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TRAIL/TRAIL-R in Hematologic Malignancies

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ABSTRACT

Defects in apoptosis are observed in many cancer cell types and contribute in a relevant way to tumorigenesis. Apoptosis is a complex and well-regulated cell death program that plays a key role in the control of cell homeostasis, particularly at the level of the hematopoietic system. Apoptosis can be initiated through two different mechanisms involving either activation of the death receptors (extrinsic pathway) or activation of a mitochondrial apoptotic process (intrinsic pathway). Among the various death receptors a peculiar role is played by TNF-related apoptosis-inducing ligand (TRAIL)-receptors (TRAIL-Rs) and their ligand TRAIL. TRAIL recently received considerable interest for its potent anti-tumor killing activity, sparing normal cells. Here, we will review the expression and the abnormalities of TRAIL/TRAIL-R system in hematologic malignancies. The large majority of primary hematologic tumors are resistant to TRAIL-mediated apoptosis, basically due to the activation of anti-apoptotic signaling pathway (such as NF-κB), overexpression of anti-apoptotic proteins (such as FLIP, Bcl-2, XIAP) or expression of TRAIL decoy receptors or reduced TRAIL-R1/-R2 expression. Strategies have been developed to bypass this TRAIL resistance and are based on the combination of TRAIL with chemotherapy or radiotherapy, or with proteasome or histone deacetylase or NF-κB inhibitors. The agents used in combination with TRAIL either enhance TRAIL-R1/-R2 expression or decrease expression of anti-apoptotic proteins (c-FLIP, XIAP, Bcl-2). Many of these combinatorial therapies hold promise for future developments in treatment of hematologic malignancies. J. Cell. Biochem. 110: 21–34, 2010.

KEY WORDS: LEUKEMIA; LYMPHOMA; APOPTOSIS; TRAIL

Drug resistance is a major challenge for cancer treatment. One mechanism through which cancer cells develop resistance to cytotoxic agents and radiation is related to apoptosis. Apoptosis is a complex and well-regulated process of cell death pre-programmed inside the cell that plays a key role in the control of cell homeostasis, differentiation and survival. Apoptosis can be initiated essentially through two different mechanisms involving either activation of death receptors on the cell membranes (*extrinsic pathway*) or activation of a series of cellular events primarily processed at mitochondria (*intrinsic pathway*).

Apoptosis plays an important role in tumorigenesis and cancer treatment. Defects in apoptosis are observed in many cancer cell types and contribute in a relevant way to the expansion of the malignant cells. Furthermore, since the death of tumor cells induced by chemotherapy and radiotherapy is largely mediated by activation of apoptosis, mechanisms of inhibition of apoptosis operating in cancer cells will make these cells resistant to anti-tumor treatment. Particularly, molecular changes of the apoptotic machinery that cause a dysregulation of apoptosis, including activation or enhanced expression of antiapoptotic factors (such as BCL-2, BCL-X_L), inactivation of master pro-apoptotic regulators (p53 or p53 pathway), enhanced expression or activity of pro-survival factors, including hematologic malignancies. Furthermore, abnormalities of the death receptor pathway, involving either FasL or TRAIL and their receptors, play a role in the genesis of some solid and hematologic tumors.

Herein, I will review the abnormalities of the TRAIL/TRAIL-R system in the various hematologic malignancies. Furthermore, I will discuss therapeutic potential strategies or interventions that can lower the threshold for TRAIL-mediated apoptosis of leukemia and lymphoma cells that could became useful approaches to treat lymphomas or leukemias.

TRAIL AND TRAIL-Rs: STRUCTURAL FEATURES

TRAIL was discovered through its high sequence homology to FasL and TNF (Table I). TRAIL is a type II transmembrane protein anchored to the cell membrane, with a carboxyl terminus located extracellularly and containing the receptor binding domain. Membrane-bound TRAIL can be cleaved to a soluble TRAIL species through the action of metalloproteases. Importantly, the soluble TRAIL possesses biological activity as well as the membrane-bound form. TRAIL is able to form trimers, like all the other ligands of the

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TABLE I. Biochemical, Molecular Properties, Nomenclature, and Ligands of the Main Receptors of the TNF Receptor Superfamily

Receptor	Chromosome location	Gene nomenclature (HUGO)	Ligand	Chromosome location	Gene nomenclature (HUGO)	Adaptor
TNFR1	12p13.2	TNFRSF1A	TNF, LTα	6p21.3	TNF, TNFB	TRADD
CD95 (Fas) FAS1 APO-1	10p24.1	FAS	CD95L FASL CD718	1q23	FASLG	FADD
DR3 TR3 TRAMP	1p36.2	TNFSRF25	TLA1A TL1	9q32	TNFSF 15	TRADD
DR4 TRAIL-R1 CD261 APO-2	8p21	TNFSR10A	Apo2L/TRAIL CD253	3q26	TNFSF 10	FADD
DR5 TRAIL-R2 CD262 KILLER	8p22-p21	TNFSR10B	Apo2L/TRAIL CD253	3q26	TNFSF 10	FADD
DcR1 TRAIL-R3 CD263 TRID	8p22-p21	TNFSR10C	Apo2L/TRAIL CD253	3q26	TNFSF 10	FADD
DcR2 TRAIL-R4 CD264 TRUNDD	8p21	TNFSR10D	Apo2L/TRAIL CD253	3q26	TNFSF 10	FADD

TNF family. TRAIL activity is dependent on the integrity of a cysteine residue (Cys 230), able to bind a zinc ion.

TRAIL is able to bind to five different membrane receptors, called TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 [LIT, Decoy Receptor (DcR)1], TRAIL-R4 [TRUNDD, DcR2] and Ostoprotegerin (OPG) (Table I). TRAIL-R1 and R2 contain a cytoplasmic death domain capable of recruiting apoptosis signaling molecules and inducing cell death. In contrast, TRAIL-R3 and R4 do not contain in their cytoplasmic tails functional death domains and act as decoy receptors, through a sequestering action on TRAIL, thus preventing its binding to TRAIL functional TRAIL-R1 and R2 receptors. OPG is also able to bind TRAIL, but with a very low affinity, thus indicating that its role as a TRAIL receptor under physiologic conditions is highly unlikely.

The peculiar property of TRAIL-R1 and R2 consists in the presence of a death domain (DD) in their intracellular portion: the DD consists in six to seven α-helices motif able to bind the other DD via homotypic interactions. TRAIL-R3 does not possess a DD; its intracellular domain acts as a glycosylphosphatidylinositol (GPI) anchor. TRAIL-R4 possesses in its intracellular portion a truncated DD which does not induce death signaling.

The physiologic role of TRAIL and its receptors in tumor immunosurveillance is supported by gene knockout experiments. Particularly, TRAIL knockout mice displayed an increased susceptibility to tumor initiation and metastasis [Cretney et al., 2002]. Studies carried out in TRAIL-R-deficient mice also support a role for TRAIL as a tumor suppressor: in one study TRAIL-R-deficient mice showed an increased tendency to various types of experimental tumorigenesis [Finnberg et al., 2008], while in the other study TRAIL-R deficiency in mice enhanced lymph node metastasis without affecting primary tumor development [Grosse-Wilde et al., 2008].

TRAIL-R1 AND R2 SIGNALING

TRAIL-R1 and R2 exist as preassembled receptor oligomers on the cell surface. TRAIL binding to these receptors causes a conformational change in the preassembled receptor, that leads to the recruitment of the adaptor protein Fas-ssociated death domain (FADD) and the initiator caspases 8 and 10 as pro-caspases, forming a death-inducing signaling complex (DISC) (Fig. 1). This triggers activation of the apical caspases, inducing their autocatalytic processing and release into the cytoplasm, where they activate the effector caspases 3, 6, and 7 (Fig. 1).

