Molecular Pathways

Death Receptor Agonists as a Targeted Therapy for Cancer

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Abstract

Apoptosis is integral to normal, physiologic processes that regulate cell number and results in the removal of unnecessary or damaged cells. Apoptosis is frequently dysregulated in human cancers, and recent advancements in our understanding of the regulation of programmed cell death pathways has led to the development of novel agents to reactivate apoptosis in malignant cells. The activation of cell surface death receptors by tumor necrosis factor–related apoptosis-inducing ligand (Apo2L/TRAIL) and death receptor agonists represent an attractive therapeutic strategy to promote apoptosis of tumor cells through the activation of the extrinsic pathway. The observation that Apo2L/TRAIL can eliminate tumor cells preferentially over normal cells has resulted in several potential therapeutics that exploit the extrinsic pathway, in particular, the soluble recombinant human (rh)Apo2L/TRAIL protein and agonist monoclonal antibodies that target death receptors 4 or 5. Many of these agents are currently being evaluated in phase 1 or 2 trials, either as a single agent or in combination with cytotoxic chemotherapy or other targeted agents. The opportunities and challenges associated with the development of death receptor agonists as cancer therapeutics, the status of ongoing clinical evaluations, and the progress toward identifying predictive biomarkers for patient selection and pharmacodynamic markers of response are reviewed. *Clin Cancer Res; 16(6); 1701–8. ©2010 AACR.*

Background

Cancer occurs when cells accumulate mutations that enable them to escape the mechanisms that normally restrict their ability to survive and proliferate. One of the mechanisms by which inappropriate cells are removed is through apoptosis or programmed cell death. This evolutionarily conserved pathway may be triggered in response to damage to key intracellular structures or the presence or absence of extracellular signals that provide normal cells within a multicellular organism with contextual information (1). Members of the tumor necrosis factor (TNF) superfamily of cytokines represent key extracellular regulators of the apoptotic process (2). In particular, tumor necrosis factor–related apoptosis-inducing ligand (Apo2L/TRAIL), which was cloned on the basis of its sequence homology to TNF and CD95 ligand (FasL), is being investigated for its therapeutic potential to induce tumor cell death (3, 4).

Apo2L/TRAIL binds to five TNF receptor superfamily members. Two of these receptors, death receptor 4 (DR4, TRAIL-R1, TR1) and death receptor 5 (DR5, TRAIL-R2, TR2) contain a cytoplasmic death domain and are capable

of transducing an apoptotic signal when engaged with their ligand (5–7). Three other TRAIL receptors, decoy receptor 1 (DcR1), decoy receptor 2 (DcR2), and soluble osteoprotegerin (OPG), lack the ability to initiate an apoptotic signal and are thought to function as inhibitory receptors (8–11).

Binding of homotrimeric Apo2L/TRAIL to DR4 and DR5 induces oligomerization of the receptors and initiation of a pathway mediated by proteases called caspases (2, 12). This pathway, sometimes called the extrinsic pathway to denote its responsiveness to extracellular signals, is initiated by the recruitment of the initiator caspase-8 and caspase-10 through the adaptor protein FADD to generate a death-inducing signaling complex (DISC; refs. 2, 12). The initiator caspases in turn activate the downstream effector caspase-3, caspase-6, and caspase-7, which cleave a variety of cellular substrates to execute the apoptotic program (Fig. 1; refs. 1, 2).

In some cells, called type 1, the DR-initiated extrinsic pathway generates a signal strong enough to initiate apoptosis by itself. However, in the majority of cells, called type 2, the DR-initiated signal needs to be amplified to induce apoptosis. This amplification can be achieved by a cross-talk between the extrinsic pathway and the Bcl-2–regulated mitochondrial, or intrinsic, pathway (2). One mechanism by which the extrinsic pathway recruits the intrinsic pathway involves caspase-8–mediated cleavage of the proapoptotic BH3-only Bcl-2 family member, Bid, to generate active truncated Bid. Active truncated Bid antagonizes the function of prosurvival Bcl-2 family members, such as Bcl-2, Bcl-XL, and Mcl-1, triggering a sequence of events that culminates in the release of

