 (2003).
204. Shankar, S., Chen, X. & Srivastava, R. K. Effects of sequential treatments with chemotherapeutic drugs followed by TRAIL on prostate cancer *in vitro* and *in vivo*. *Prostate* **62**, 165–186 (2005).
205. Wang, X. *et al.* 17-Allylamino-17-demethoxygeldanamycin synergistically potentiates tumor necrosis factor-induced lung cancer cell death by blocking the nuclear factor-κB pathway. *Cancer Res.* **66**, 1089–1095 (2006).
206. Kim, K. M., Song, J. J., An, J. Y., Kwon, Y. T. & Lee, Y. J. Pretreatment of acetylsalicylic acid promotes tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by down-regulating BCL-2 gene expression. *J. Biol. Chem.* **280**, 41047–41056 (2005).
207. Bai, J. *et al.* Predominant Bcl-XL knockdown disables antiapoptotic mechanisms: tumor necrosis factor-related apoptosis-inducing ligand-based triple chemotherapy overcomes chemoresistance in pancreatic cancer cells *in vitro*. *Cancer Res.* **65**, 2344–2352 (2005).
208. Deeb, D. *et al.* Curcumin sensitizes prostate cancer cells to tumor necrosis factor-related apoptosis-inducing ligand/Apo2L by inhibiting nuclear factor-κB through suppression of IκBα phosphorylation. *Mol. Cancer Ther.* **3**, 803–812 (2004).
209. Chawla-Sarkar, M. *et al.* Suppression of NF-κB survival signaling by nitrosylcobalamin sensitizes neoplasms to the anti-tumor effects of Apo2L/TRAIL. *J. Biol. Chem.* **278**, 39461–39469 (2003).
210. Mitsiades, C. S. *et al.* TRAIL/Apo2L ligand selectively induces apoptosis and overcomes drug resistance in multiple myeloma: therapeutic applications. *Blood* **98**, 795–804 (2001).
211. Ricci, M. S. *et al.* Reduction of TRAIL-induced Mcl-1 and cIAP2 by c-Myc or sorafenib sensitizes resistant human cancer cells to TRAIL-induced death. *Cancer Cell* **12**, 66–80 (2007).
212. Lagneaux, L. *et al.* Valproic acid induces apoptosis in chronic lymphocytic leukemia cells through activation of the death receptor pathway and potentiates TRAIL response. *Exp. Hematol.* **35**, 1527–1537 (2007).
213. Han, H. *et al.* 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) sensitizes human leukemic HL-60 cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis through Akt downregulation. *Apoptosis* **12**, 2101–2114 (2007).
214. Shrader, M. *et al.* Gefitinib reverses TRAIL resistance in human bladder cancer cell lines via inhibition of AKT-mediated X-linked inhibitor of apoptosis protein expression. *Cancer Res.* **67**, 1430–1435 (2007).
215. Chen, X. *et al.* Constitutively active Akt is an important regulator of TRAIL sensitivity in prostate cancer. *Oncogene* **20**, 6073–6083 (2001).
216. Rosato, R. R., Dai, Y., Almenara, J. A., Maggio, S. C. & Grant, S. Potent antileukemic interactions between flavopiridol and TRAIL/Apo2L involve flavopiridol-mediated XIAP downregulation. *Leukemia* **18**, 1780–1788 (2004).

Competing interests statement

The authors declare competing financial interests: see web version for details.

Acknowledgements

R.W.J. and M.J.S. are supported by a National Health and Medical Research Council of Australia (NH&MRC) Program Grant. M.J.S. is supported by a NH&MRC Senior Principal Research Fellowship and R.W.J. by a Pfizer Fellowship and grants from the Leukaemia Foundation of Australia, the Bennelong Foundation and the Cancer Council Victoria. A.J.F. is supported by The Cancer Research Institute Predoctoral Emphasis Pathway in Tumour Immunology. We thank A. Ashkenazi, G. Begley and R. Humphries for helpful discussions.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BUB1 | *FUT3* | *FUT6* | *GALNT14* | *GALNT3* | *GSK3A* | *GSK3B* | *MLL* | *MYCN* | *PAK1* | *Trp53*

National Cancer Institute: http://www.cancer.gov/
bladder cancer | breast cancer | chronic lymphocytic leukaemia | chronic myeloid leukaemia | colorectal cancer | fibrosarcoma | head and neck cancer | lung cancer | lymphoma | neuroblastoma | non-Hodgkin lymphoma | prostate cancer |

National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary/
AMG 655 | apomab | bortezomib | cisplatin | doxorubicin | folinic acid | fluorouracil | gefitinib | gemcitabine | irinotecan | lexatumumab | mapatumumab | rapamycin | rituximab | vorinostat

UniProtKB: http://www.uniprot.org
APAF1 | BAK | BAX | BCL2L10 | BID | BIRC2 | BIRC3 | BIRC4 | caspase 3 | caspase 7 | caspase 8 | caspase 10 | CFLAR | DIABLO | ERK1 | ERK2 | FADD | FASLG | FRAP1 | IL21 | MYC | NEMO | perforin 1 | REL | RELA | RIP | TNFSF10 | TNFRSF10A | TNFRSF10B | TNFRSF10D | TNFRSF11B | TNFRSF11B | TNFRSF1A | TNFRSF6 | TRADD | TRAF2

FURTHER INFORMATION

R. W. Johnstone's homepage: http://www.petermac-research.org/default.php?doc_id=31&title=Gene%20Regulation

ALL LINKS ARE ACTIVE IN THE ONLINE PDF