A recent study has elucidated in more detail the mechanism of DISC formation. DISC formation implies the binding of a death ligand to its specific death receptor, their oligomerization at the level of specialized regions of the cell membrane (lipid rafts) favored by the interaction of the various death receptor molecules and then favoring their open conformation with exposure of DD able to interact with FADD: this process progresses through stepwise receptor clustering, with open death receptor molecules interacting via their globular domains, linked by a multitude of weak Fas/FADD bridges, leading to overall stable DISC clusters [Scott et al., 2009].

The active caspase 8 dimer is maintained stable to activate its downstream targets through a process that involves polyubiquitination: caspase-8 is polyubiquitinated at the DISC by the neddylated form of CUL3/RBX1, thus permitting p62 binding; autocatalytic processing of caspase-8 separates its recruitment and catalytic domains; the catalytic domain is transported by p62 to ubiquitin-rich aggregosomes in the cytosol, where it remains in an active form through the stabilization of its dimeric conformation [Jin et al., 2009].

DISC formation is modulated by several inhibitory mechanisms; among them a peculiar role is played by cellular FLICE Inhibitory Protein c-FLIP, which associates with DISC by interacting with FADD to block initiator caspase activation (Fig. 1). Several c-FLIP variants are generated by alternative mRNA splicing and three of them are expressed at the protein level: c-FLIP long c-$FLIP_L$, c-FLIP short c-$FLIP_S$ and c-$FLIP_R$. All these three isoforms possess two death effector domains, but only c-$FLIP_L$ possesses also a caspase-like domain. However, since c-$FLIP_L$ lacks a critical cysteine residue has no cysteine protease activity. While the inhibitory role of c-$FLIP_S$ on caspase-8 activation is well established, c-$FLIP_L$ in addition to the above mentioned antiapoptotic effects, may have also in some cell types a pro-apoptotic function (this last effect being mediated through the formation of caspase-8: c-$FLIP_L$ heterodimers).

In addition to its pro-apoptotic function through the extrinsic apoptotic pathway, TRAIL has been shown in some cell types to initiate mitogenic and prosurvival signals, including activation of nuclear factor (NF)-κB, protein kinas B (PKB or AKT) and mitogen activated protein kinases (MAPKs). This nonapoptotic TRAIL signaling is particularly evident in cells resistant to the cytotoxic effect of TRAIL. The proinflammatory nature of the non-apoptotic TRAIL response is of special relevance because it might boost the metastatic and invasive potential of apoptosis-resistant tumor cells.

A recent study suggested an intriguing relation between TRAIL/TRAIL-R pathway and Par-4/GRP78. Prostate apoptosis response-4

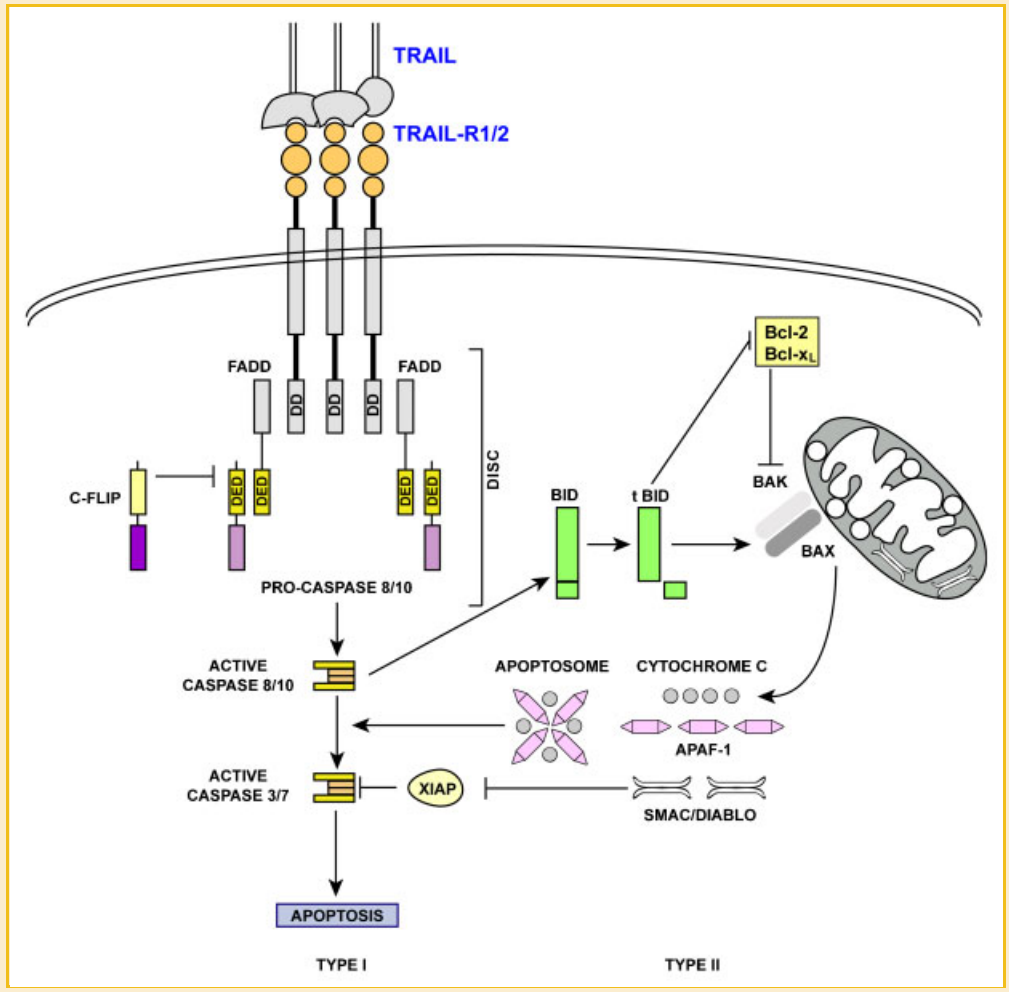

Fig. 1. Schematic overview of the TRAIL apoptosis pathway. Binding of a TRAIL trimer to TRAIL-R1 or TRAIL-R2 leads to receptor trimerization and formation of the death-inducing signaling complex (DISC) through first FADD recruitment, which interacts with TRAIL-R via the death domains (DD), and then caspase 8/10 which interact with FADD via their death effector domains (DED). As a consequence of these events, caspase-8 (or/and caspase-10) is activated. The amount of caspase-8 activity generated in type I cells is sufficient to activate effector caspases (caspase-3 and -7) and to trigger apoptosis. In type II cells, however, apoptotic commitment require the amplification of the apoptotic signaling through activation of a mitochondrial apoptotic response triggered by caspase-8-dependent cleavage of the Bid protein to its active form, t-Bid.

(Par-4) is a proapoptotic protein acting both at the level of cytoplasm and nucleus. Par-4 was shown to be secreted by normal and cancer cells: the extent of Par-4 secretion was increased by endoplasmic reticulum stress-inducing agents and by TRAIL [Burikhanov et al., 2009]. This secreted Par-4 molecular species is able to induce apoptosis through binding to the stress response protein, Glucose Regulated Protein-78 (GRP78), expressed on the surface of cancer cells. The interaction between Par-4 and its receptor GRP78 leads to activation of the FADD/Caspase-8/Caspase-3 activation [Burikhanov et al., 2009]. Intriguingly, TRAIL-mediated apoptosis of prostate cancer cells seems to be dependent on Par-4 signaling via GRP78 receptor, as suggested by the observation that GRP78 inhibitory antibodies markedly inhibit TRAIL-mediated apoptosis [Burikhanov et al., 2009].