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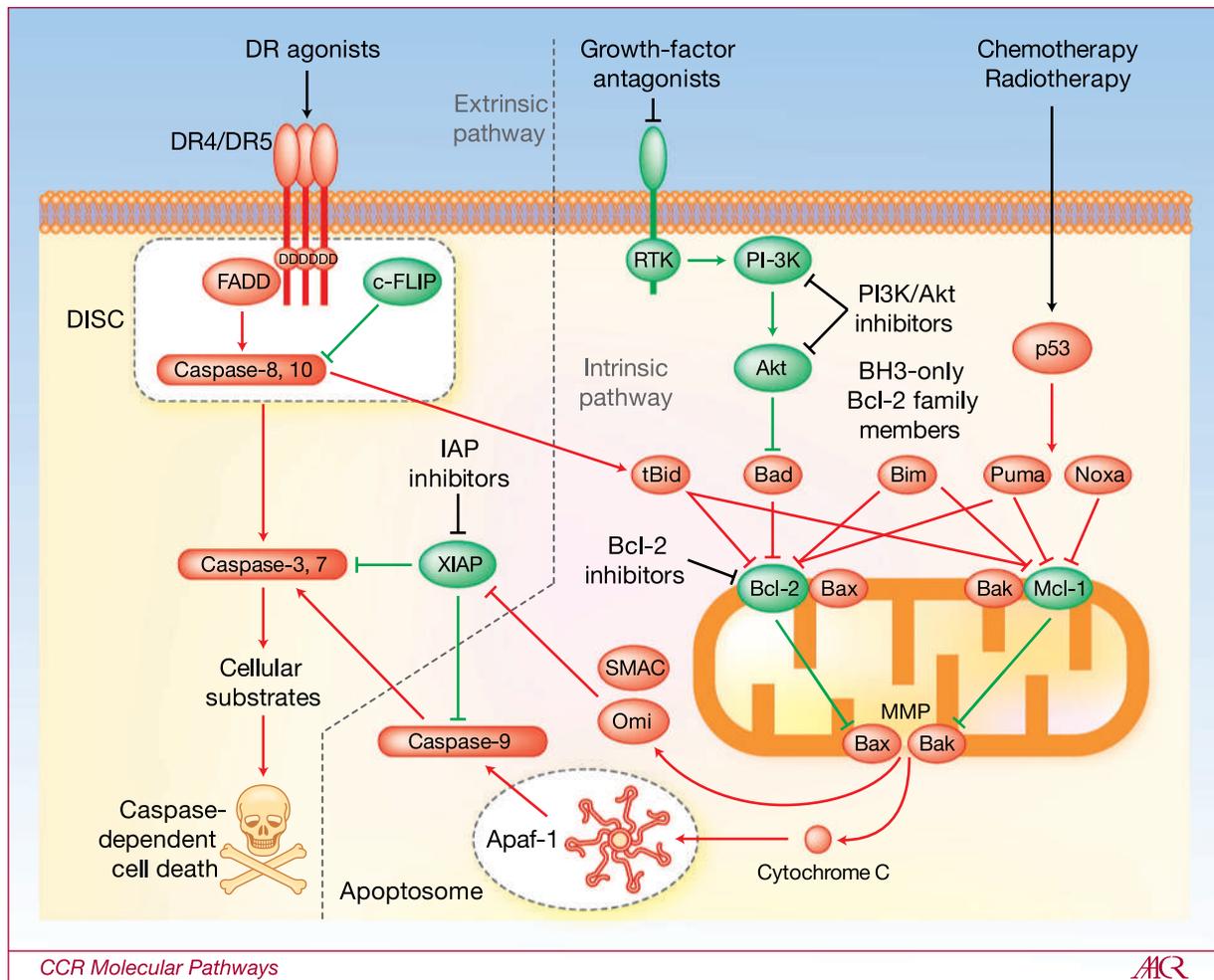*American Association for Cancer Research*