In contrast to TRAIL-R1 and TRAIL-R2, TRAIL-R3 and TRAIL-R4 failed to induce apoptotic cell signaling. TRAIL-R3 and TRAIL-R4 are able to bind TRAIL, even with an affinity lower than TRAIL-R1 and TRAIL-R2, but fail to recruit FADD and to induce downstream cell signaling events leading to apoptosis. TRAIL-R3 and TRAIL-R4 inhibit TRAIL-induced apoptosis by distinct mechanisms: TRAIL-R3 acts merely as a competitor for TRAIL binding, preventing TRAIL-R1/TRAIL-R2-associated DISC assembly, while TRAIL-R4 impairs TRAIL DISC processing and initiator caspase activation [Merino et al., 2006].

TRAIL AS AN ANTI-CANCER AGENT

The fact that TRAIL induces apoptosis in a large range of tumor cell lines, largely sparing normal cells, has lead to the proposal to use recombinant TRAIL as an agent able to specifically induce death of tumor cells. From the initial in vivo experiments in animals grafted with human tumor cells rapidly emerged a superiority of TRAIL over other death ligands: the administration of TRAIL was safe, while the

other death ligands (such as FasL or TNF-α) cause rapid death by inducing shock and/or liver toxicity. Furthermore, these studies showed a significant in vivo anti-tumor effect of TRAIL [Walczak et al., 1999].

A further element of stimulation for the study of TRAIL as an anticancer drug comes from additional observations showing that TRAIL exerts its antitumor activity also in p53-negative tumor cells, synergizes with chemotherapy or radiotherapy.

An untagged version of recombinant human soluble TRAIL has been taken into clinic (AMG951, *Dulanermin*). This soluble TRAIL administered in vivo displays a short half-life (30–60 min). AMG951 was tested i9n therapy: 13% of 51 evaluable patients with solid tumors and hematologic malignancies displayed stable disease and 1 patient reported a partial response [Herbst, 2006]. AMG951 was tested in two additional studies, showing clinical responses only in a minority of patients [Ling, 2006; Pan, 2007]. In these three phase I studies no adverse events related to AMG951 administration have been observed. Interestingly, one additional study investigated the combination of AMG951 with rituximab in patients with low-grade non Hodgkin lymphoma: on 5 evaluable patients, 2 displayed a complete response, 1 a partial response and 2 stable disease [Yee et al., 2007]. An ongoing study was finalized to the evaluation of A;MG951 in patients with non-small cell lung cancer treated with chemotherapy +/− bevacizumab (NCT 00508625).

In addition to soluble TRAIL, agonistic monoclonal antibodies (mAbs) anti-TRAIL-R1 or anti-TRAIL-R2 may be used to trigger a TRAIL-mediated cytotoxic response (Fig. 2). An advantage of these mAbs over TRAIL is represented by their long half-life (14–21 days), compared to the short half-life of soluble recombinant TRAIL.

Several agonistic anti-TRAIL-R mAbs have been produced and introduced into clinical trials. The most important among these are MAPATUMAMAB (anti-TRAIL-R1, Human Genome Science), LEXATUMAMAB (anti-TRAIL-R2, Human Genome Sciences) AMG655 (anti-TRAIL-R2, Amgen) and APOMAB (anti-TRAIL-R2, Genentech) (Fig. 2). The antibodies have been used in clinical trials. MAPATUMAMAB was used in three different phase I clinical studies, showing that its administration up to 20 mg per kg was safe, but induced only limited anti-tumor effects [Hotte, 2004; Le, 2004; Tolcher et al., 2007]. Two phase II studies have been initiated with MAPATUMAMAB, one in non Hodgkin lymphoma (NHL) [Younes et al., 2005] and the other in non-small-cell lung carcinoma [Greco et al., 2008]: in the NHL trial a significant anti-tumor activity was observed in a minority of patients.

Several clinical studies have been carried out using the agonistic anti-TRAIL-R2 mAb LEXATUMAMAB showing that its administration is safe up to 10 mg per kg and, in some patients, induced a prolonged stable disease [Plummer et al., 2007; Patnaik et al., 2006]. LEXATUMAMAB was also administered in combination with chemotherapeutic drugs [Sikic et al., 2007]. Initial phase I studies have been carried out with AMG 655 [LoRusso et al., 2007] and APOMAB [Camidge et al., 2007].

The clinical utility of TRAIL or agonistic anti-TRAIL-R1/-R2 mAbs is limited to TRAIL-sensitive tumors. The sensitivity of primary tumor cells to TRAIL is highly variable in the various tumors and also within the tumor histotype [Pasquini et al., 2006]. Therefore in future studies it will be important either to combine TRAIL-R targeting with agents able to increase the sensitivity of tumor cells to TRAIL or to develop diagnostic tools able to determine TRAIL sensitivity of tumor clinical samples.

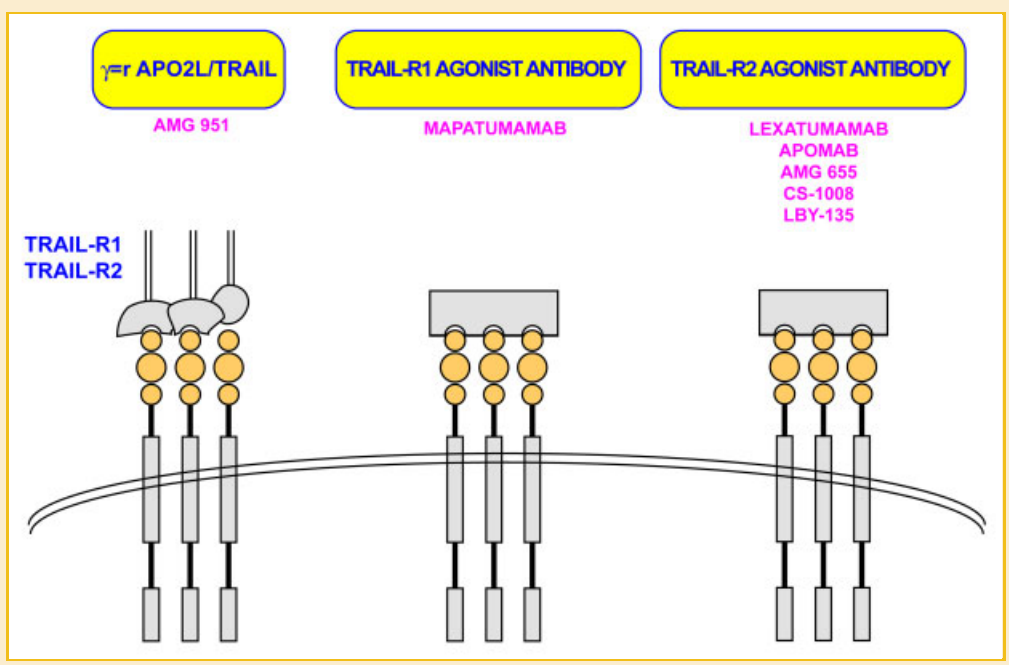

Fig. 2. Pro-apoptotic TRAIL-R1 and TRAIL-R2 agonists. Two types of pro-apoptotic TRAIL-R1/TRAIL-R2 agonists have been identified: (i) ligand and (ii) agonistic antibodies. Recombinant human TRAIL binds to TRAIL-R1 and TRAIL-R2, while the monoclonal agonist antibodies are selectively specific for either TRAIL-R1 or TRAIL-R2.

THE ROLE OF TRAIL/TRAIL-R IN HEMATOLOGIC MALIGNANCIES

MULTIPLE MYELOMA

Gene expression studies have shown that TRAIL and TRAIL-R1/-R2 are overexpressed in multiple myeloma cells (MMC) compared to normal plasmocytes [Jordan et al., 2009]. This TRAIL/TRAIL-R expression pattern could induce an autocrine apoptotic mechanism. However, this apoptotic loop is inhibited by the concomitant elevated expression of TRAIL decoy receptors in MMCs, particularly TRAIL-R4 and osteoprotegerin (OPG) [Shipman and Croucher, 2003]. TRAIL induces apoptosis of MM cell lines and of primary MMCs in vitro and reduces tumor burden in subcutaneous models of myeloma growth in vivo [Gazitt, 1999; Mitsiades et al., 2001]. Oligomerized TRAIL induces myeloma cell death much more efficiently than non-oligomerized TRAIL trimers [Berg et al., in press].

According to these findings it was suggested that recombinant soluble TRAIL could be evaluated as an anti-myeloma agent. In this context, agonistic anti-TRAIL-R1/-R2 mAbs could have an advantage over soluble TRAIL because they induce apoptosis of MMCs and bypass the inhibition of TRAIL-induced apoptosis by decoy receptors, such as soluble OPG [Menoret et al., 2006; Locklin et al., 2007]. However, MMCs may develop resistance to TRAIL not only through the inhibitory effects of decoy receptors, but also through an elevated expression of c-FLIP. Indeed, Mitsiades and coworkers reported that MMC resistance to TRAIL was associated with a low pro-caspase-8/c-FLIP ratio [Mitsiades et al., 2002]. However, these results were not supported by other observations [Spencer et al., 2002]. Recently, it was shown that IL-6 secreted by bone marrow stroma confers resistance to TRAIL of MMCs in association with the induction of increased c-FLIP levels [Perez et al., 2008]. This finding is not surprising because IL-6 is an important survival factor in myeloma via activation of major signaling pathways. Previous studies have shown that IL-6 protects against dexamethasone and FAS-mediated apoptosis. Importantly, IL-6 neutralizing Ab was able to partially overcome resistance to TRAIL-mediated apoptosis [Perez et al., 2008].

A recent study provided a particularly interesting observation based on the use of adenovirus transduced $CD34^+$ cells expressing membrane-bound TRAIL ($CD34\text{-}TRAIL^+$ cells). Using a MM model in immunodeficient mouse, it was reported a significantly greater effectiveness for $CD34\text{-}TRAIL^+$ cells in inducing tumor cell apoptosis over sTRAIL: this phenomenon seems to be due to the capacity of $CD34\text{-}TRAIL^+$ cells, but not of sTRAIL, to induce a damage of tumor vasculature [Lavazza et al., in press].

The proteasome inhibitor bortezomib (Velcade) has emerged in the last years as a very useful drug for the treatment of relapsed and refractory MM, as well as in elderly patients not suitable for bone marrow transplantation. For these reasons it seemed particularly interesting to explore the capacity of TRAIL to potentiate the cytotoxic effects of bortezomib on MMCs. Co-treatment of myeloma cells with bortezomib and TRAIL resulted in enhanced apoptosis [Balsas et al., 2008]. Bortezomib treatment did not significantly alter plasma membrane amount of TRAIL-R1 and TRAIL-R2, but increased TRAIL-induced caspase-8 and caspase-3 activation [Balsas et al., 2008]. Importantly, bortezomib + TRAIL co-treatment allowed to bypass stroma-mediated TRAIL resistance of MMCs [Perez et al., in press].

Another strategy to increase the susceptibility of MMCs to TRAIL consists in the contemporaneous addition of TRAIL with synthetic small molecule SMAC-mimicking compounds. Second mitochondria-derived activator of caspases (SMAC) was identified as a proapoptotic factor released from mitochondria into the cytosol triggered by multiple apoptosis stimuli. Upon stimulation, the released SMAC physically interacts with XIAP through the N-terminal four conserved amino acid residues that bind the BIR3 domain of XIAP, and eliminate the inhibitory effect of XIAP on caspase activation. Due to the potent pro-apoptotic effects of SMAC, synthetic small molecule SMAC-mimicking compounds (SMAC-mimetics) are being developed to sensitize apoptosis-resistant cancer cells to various apoptotic stimuli, including those mediated by TRAIL. SMAC mimetics greatly potentiated the apoptotic effects of TRAIL on MMCs [Chauhan et al., 2007].

Perifosine, an AKT/PKB inhibitor, is an agent that is currently tested in several clinical trials in patients with relapsed MM. Recent studies suggest that this drug could exert its cytotoxic effects on MMCs through multiple mechanisms, involving also TRAIL-Rs. In fact, perifosine was shown to induce up-regulation of TRAIL-R1/TRAILK-R2, to induce migration of these receptors in lipid rafts and to synergistically enhance TRAIL-mediated apoptosis [Gapate and Mollinedo, 2007; David et al., 2008].

CHRONIC MYELOGENOUS LEUKEMIA

CML is a clonal myeloproliferative expansion of transformed hematopoietic progenitor cells characterized by Philadelphia chromosome (Ph1) generated by the chromosomic translocation t(9;22)(q34;q11). Approximately 20% of aduly and 5% of childhood acute lymphoblastic leukemia are also Ph1 positive. The Ph1 results in the juxtaposition of Bcr and Abl genes, and this generates a chimeric protein termed BCR-ABL with a marked tyrosine kinase. The tyrosine kinase inhibitor (TKI) is now the first-line treatment for chronic-phase of CML, and several newer TKIs, such as desatinib and nilotinib, have been added to the pharmacologic instruments anti-CML. Despite the proven efficacy of TKIs to induce hematologic and cytogenetic remission, the majority of patients still have molecularly detectable disease: this is largely due to the incapacity of these agents to kill quiescent $CD34^+$ leukemic stem cells.

Initial studies have shown that a part of Ph1-positive leukemic cell lines is sensitive to the pro-apoptotic effects of TRAIL [Uno et al., 2003]. Some of these cell lines are resistant or scarcely sensitive to TRAIL-mediated apoptosis. However, their sensitivity to TRAIL could be considerably increased by cotreatment with imatinib [Nimmanapalli et al., 2001]. TRAIL could also induce cell death in the Ph^+ cell lines that are refractory to imatinib [Uno et al., 2003]. Sensitization of imatinib-resistant CML cells to TRAIL-induced apoptosis is mediated through down-regulation of BCR-ABL and of c-FLIP [Park et al., 2009a].

Few studies have explored TRAIL-R expression on CML cells. Studies on cell lines have shown the preferential expression of TRAIL-R2 with respect to TRAIL-R1 on the majority of these cell lines [Uno et al., 2003]; a similar pattern of expression was observed

on fresh leukemic cells, both during the chronic phase and blast crisis [Uno et al., 2003]. Analysis of the coding sequences of TRAIL-R1 and TRAIL-R2 showed that these receptors were not mutated in CML [Liu et al., 2005].

The mechanism of resistance of CML cells to TRAIL has been explored providing evidence that the antiapoptotic heat shock protein (HSP) 70 could be involved in this phenomenon. In fact, TRAIL resistant CML cell lines were shown to overexpress HSP 70: the inhibition of HSP 70 expression sensitizes these cell lines to the antiapoptotic effect of TRAIL [Guo et al., 2005]. This effect could be explained by the capacity of HSP 70 to bind TRAIL-R1 and TRAIL-R2 and to inhibit their death signaling [Guo et al., 2005]. TRAIL resistance may be bypassed by co-treatment of CML cells with an histone deacetylase inhibitor and TRAIL, through a mechanism involving BCR-ABL downregulation and activation of a caspase-dependent mitochondrial pathway [Park et al., 2009b].