Fig. 1. Integration of information by the extrinsic and intrinsic apoptotic pathways drives cell survival decisions. The extrinsic pathway is triggered upon binding of DR agonists to the proapoptotic DR4 and/or DR5 receptors on the surface of a target cell. This results in the formation of a death-inducing signaling complex (DISC) and the activation of the initiator caspase-8 and caspase-10. These apical caspases activate the downstream effector caspase-3, caspase-6, and caspase-7, which cleave a broad range of cellular substrates to mediate apoptotic cell death. The intrinsic, or Bcl-2–regulated mitochondrial pathway, is responsive to a variety of cellular stresses, including conventional chemotherapeutics and targeted agents that antagonize growth and survival pathways. For example, in response to DNA-damaging agents, the tumor suppressor p53 initiates the intrinsic pathway by upregulating Puma and Noxa. These proapoptotic Bcl-2 family members facilitate the release of proapoptotic factors, such as cytochrome c and Smac/DIABLO, from the mitochondrion. The extrinsic and intrinsic pathways communicate with one another and, in many cells, work together to regulate cell survival. For example, caspase-8 can cleave and activate the proapoptotic Bcl-2 family member Bid. Cross-talk between these pathways provides a rationale to explain the combination activity observed between DR agonists and conventional chemotherapeutics, growth-factor antagonists, Bcl-2 inhibitors, inhibitors of apoptosis, and phosphoinositide 3-kinase (PI3K) pathway inhibitors, in preclinical models (see main text for more details). IAP, inhibitors of apoptosis.

apoptotic factors from the mitochondrion (2, 12). These apoptotic factors mediate activation of caspase-9 and antagonize the activity of inhibitors of apoptosis that otherwise function to suppress caspase activity. Thus, the intrinsic pathway can function together with the extrinsic pathway to promote caspase activation and apoptosis.

The tumor suppressor p53 is also a critical regulator of the intrinsic pathway. In response to a wide variety of cellular stresses, such as DNA damage (resulting from radiation or chemotherapy) or hypoxia, p53 regulates the expression of genes that induce either cell cycle arrest or apoptosis. Key p53-responsive genes within the intrinsic pathway include *Bax*, *Puma*, *Noxa*, and *Apaf-1* (13). The

importance of p53 is emphasized by the fact that it is inactivated in about half of all human cancers, contributing significantly to resistance to conventional chemotherapies. In addition, DR5 is p53-responsive, providing a reciprocal mechanism for cross-talk between the extrinsic and intrinsic pathways.

As dysregulated apoptosis plays a key role in the pathogenesis and progression of cancer, developing agents that promote or restore apoptosis through the activation of the extrinsic pathway has emerged as an important therapeutic strategy. The primary modalities are dulanermin [recombinant human (rh)Apo2L/TRAIL], an optimized, zinc-coordinated, homotrimeric recombinant protein

consisting of amino acids 114 to 281 of the endogenous polypeptide, and agonist antibodies directed against either DR4 or DR5 (Table 1).

Several characteristics render DR agonists attractive from a therapeutic perspective. First, although DR4 and DR5 are expressed on a wide variety of normal and tumor cell types, Apo2L/TRAIL preferentially induces apoptosis of tumor cells (14). Second, the ability of DR agonists to induce apoptosis is independent of the tumor suppressor p53 (12, 15). Because p53 is frequently inactivated in human tumors, DR agonists may have the ability to induce apoptosis in tumors that have acquired either full or partial resistance to chemotherapy. Indeed, Apo2L/TRAIL has been shown to induce apoptosis and to also cooperate with chemotherapy in p53-deficient colon cancer cells (16). Third, because of the relationship between the extrinsic and intrinsic apoptotic pathways, DR agonists may display enhanced activity when combined with a variety of conventional chemotherapeutic and targeted therapeutics that antagonize cell growth and survival pathways.

These theoretical advantages have been confirmed in a large number of preclinical studies by academic and industry groups. Dulanermin and DR agonist antibodies have shown activity in vitro and in vivo against a wide variety of tumor cell lines, including lung, colon, pancreatic, non–Hodgkin lymphoma (NHL), multiple myeloma, glioma, and breast (15, 17). In addition, dulanermin has shown activity against primary tumor explants derived from patient pancreatic and colorectal tumors, suggesting that primary tumors may be responsive to DR agonists (18, 19). Combinations of DR agonists with conventional chemotherapeutics and a wide variety of targeted agents have also yielded promising preclinical results (15, 17). Thus, the combination of DR agonists and standard-of-care agents is an attractive therapeutic strategy, encompassing tumor cell selectivity, broad applicability, and flexibility.

Approaches to Targeting Death Receptors

Multiple approaches for agonizing DRs are in the preclinical or clinical development (Table 1; refs. 20, 21). Although dulanermin and agonist antibodies generate broadly similar results in many preclinical models, they have distinct characteristics that may influence their therapeutic potential. For example, the serum half-life of dulanermin is 30 to 60 minutes in humans (22). In contrast, the serum half-life of the DR agonist antibodies ranges from 6 to 21 days (20). In addition, unlike agonist antibodies, which bind specifically to a particular DR, dulanermin also binds to the decoy receptors DcR1, DcR2, and OPG.

Another distinction between these modalities concerns the manner in which they agonize their cognate receptors. A requirement for aggregation for efficient signal transduction is a characteristic shared by many TNF receptor family members (2). Structural studies indicate that Apo2L/TRAIL forms a central homotrimer around which three receptors bind, thereby inducing oligomerization of intracellular death domains (23, 24). Although some antibodies seem to be able to autonomously agonize DR4 or DR5, many DR agonist antibodies require additional cross-linking to achieve optimal activity in vitro (25–28). In syngeneic and xenograft models, this cross-linking function is likely provided by binding to FcγRs (28).

A related issue—considering that all the current investigational DR agonist antibodies are IgG1—is that antibody effector function might contribute to their mechanism of action. For example, the DR4 agonistic antibody mapatumumab has been shown to mediate antibody-dependent cellular cytotoxicity against DR4-expressing target cells in vitro (29). This ability to engage the immune system also raises the possibility that DR agonist IgG1 antibodies may bring additional immune-mediated mechanisms to bear on the tumor. In this respect, an agonist antibody directed against the mouse TRAIL DR can also induce tumor-specific effector and memory T cells (25). Whether this ability is unique to DR agonist antibodies or whether it reflects the established role of TRAIL in tumor surveillance remains to be determined.

Most of the characteristics that differentiate Apo2L/TRAIL and antibody modalities have been extensively investigated in preclinical studies. However, because some of these factors are difficult to model preclinically, a clear understanding of how they may influence efficacy in humans awaits the availability of clinical data.

Clinical-Translational Advances

Clinical development of DR 4/5 agonists. Several DR agonists are in clinical development. Clinical trials, completed and ongoing, evaluating these agents as monotherapy and in combination with other anticancer agents are shown in Table 1.

Single-agent studies. Several phase 1, monotherapy, dose-escalation, safety studies in patients with advanced solid tumors have been completed (22, 30–34). In general, these agents have been well-tolerated at the doses tested, and most agents did not reach a maximum tolerated dose. Although the safety profile of the class is still emerging, concerns of fulminant hepatotoxicity as seen with FasL (35) have not been substantiated. The pharmacokinetics of the antibody agonists maintain drug concentrations that predict activity in preclinical models, with less frequent dosing compared with dulanermin. Modest single-agent antitumor activity has been shown in patients with refractory disease, with partial responses reported in phase 1 trials of the fully human DR5 agonist antibody conatumumab [one patient with non–small cell lung carcinoma (NSCLC)] and dulanermin (one patient with chondrosarcoma; refs. 22, 36). Single-agent phase 2 trials of the fully human DR4 agonist antibody mapatumumab have been completed in NSCLC, colorectal cancer (CRC), and NHL. Two partial responses and one complete response among 40 patients with pretreated follicular NHL were observed (37); however, there were no objective responses in treatment-refractory NSCLC and CRC patients (38, 39).