As above mentioned, TKIs, although very efficacious in the treatment of CML, are unable to eradicate leukemic stem/progenitor cells. Conversely, allogeneic stem cell transplantation remains the only treatment that can consistently eradicate CML [Barrett, 2009]. The success of this regimen is mainly based on NK cell-mediated antileukemia effect. Therefore, many research efforts are devoted to the development of pharmacologic strategies able to target and to kill these cells, sparing normal hematopoietic stem cells and understand the mechanisms through which CML stem cells are eliminated through the graft-versus-leukemia effects induced by allogeneic SCT. In this context, particularly interesting is a recent report on the expression of TRAIL and its receptors on primitive quiescent $CD34^+$ cells in CML and on their susceptibility to NK cell-mediated cytotoxicity. In T cell depleted SCT CML patients it was shown that: quiescent $CD34^+$ cells are less susceptible than their cycling counterpart to lysis by NK cells from their HLA-identical sibling donors; compared with cycling populations, quiescent $CD34^+$ CML cells had higher surface expression of TRAIL-R1 and TRAIL-R2; the proteasome inhibitor bortezomib up-regulated TRAIL receptor expression on quiescent $CD34^+$ CML cells, and enhanced their susceptibility to cytotoxicity by expanded donor NK cells [Yong et al., 2009]. Interestingly, in haploidentical SCT in CML patients TRAIL expression increased on the surface of effector NK cells [Schulze et al., 2008]. An increased expression of both membrane bound and soluble TRAIL was also observed on IFNα-stimulated neutrophils and monocytes, thus suggesting that the therapeutic effect of IFNα in CML could be at least in part mediated by increased TRAIL release [Tecchio et al., 2004].

MYELODYSPLASTIC SYNDROMES

Myelodysplastic syndromes (MDSs), previously identified as pre-leukemic conditions, are heterogeneous group of clonal disorders of hematopoiesis. Three different international groups of experts [The World Heath Organization (WHO), the French American British (FAB) and the International Prognostic Scoring System (IPSS)] have proposed a classification of MDSs into different stages based on bone marrow pathology, cytogenetic features and presence of blood cytopenias [Catenacci and Schiller, 2005].

A consistent number of studies have shown that cells from patients with early stage MDS, including $CD34^+$ progenitor cells, are sensitive to apoptotic stimuli, whereas bone marrow cells from patients with advanced stages of MDS are less sensitive to apoptosis and exhibit an extensive proliferative capacity [reviewed in Kerbany and Deeg, 2007]. Several studies have shown increased rates of programmed cell death in bone marrow cells of patients with low grade/early stage MDS, a phenomenon seemingly related to extensive deregulation of the extrinsic proapoptotic pathways, including TNF-α, FAS-Ligand and TRAIL. Particularly, an over-expression of TNF-α, Fas-Ligand, TRAIL with their respective agonistic receptors, has been observed at the level of bone marrow cells of these patients. Concerning TRAIL and its receptors, Zang et al. [2001] showed that the agonistic TRAIL-R1 and TRAIL-R2 are overexpressed in MDS bone marrow cells and the ratio between these receptors and decoy receptors (TRAIL-R3 and TRAIL-R4) is higher in MDS than in normal bone marrow. TRAIL-induced apoptosis of MDS bone marrow cells derived from patients with low grade/early stage MDS, while TRAIL resistance was observed in cells from patients with more advanced disease [Zang et al., 2001].

A differential expression of c-FLIP isoforms during disease progression could play a relevant role the mechanism of TRAIL resistance. In MDS cells, the level of c-$FLIP_L$ expression correlates inversely with apoptosis, while the level of c-$FLIP_S$ expression directly correlates with apoptosis [Benesch et al., 2003]. C-$FLIP_L$ expression is high in advanced stages of disease, while c-$FLIP_S$ expression is preferentially observed in early stage disease [Benesch et al., 2003]. Experiments of enforced expression of c-$FLIP_L$ and c-$FLIP_S$ in leukemic cell lines confirmed the differential effects of these two TRAIL isoforms on TRAIL-mediated apoptosis: thus, while c-$FLIP_L$ markedly inhibited TRAIL-induced apoptosis, c-$FLIP_S$ exerted only a slight inhibitory effect on TRAIL-induced apoptosis, allowing BID cleavage and caspases-3 activation [Seal et al., 2008].

ACUTE LYMPHOBLASTIC LEUKEMIA (ALLS)

TRAIL sensitivity of ALLs was explored in detail. In an initial study Clodi et al. [2000] reported a scarce sensitivity of B-ALL cells to TRAIL-mediated apoptosis, not correlated with TRAIL-R1/-R2 expression; furthermore, ALL blasts express TRAIL protein, but fail to kill target cell lines in a TRAIL-dependent manner. In a second report, Wuchter et al. confirmed the limited sensitivity of B-ALL and T-ALL cells to TRAIL, providing evidence that only 27% of B-ALL and 15% of T-ALL are sensitive to TRAIL-mediated apoptosis [Wuchter et al., 2001]. TRAIL-R1 and TRAIL-R2 expression in ALLs is heterogeneous, but does not correlate with TRAIL sensitivity [Wuchter et al., 2001]. In a third study, Ehrhardt et al. have also explored TRAIL sensitivity of primary ALL cells showing that about 50% of cases are sensitive to the proapoptotic effects of TRAIL; however, surprisingly, TRAIL attenuated spontaneous apoptosis and, in some cases, induced also proliferation. The stimulatory effect on proliferation of TRAIL in these cases seems to be mediated through NF-κB activation [Ehrhardt et al., 2003].

The mechanism of TRAIL resistance was explored in TRAIL-resistant ALL cell lines showing that this phenomenon could be mediated through phosphorylation (activation) of AKT and phosphorylation (inactivation) of PTEN [Dida et al., 2008]. Several strategies have been proposed to bypass the resistance of ALL cells to TRAIL. In this context, particularly relevant was a study from Fakler

et al. [2009] showing that XIAP inhibitors may overcome TRAIL resistance of ALL cells. This study was based on the observation that high levels of inhibitor of apoptosis proteins (IAPs) represent a key antiapoptotic mechanism in many cancers, including acute leukemias [Schimmer et al., 2006]. Among the various IAP family members, particularly relevant is X-linked inhibitor of apoptosis (XIAP) for its antiapoptotic function [Eckelman et al., 2006]. According to these findings, XIAP was identified as a potential target for ALL therapy: ideal targets of XIAP are represented by SMAC peptides that antagonize XIAP. Fokler et al. [2009] showed that two of these peptides are able to considerably potentiate the proapoptotic effect of TRAIL on ALL cells and to inhibit clonogenic survival of these cells; furthermore, the TRAIL and XIAP inhibitors combination in vivo significantly reduces leukemic burden in a mouse model of pediatric ALL engrafted in NOD/SCID mice. Other evidences suggest that c-IAP-1 and c-IAP-2 are also important targets for T-ALL therapy. Thus, incubation of T-ALL blasts with A490, a JAK-2 inhibitor, inhibited Stat3 phosphorylation, greatly reduced c-IAP-1 and c-IAP-2 levels and enhanced TRAIL-induced apoptosis [Lanuti et al., 2009]. Another approach was proposed for B-ALL, based on the use of a TRAIL fusion protein designated scFvCD19:sTRAIL, consisting of a CD19-speciifc single-chain Fv antibody fragment (scFv) fused to the soluble extracellular domain of TRAIL (sTRAIL). scFvC19:sTRAIL induced apoptosis of primary B-ALL cells, consistently potentiated by concomitant addition of the histone deacetylase inhibitor valproic acid [Stiegmailer et al., 2008].

CHRONIC LYMPHOCYTIC LEUKEMIA

Initial studies have shown that CLL cells are resistant to apoptosis mediated by Fas Ligand. Subsequent studies have shown that primary CLL cells are resistant to TRAIL-mediated apoptosis [MacFarlane et al., 2002]. CLL cells express low levels of TRAIL-R1 and TRAIL-R2, but do not express TRAIL-R3 and TRAIL-R4 [MacFarlane et al., 2002]. The mechanism of TRAIL resistance occurs upstream caspases-8 activation and seems to involve both low TRAIL-R1/TRAIL-R2 expression and high c-FLIP$_L$ expression [MacFarlane et al., 2002]. These findings were in large part confirmed in a second study carried out on 44 primary CLLs: 11/44 of these cases were moderately sensitive to the proapoptotic effects of TRAIL; in 19/44 cases TRAIL had no effect; in 14/44 cases TRAIL increased leukemic cell survival [Secchiero et al., 2005]. Interestingly, all CLL cases were shown to express membrane TRAIL at a level higher than normal lymphocytes: the addition to CLL cultures of a TRAIL-R1-Fc chimera, which binds at high affinity to surface TRAIL, significantly decreased the percentage of viable cells, thus suggesting that surface TRAIL may play a role in promoting CLL cell survival [Secchiero et al., 2005].