Table 1. Summary of DR agonists in clinical development

DR agonist	Indication	Phase and drug combination	Sample Size	Results	Reference
Conatumumab (AMG 655) fully human monoclonal antibody DR5 agonist	Advanced solid tumors	Phase 1: single agent	37	No DLT; no MTD up to 20 mg/kg Q2W; 1 PR in NSCLC	(36)
	NSCLC	Phase 1b: paclitaxel/carboplatin	12	1 DLT; no MTD up to 15 mg/kg Q3W; 1 CR, 3 PR	(44)
		Randomized phase 2: paclitaxel/carboplatin	150	—	—
	Lymphoma	Phase 1b: bortezomib or vorinostat	27	2 DLTs; no MTD up to 15 mg/kg Q3W; 3 CR	(51)
		Single arm phase 2: bortezomib	20	—	—
	Soft tissue sarcoma	Phase 1b: doxorubicin	6	No DLT; no MTD up to 15 mg/kg Q3W; 2 PR	(40)
		Randomized phase 2: doxorubicin	120	—	—
	CRC	Phase 1b: mFOLFOX/ bevacizumab	12	No DLT; no MTD up to 10 mg/kg Q2W; 6 PR	(47)
		Randomized phase 2: mFOLFOX/bevacizumab	180	—	—
	CRC	Randomized phase 2: FOLFIRI	150	—	—
	Pancreatic cancer	Phase 1b: gemcitabine	13	No DLT; no MTD up to 10 mg/kg Q2W; 2 PR	(41)
		Randomized phase 2: gemcitabine	120	—	—
	Advanced solid tumors	Phase 1b: AMG 479 (IGF-IR antagonist)	108	—	—
		Single arm phase 2: AMG 479		—	—
	CRC	Phase 1b: panitumumab	6	No DLT; no MTD up to 10 mg/kg Q2W	(45)
		Single arm phase 2: panitumumab	47	No objective responses	(46)
CS-1008 humanized monoclonal antibody DR5 agonist	Advanced solid tumors	Phase 1: single agent	17	No DLT; no MTD up to 8 mg/kg QW; no objective responses	(32)
	NSCLC	Randomized phase 2: paclitaxel/carboplatin	100	—	—
	CRC	Randomized phase 2: irinotecan	80	—	—
	Ovarian	Single arm phase 2: paclitaxel/carboplatin	40	—	—
	Pancreatic cancer	Single arm phase 2: gemcitabine	60	—	—
Dulanermin (rhApo2L/ TRAIL) Proapoptotic receptor agonist	Advanced solid tumors	Phase 1: single agent	58	2 DLTs; no MTD up to 15 mg/kg/d for 5d Q3W; 1 PR in chondrosarcoma*	(22)
	NHL	Phase 1b: rituximab	7	No DLT; no MTD up to 8 mg/kg/d for 5d Q3W; 2 CR, 1 PR	(51)
		Single arm phase 2: rituximab	132	—	—

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Table 1. Summary of DR agonists in clinical development (Cont'd)