Various strategies have been proposed to improve the sensitivity of CLL cells to TRAIL-mediated apoptosis. Thus, MacFarlane et al. [2005] have shown that histone deacetylase inhibitors (HDACis) greatly potentiated the proapoptotic activity of TRAIL against CLL cells: importantly, >90% of primary CLL cells are sensitive to the combination TRAIL + histone deacetylase inhibitors. The apoptotic triggering to CLL cells seems to be almost exclusively mediated through TRAIL-R1 [MacFarlane et al., 2005]. These observations were confirmed in a more recent report, suggesting that the stimulatory effect of HDACis on TRAIL-induced apoptosis could be mediated through TRAIL-R upmodulation and Bcl-2 downmodulation [Norian et al., in press]. Other studies focused on proteasome inhibitors and on their effects on Bax protein. Bax is a short lived protein in CLL cells and is expressed at low levels in these cells: in TRAIL-resistant CLL cells the addition of TRAIL induced a rapid degradation of mitochondrial Bax. In CLL cells proteasome inhibitors induced Bax accumulation and sensitized to TRAIL-mediated apoptosis. Given the well-established therapeutic potential of proteasome inhibitors for treatment of B-cell malignancies, the TRAIL and proteasome inhibitors combination seems particularly promising for CLL treatment [Liu et al., 2008]. Another strategy to bypass the TRAIL resistance of CLL cells consisted in the combination of either chlorambucil or fludabarine (the two drugs used for CLL treatment) with TRAIL: both these drugs stimulate TRAIL-R1 and -R2 expression and potentiate the sensitivity of CLL cells to TRAIL-mediated apoptosis [Johnston et al., 2003]. Interestingly, blocking of TRAIL-Rs reduced the effect of chlorambucil and fludabarine on CLL cells. Nongenotoxic activation of p53 using nutilin-3 induces, like fludabarine and chlorambucil, TRAIL-R expression on the surface of CLL cells [Secchiero et al., 2006].

Recently, it was proposed that the combination of XIAP inhibitors with TRAIL acts as a potent inducer of CLL apoptosis, including resistant forms and poor prognostic subgroups of CLLs [Loeder et al., 2009]. Importantly, the cooperative interaction of XIAP inhibitor and TRAIL is even evident in distinct subgroups of patients with poor prognostic features, such as TP53 mutation and chemotherapy-refractory disease [Loeder et al., 2009].

Lenalinomide is an oral immunomodulatory agent with clinical activity in various hematologic diseases, including CLL. The exact mechanism through which lenalinomide exerts its immunomodulatory activity is uncertain. A recent report showed that this agent promoted CD154 antigen expression on CLL cells, increased TRAIL-R2 and p73 expression and sensitized these cells to TRAIL-mediated apoptosis [Lapalombella et al., in press].

LYMPHOMAS

Genetic alterations of the TRAIL-R pathway in non-Hodgkin's lymphomas. Genetic alterations in components of the TRAIL receptor pathway have been identified only in a small subset of tumors. For example, somatic mutations of TRAIL-R1 and TRAIL-R2 genes were found in a small proportion of non-Hodgkin's lymphoma [Lee et al., 2001]. Interestingly, TRAIL-R1 and TRAIL-R2 genes map to chromosome 8p21-22 [Marsters et al., 1997], which is a frequent site of allelic deletions in many types of human tumors including non-Hodgkin's lymphoma [Monni et al., 1996; Mitelman et al., 1997]. In a series of 117 human non-Hodgkin's lymphoma, eight tumors (6.8%) were found to have two TRAIL-R1 gene mutations or six TRAIL-R2 gene mutations [Lee et al., 2001]. Six of these mutations (2 TRAIL-R1 and 4 TRAIL-R2) were detected in the death domains and one nonsense mutation of TRAIL-R2 was detected just before the death domain [Lee et al., 2001]. This study suggests that somatic mutations of TRAIL-R1 and TRAIL-R2 genes may play a role in the pathogenesis of some non-Hodgkin's lymphoma [Lee et al., 2001]. Subsequent studies have delineated a common region of

deletion on chromosome 8 of approximately 600 kb occurring in some B NHLs: TRAIL-R1 and TRAIL-R2 are the targets of this deletion [Rubio-Moscardo et al., 2005]. It was suggested that these genes may act as a dosage-dependent suppressor genes by impairing the TRAIL apoptotic pathway in B-cell lymphoma.

Adult T cell leukemia lymphoma (ATLL). ATLL is a neoplasm of mature T-lymphocytes that is etiologically associated with human T-lymphocytotropic virus type I (HTLV-1) and in classified into four clinical subtypes, including acute, chronic, smoldering and lymphoma. Primary ATLL cells and ATLL cell lines express TRAIL-Rs, but are usually resistant or scarcely sensitive to TRAIL-mediated apoptosis [Hasegawa et al., 2004]. Furthermore, ATLL cells express membrane-bound TRAIL whose expression is induced by Tax via NF-κB activation [Matsuda et al., 2005].

ATLL cells are resistant also to FasL/CD95-mediated apoptosis [Zehender et al., 2001]. The mechanism through which Tax induces FasL and TRAIL resistance seems to be related to the induction of elevated levels of expression of c-FLIP$_L$ and c-FLIP$_S$ [Krueger et al., 2006], through a molecular mechanism involving NF-κB activation [Okamoto et al., 2006]. In line with these observations, NF-κB inhibitors considerably increase the sensitivity of ATLL cells to TRAIL [Matsuda et al., 2005].

Cutaneous T-cell lymphoma. Cutaneous T-cell lymphomas (cTCLs) are a clinically and biological distinct group of NHLs characterized by clonal proliferation of skin-homing malignant T lymphocytes. Mycosis fungoides and Sezary syndrome are the two most common cTCL types. Importantly, cTLC cells overexpress TRAIL and the assessment of its expression, together with that of other genes (Stat4, GATA-3, CD1D, PLS3) is of diagnostic importance [Nebozhyn et al., 2006]. In addition to TRAIL, other genes of the TNF family are deregulated in cTCLs [Tracey et al., 2003].

CTCL cell lines exhibited a pronounced resistance to death ligands (TRAIL and FasL), compared to T-ALL cell lines [Braun et al., 2007]. The mechanism of resistance of these cells to death ligands was not clearly established: however, it is important to note that all these cell lines show overexpression of c-FLIP [Braun et al., 2007].

Studies on primary tumor cells from patients with Sézary syndrome showed that they are resistant in 16/16 cases to TRAIL-mediated apoptosis and have lost in all cases TRAIL-R2 expression [Contassot et al., 2008]. Furthermore, these cells are frequently resistant to FasL-mediated apoptosis and display elevated c-FLIP expression [Contassot et al., 2008].

Indolent lymphomas of mature B lymphocytes. The lymphomas of B lymphocytes are a biologically diverse group of B cell derived neoplasms that include B cell small lymphocytic lymphoma, follicular lymphoma, mantle cell lymphoma, lymphoplasmacytic lymphoma and extranodal marginal lymphomas.