DR agonist	Indication	Phase and drug combination	Sample Size	Results	Reference
	CRC	Phase 1b: FOLFOX/ bevacizumab	23	—	—
	CRC	Phase 1: irinotecan/ cetuximab or FOLFIRI± bevacizumab	37	Irinotecan/cetuximab: 5 DLTs; no MTD up to 8 mg/kg/d for 5d Q3W; 3 PR; FOLFIRI + bevacizumab: no DLT; no MTD up to 9 mg/kg/d for 3 d Q2W*	(50)
	NSCLC	Phase 1b: paclitaxel/ carboplatin± bevacizumab	24	No DLT; no MTD up to 20 mg/kg/d for 2 d Q3W; 1 CR, 13 PR	(49)
		Randomized phase 2: paclitaxel/carboplatin± bevacizumab	213	—	—
Lexatumumab fully human monoclonal antibody DR5 agonist	Advanced solid tumors	Phase 1: single agent	31	1 DLT; no MTD up to 10 mg/kg Q2W; no objective responses	(34)
	Advanced solid tumors	Phase 1: single agent	22	3 DLTs; MTD of 10 mg/kg Q3W; no objective responses*	(63)
	Advanced solid tumors/ lymphoma	Phase 1: ± IFN γ	68	—	—
	Advanced solid tumors	Phase 1: gemcitabine, pemetrexed, doxorubicin, or FOLFIRI	41	No MTD up to 10 mg/kg Q2W or Q3W; PRs observed	(48)
Mapatumumab fully human monoclonal antibody DR4 agonist	Advanced solid tumors	Phase 1: single agent	49	3 DLTs; no MTD up to 10 mg/kg; no objective responses	(33)
	Advanced solid tumors	Phase 1: single agent	41	No DLT; no MTD up to 20 mg/kg Q4W; no objective responses	(31)
	Advanced solid tumors	Phase 1: gemcitabine/ cisplatin	49	5 DLTs; no MTD up to 30 mg/kg; 12 PR	(43)
	Advanced solid tumors	Phase 1: paclitaxel/ carboplatin	27	2 DLTs; no MTD up to 20 mg/kg Q3W; 5 PR	(42)
	Hepatocellular carcinoma	Phase 1: sorafenib	18	—	—
		Single arm phase 2: sorafenib		—	—
	NHL	Phase 2: single agent	40	1 CR, 2 PR	(37)
	Multiple myeloma	Randomized phase 2: bortezomib	100	—	—
	CRC	Phase 2: single agent	38	No objective responses	(39)
	NSCLC	Phase 2: single agent	32	No objective responses	(38)
	NSCLC	Randomized phase 2: paclitaxel/carboplatin	105	—	—
PRO95780 fully human monoclonal antibody DR5 agonist	Advanced solid tumors	Phase 1: single agent	50	1 DLT; no MTD up to 20 mg/kg Q2W; no objective responses	(30)
	CRC	Phase 1: cetuximab/ irinotecan or FOLFIRI/ bevacizumab	24	—	—
	CRC	Phase 1b: FOLFOX/ bevacizumab	6	—	—

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Table 1. Summary of DR agonists in clinical development (Cont'd)

DR agonist	Indication	Phase and drug combination	Sample Size	Results	Reference
	NSCLC	Randomized phase 2: paclitaxel/carboplatin/ bevacizumab	*128*	—	—
	NHL	Single arm phase 2: rituximab	*49*	—	—

NOTE: A description of trials for which no results are reported can be found at ClinicalTrials.gov (http://clinicaltrials.gov). Sample size in italics denotes estimated sample size.

Abbreviations: NSCLC, non-small cell lung cancer; CRC, colorectal cancer; NHL, non-Hodgkin lymphoma; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; Q2W, every 2 wk; CR, complete response; Q3W, every 3 wk; PR, partial response; QW, once per week; Q4W, every 4 wk.

*Data updated in presentation.

Combination Studies

Combinations with chemotherapy or targeted agents may enhance the antitumor activity of the class through cross-talk between the intrinsic and extrinsic pathways. Several phase 1b safety studies of DR agonists in combination with chemotherapy and/or targeted agents have also been reported in studies of advanced solid tumors and specific tumor histologies (40–52). DR agonists were safely combined with standard doses of cancer therapeutics in small cohorts of patients. These combinations include single-agent cytotoxics (gemcitabine, doxorubicin, and pemetrexed), cytotoxic combinations (FOLFIRI, carboplatin/paclitaxel, and cisplatin/gemcitabine), targeted agents (rituximab, panitumumab, bortezomib, and vorinostat), and cytotoxic-targeted agent combinations (FOLFOX/bevacizumab, carboplatin/paclitaxel/bevacizumab, and irinotecan/cetuximab).

Importantly, in these studies, the combinations did not seem to significantly further sensitize normal cells to apoptosis, and no significant drug-to-drug pharmacokinetic interactions were reported. Efficacy in these small, single-arm trials is difficult to formally assess in combination with another active agent. Ongoing, randomized phase 2 studies will provide a more formal evaluation of the safety and clinical efficacy of the DR agonists in a variety of combinations and tumor types. The results of these trials are anticipated over the next 1 to 2 years.

Translational Implications

In addition to evaluating rational combinations as a means to sensitize tumor cells, identifying predictive biomarkers that define a patient population likely to benefit as well as biomarkers that can be measured and evaluated objectively as indicators of pharmacodynamic response is important to the successful clinical development of any therapeutic agent. Identification of such markers for DR agonists has been challenging. Because the apoptotic ma-chinery integrates information from many sources to make life or death decisions, it is not surprising that sensitivity to DR agonists is multifactorial. Although some expression of DR4 or DR5 is required for responsiveness, the level of receptor expression does not correlate well with sensitivity to DR agonists (12). In this respect, the status of intracellular components involved in signaling, such as the ratio of caspase-8 to c-FLIP, as well as the levels of antiapoptotic regulators that influence the cell's threshold sensitivity to apoptotic stimuli, such as Bcl-2 family members and inhibitors of apoptosis, play an important role in determining a cell's response to DR agonists (12, 21).

Although changes in the level of these apoptotic regulators are associated with alterations in sensitivity in certain cell lines (12, 21), identifying molecular signatures with broad predictive value has been difficult. Enzymes that O-glycosylate DR4 and DR5 may prove to be useful stratification markers (53). High expression of GALNT14 was found to correlate with sensitivity to Apo2L/TRAIL in pancreatic, NSCLC, and malignant melanoma cell lines, whereas GALNT3, FUT3, and FUT6 levels significantly correlated with sensitivity in colorectal cell lines. The demonstration of an association between levels of these enzymes and O-glycosylation of DR4 and DR5 suggests a possible functional link between these enzymes and receptor clustering. Cell panel screening has also generated a potential stratification hypothesis for breast cancer. Cell lines that lack expression of estrogen, progesterone, and HER-2 receptors, and those that carry gene signatures consistent with the mesenchymal phenotype, have been found to be particularly sensitive to Apo2L/TRAIL (54). However, whether any of these markers show positive predictive value in patients remains to be determined.

Similar or novel determinants of sensitivity may exist in primary human tumors. In NSCLC for example, DR expression has been shown in most paraffin-embedded primary tumor samples (55). However, recent reports suggest that this signal is located predominantly in the cytoplasm and not the cell membrane (56). In a recent report of 2

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study of mapatumumab with paclitaxel/carboplatin in patients with solid tumors including NSCLC, the majority of patient tumor samples were negative for membranous DR4 by immunohistochemistry (42). Therefore, DR expression may be heterogeneous in primary human tumors. Procaspase-8 and c-FLIP are important regulators of the extrinsic pathway and are differentially expressed in some NSCLC tumor samples (57). GALNT14 is also differentially expressed in primary NSCLC and has been correlated with TRAIL sensitivity in NSCLC cell lines (39). Because many DR agonists are being evaluated in NSCLC, the examination of the DR pathway in primary NSCLC tumor samples from these trials and correlation with clinical outcomes may help generate hypotheses for patient selection in future trials.

The identification and implementation of reliable pharmacodynamic markers could be important in selecting dose and indication, real-time monitoring of drug efficacy, and prediction of clinical outcomes (58, 59). Serum-based biomarkers of apoptosis have been described, including monitoring of circulating tumor cells and measurement of active caspase-3 and caspase-cleaved cytokeratin-18 (60, 61). Serum caspase-3 levels were found to be transiently elevated in a subset of colorectal, sarcoma, and NSCLC patients treated with dulanermin in a phase 1a trial (62). Although not predictive of response, these preliminary findings provided proof of concept that apoptosis markers can be detected in patient serum and support the use of serum-based apoptosis assays as a means to monitor DR agonist activity in a clinical setting.

DR agonists represent a new class of therapeutics that selectively target apoptosis. Early clinical trials of these agents have established the safety of the approach and showed proof of concept antitumor activity. Forthcoming data from phase 2 trials will help define their clinical activity in a variety of tumor types and in different combinations. Although much has been learned about the biology of the TRAIL pathway, further work will be needed to understand factors that might limit response, overcome mechanisms of tumor-cell resistance, and identify the patients most likely to benefit from these therapies.

Disclosure of Potential Conflicts of Interest

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Death Receptor Agonists as a Targeted Therapy for Cancer

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