Follicular lymphomas. Follicular lymphomas (FLs) are indolent non-Hodgkin lymphomas, very frequently characterized by a t(14;18) chromosomal translocation resulting in overexpression of the antiapoptotic Bcl-2 protein. This phenomenon greatly contributes to the survival advantage of B follicular lymphoma cells, but it is not sufficient to explain the growth advantage of these cells over the normal counterpart. Therefore, additional events contribute to the growth advantage of FLs. Given this background, it was particularly interesting to explore TRAIL sensitivity of FL cells. Primary FL B cells and germinal center-derived B lymphoma cell lines are sensitive to TRAIL-induced apoptosis [Travert et al., 2008]. The apoptotic signaling in these cells is mainly mediated by TRAIL-R1 [Travert et al., 2008]. However, when TRAIL sensitivity of these cells was studied in a more physiologic context, trying to mimic the germinal center microenvironment, it appears evident that FL B cells became TRAIL-resistant. GC microenvironment was mimicked through CD40L/CD40 costimulation. Therefore, it appeared evident that CD40 signaling protects tumor FL B cells from TRAIL-induced apoptosis via NF-κB activation [Travert et al., 2008]. Another study, however, has shown that CD40 stimulation either protected from TRAIL- or drug-induced apoptosis or induced apoptosis itself, depending on the maturation stage of FL B cells: in malignant germinal center B cells CD40 signaling leads to survival, while in GC B cells from early maturation stages CD40 signaling leads to apoptosis [Nuutinen et al., 2009]. These results indicate that inhibition of the NF-κB pathway using blocking anti-CD40 antibodies together with TRAIL should be considered as a therapeutic strategy for FL treatment.

Mantle cell lymphoma. Mantle cell lymphoma (MCL) is a B lymphoid neoplasm with a mature phenotype, genetically characterized by the t(11;14)(q13;q32) translocation leading to cyclin D1 overexpression with the consequent deregulation of cycle at the G_1/S checkpoint. This is an aggressive lymphoma, associated with an average overall survival of around 3 years. The studies carried out on this lymphoma have shown a possible role of the TRAIL/TRAIL-R system in its pathogenesis.

TRAIL-R1 and TRAIL-R2 are usually not mutated in MCL patients. However, the TRAIL-R1 death domain A1322G polymorphism was significantly more frequent in MCL patients than in a sex and age-adjusted healthy population [Fernandez et al., 2004].

TRAIL-R1 and TRAIL-R2 have been mapped to chromosome 8p21-22, a region frequently lost in MCL [Martinez-Climent et al., 2001]. A deleted region of 1.5 Mb in 8p21.3 encompasses the TRAIL receptor cluster gene and occurs in about 25% of MCL patients [Rubio-Moscardo et al., 2005]. This deletion is associated with downregulation of TRAIL-R2 and, at clinical level, with leukemic dissemination and bone marrow disease [Rubio-Moscardo et al., 2005]. Other studies were focused to the analysis of TRAIL sensitivity of MCL cell lines and primary MCL cells. These studies showed that TRAIL can trigger apoptosis in a majority of MCL cell lines and primary cultures [Roué et al., 2007]. Low sensitivity or resistance to TRAIL-induced apoptosis was associated with high c-FLIP expression [Roué et al., 2007]. IκB kinase inhibitors, but not proteasome inhibitors, increase the sensitivity of MCL cells to TRAIL by lowering c-FLIP levels [Roué et al., 2007].

Strategies to bypass TRAIL resistance of indolent NHLs. Different strategies have been proposed to bypass resistance of NHL cells. These different strategies were all based on the combination of TRAIL with another anti-lymphoma agent. Some studies were

devoted to the analysis of the combination of TRAIL with rituximab, a chimeric mAb targeting the CD20 antigen present on B lymphocytes and most of B-cell lymphomas. Lymphoma animal models have supported the efficacy of the combination of either agonistic anti-TRAIL-R1 mAbs [Maddipatla et al., 2007] or recombinant TRAIL [Daniel et al., 2007] with Rituximab. Both drug combinations were more effective in controlling lymphoma growth in vivo than either single drug. These observations have a possible clinical application because the combinations of Rituximab plus chemotherapeutic agents, although have a considerable antitumor activity in some patients, in other patients induce only a limited antitumor response or are associated with an elevated toxicity.

Another strategy consists in associating anti-TRAIL-R mAbs with a proteasome inhibitor, bortezomib. The proteasome inhibitor Bortezomib has pleiotropic effects and may enhance TRAIL signaling by different mechanisms, including TRAIL-R upmodulation, c-FLIP downmodulation, inhibition of NF-κB activation, and perturbation of the expression of some members of the Bcl-2 family. The combination of agonistic anti-TRAIL-R mAbs and bortezomib resulted in increased apoptosis of NHL cells and enhanced killing of NHL cells in a severe immunodeficient mouse/human NHL cell line xenograft system [Smith et al., 2007].

A particular strategy was proposed to bypass TRAIL resistance of follicular B lymphoma cells, based on the use of NF-κB inhibitors or blocking anti-CD40 Abs together with TRAIL [Travert et al., 2008].

Aggressive lymphomas. Diffuse large B-cell lymphomas (DLBCLs) and Burkitt Lymphoma (BL) account for the majority of aggressive lymphomas in adults and children. DLBCLs are very heterogeneous from a biologic point of view and exhibit a variable clinical course; in contrast, BL is genetically homogeneous.

DLBCLs. TRAIL sensitivity was explored in DLBCL primary samples. Twelve out of a total of 22 DLBCL samples were sensitive to hsTRAIL/Apo2L. These sensitive lymphomas included seven clinically chemotherapy-refractory lymphomas. Furthermore, hsTRAIL/Apo2L induced apoptosis in DLBCL cells and in B-cell lines that showed high expression levels of inhibitors of the intrinsic apoptosis pathway: Bcl-2 and/or X-linked inhibitor of apoptosis (XIAP). hsTRAIL/Apo2L-sensitive lymphoma cells showed expression of the TRAIL receptors R1 and/or R2 and absence of R3 and R4 [Cillessen et al., 2006].

The mechanism of TRAIL resistance of DLBCL could be related to the frequent expression of c-FLIP observed in this lymphoma type: in fact, 44% of primary cases were distinctly c-FLIP positive at an immunochemistry analysis [van Houdt et al., 2007]. TRAIL/TRAIL-R expression in DLBL was correlated with disease prognosis. Microarray expression profiling of apoptosis related genes predicts clinical outcome in patients with primary nodal DLBCL. Unsupervised cluster analysis using genes involved in apoptosis resulted in three separate DLBCL groups. One group with poor clinical outcome was characterized by high expression levels of pro-and anti-apoptotic genes involved in the intrinsic apoptosis pathway. A second group, also with poor clinical outcome, was characterized by high levels of apoptosis inducing cytotoxic effector genes, including TRAIL, possibly reflecting a cellular cytotoxic immune response. The third group showing a favorable outcome was characterized by low expression levels of genes characteristic for both other groups [Muris et al., 2007]. These results suggest that chemotherapy refractory DLBCL are characterized either by an intense cellular cytotoxic immune response or by constitutive activation of the intrinsic mediated apoptosis pathway with concomitant downstream inhibition of this apoptosis pathway.

In a series of DLBCL 24 p53 mutations in 113 cases (21%) were identified. Twelve (50%) of the 24 cases had mutations localized to the DNA binding codons in the core domain of *TP53*. The presence of any *TP53* mutation correlated with poor overall survival (OS; $P < 0.044$), but DNA-binding mutations were the most significant predictor of poor OS ($P < 0.001$). Gene expression analysis showed that *TRAIL* receptor-2 (*DR5*) was the most differentially underexpressed gene in the *TP53* mutated cases [Young et al., 2007].

Burkitt's lymphoma. Chromosomal translocations that juxtapose one of three immunoglobulin loci to the c-myc protooncogene are the hallmark of Burkitt lymphomas, the Epstein Barr virus (EBV), whether they carry or not Ig/myc translocations occurs as accidents of normal B lymphocyte differentiation. Two initial studies have explored TRAIL sensitivity of BL cells. In one study TRAIL sensitivity of FasL sensitive and FsL resistant cell, lines was explored, showing that usually the former ones are also sensitive to TRAIL, while the latter ones are also resistant to TRAIL [Hussain et al., 2003]. In the other study TRAIL sensitivity was explored in 12 BL cell lines, showing that 5/12 of these lines are TRAIL sensitive. Importantly, TRAIL sensitivity did not correlate with EBV status [Mouzakiti and Packham, 2003].

The mechanisms responsible for TRAIL resistance of BL cells have been explored. The constitutive NF-κB activation was identified as one of the main factors conferring TRAIL resistance to BL cells [Tafuku et al., 2006]. In line with this observation, suppression of constitutive NF-κB activity restores sensitivity of BL cells to TRAIL [Hussain et al., 2008].

Radiotherapy, TRAIL, and lymphomas. Radiotherapy is one of the therapeutic strategies used in the treatment of NHLs. Experiments have been carried out in order to define a possible synergism between radiotherapy and TRAIL on induction of B-cell lymphoma cell death. Radiation-induced apoptosis almost completely relies on mitochondrial pathways with caspase activation occurring secondarily to mitochondrial damage. Experiments carried out on Jurkat cell line showed that a combination of TRAIL and ionizing radiation was highly synergistic and greatly potentiated the efficacy of both apoptosis induction and eradication of clonogenic cells [Belka et al., 2001]. These observations were confirmed in a lymphoma model in vivo using p53 mutant and Bcl-2 overexpressing cells, showing that combination treatment with radiotherapy and TRAIL markedly inhibited tumor growth [Wissink et al., 2006]. Interestingly, TRAIL expression is stimulated by irradiation during radiotherapy treatment of lymphoma patients [Unnithan and Macklis, 2004].

Hodgkin's lymphoma. HL is a common human lymphoma that seems to be derived from germinal center B cells. The disease is characterized by the presence of rare tumor cells called Red Stenberg that, in spite the loss of their B cell phenotype including Ig expression, evade the control of the immune system and survive. A resistance of these cells to death ligands could explain their capacity to escape cell death by cytotoxic T lymphocytes. In vitro studies, carried out on Hodgkin's lymphoma cell lines, have shown that the Red Stenberg cells do not undergo apoptotic cell death after death receptor stimulation [Re et al., 2000], due to the almost constant c-FLIP overexpression observed in these cells [Mathgas et al., 2004].

These observations indicate that resistance of Red Stenberg tumor cells to death receptor stimulation is due to functional inhibition mediated by a strong, NF-κB dependent up-regulation of c-FLIP proteins. The manipulation of death receptor systems by pharmacological agents that down-regulate NF-κB activity and subsequently c-FLIP expression could offer a new therapeutic tool in Hodgkin's disease treatment.

ACUTE MYELOID LEUKEMIAS

Initial studies carried out on a limited number of cases have shown a low sensitivity of AMLs to the apoptotic effects of TRAIL [Jones et al., 2003]. In contrast, some continuous cell lines derived from AML were observed to have a pronounced sensitivity to the TRAIL-mediated apoptotic effects [Wen et al., 2000]. Furthermore, in some of these cell lines, TRAIL was shown to act as an inducer of monocytic differentiation [Secchiero et al., 2002]. Riccioni et al. [2005] reported the analysis of large panel of primary AMLs showing that these cells were invariably resistant to TRAIL and frequently express TRAIL decoy receptors (TRAIL-R3 and TRAIL-R4). TRAIL-R1 and TRAIL-R2 expression was typically observed in AMLs exhibiting monocytic features [Riccioni et al., 2005]. Inukai et al. [2006] have confirmed the expression of all four TRAIL receptors, including decoy receptors on AML s (Monocytic and myelomonocytic) with MLL rearrangement and their resistance to TRAIL-mediated apoptosis. It was suggested that TRAIL resistance of these AMLs could contribute to their rapid clonal expansion and poor sensitivity to the graft versus leukaemia (GVL) effect [Inukai et al., 2006].

Several studies were devoted to the study of TRAIL sensitivity of acute promyelocytic leukemias (APLs). These AMLs were characterized by their peculiar sensitivity to the differentiation promoting effects of retinoic acid. Initial studies have documented that APL blasts are sensitive to TRAIL-mediated apoptosis and that retinoic acid induces TRAIL expression and thereafter killing of the leukemic cells [Altucci et al., 2001]. However, these findings were not confirmed by other studies. Thus, Riccioni et al. [2005] studied 17 primary APLs and observed that these AMLs before, during and after retinoic acid treatment are TRAIL resistant; the induction of granulocytic maturation of APL cells by retinoic acid was associated with a marked decline of TRAIL expression. The mechanisms responsible for TRAIL resistance of AML blasts were not explored in detail. In this context, particularly interesting was a report from Tourneur et al. [2004] showing that 37% of AML blasts exhibit a low/absent FADD expression. The low/absent FADD expression was a negative prognostic factor. Several drugs have been tested in combination with TRAIL to determine whether they may help to bypass TRAIL resistance of AML blasts. A series of studies analyzed the effect of histone deacetylase inhibitors (HDACIs). In a first study, the effect of HDACIs on AMLs expressing PML-RARα or AML1/ETO was analyzed: these inhibitors were shown to induce apoptosis of leukemic blasts through a mechanism involving activation of the death receptor pathway [Insinga et al., 2005]. In paralle, Nebbiosio et al. [2005] showed that HDACIs induced apoptosis of AML blasts, apparently related to their capacity to upregulate TRAIL and TRAIL-R2 expression on leukemic blasts. AKT inhibitors synergize with TRAIL in inducing apoptosis on AML blasts: two effects of AKT inhibitors, upregulation of TRAIL-R2 and downregulation of c-FLIP and XIAP, seem to play a significant role in enhancing the sensitivity of AML blasts to TRAIL [Tazzari et al., 2008]. Phorbol esters also potentiate the TRAIL sensitivity of AML blasts through a mechanism not involving a modification of TRAIL-R expression, but apparently through PKCε downregulation [Gobbi et al., 2009]. Finally, proteasome inhibitors, such as bortezomib, greatly enhanced the sensitivity of primary AML blasts to TRAIL through a mechanism seemingly involving c-FLIP downmodulation [Riccioni et al., 2007].

CONCLUSIONS AND FUTURE PERSPECTIVES

The data above reviewed provided support to the concept that the large majority of primary tumor cells derived from hematologic neoplasia, as well as from solid cancers are usually resistant to TRAIL-mediated apoptosis. The mechanisms of TRAIL resistance of cancer cells are multifactorial and involve either deficient TRAIL-R1/TRAIL-R2 expression, enhanced levels of TRAIL decoy receptors, increased expression of inhibitors of TRAIL-mediated DISC formation such as c-FLIP, increased levels of anti-apoptotic proteins such as XIAP or Bcl-2 or other unknown mechanisms.

Various types of combination therapies considerably enhance TRAIL-mediated apoptosis. TRAIL targets the extrinsic apoptosis pathway, while chemotherapy and radiotherapy mainly inhibit cell growth and activate the intrinsic apoptotic pathway. Combination of either radiotherapy or chemotherapy with TRAIL can activate both these two apoptotic pathways and can increase their anti-apoptotic effects: the synergistic effect between the two treatments results from enhanced expression of TRAIL-R1/TRAIL-R2 induced by chemo-therapy or radio-therapy and by cross-talk between the extrinsic and intrinsic apoptotic pathways mediated by caspases-8 induced Bid activation (tBid). In addition to radiotherapy and chemotherapy, combinatorial treatments involving other drugs, such as proteasome inhibitors, NF-κB inhibitors, HDAC inhibitors overcome tumor cell resistance to single agent therapies. These agents either upregulate TRAIL-R1/TRAIL-R2 expression on tumor cells or downregulate the expression of inhibitors of TRAIL-mediated apoptotic triggering, such as c-FLIP.

Many of these co-treatments involving either recombinant TRAIL or agonistic anti-TRAIL-R1/-TRAIL-R2 antibodies hold promise for the application in the treatment of several hematologic malignancies.

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