

Targeting the Cell Death-Survival Equation

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This issue of *CCR Focus* comprises four representative articles that closely fit the research interests of Stanley J. Korsmeyer, to whom this volume is dedicated. The first article, and perhaps the most related to Korsmeyer's work, is a review by Nika Danial of the structure and function of the ever growing BCL-2 family of proteins and their potential role in disorders as apparently disparate as cancer and diabetes (1). The second article by Amaravadi and Thompson broadens the discussion of cell death to include a penetrating analysis of the roles of necrosis and autophagy in cancer treatment (2). Our current understanding of autophagy and necrosis could not have been as adequately characterized without the generation of cells deficient in proapoptotic Bcl-2 family members (3, 4). This is followed by a third article by Rixe and Fojo, who emphasize that effective cancer treatment can occur by inducing cytostasis rather than cytotoxicity, because cytostasis is often poorly tolerated in tumor cells and, in fact, if sustained, should lead to cell death (5). They point out that we must be careful not to attribute to cytostasis what is merely insufficiently effective therapy. Finally, Verdine and Walensky return to apoptosis and show that the alpha helices of BH3 death domains can be made into drugs by means of peptide stapling and actually induce apoptosis in cancer cells (6).

Each of these four reviews make several cogent shared points, even though each deals with substantively different aspects of the process by which cell death is governed in nature.

First, the processes governing cell death involve complex interlaced metabolic pathways that form an intricate system of checks and balances. It follows that these systems present many opportunities for therapeutic intervention, but many distinct challenges as well. The cell protects itself from dying. If one wishes to promote the death of unwanted cells, one must develop agents that can defeat multiple pathways of cellular self-preservation, in addition to stimulating the prodeath pathways.

Second, we know relatively less about the factors ensuring the proper balance of prodeath and antideath pathway activities in many normal tissues. Some of these can be inferred by the incidental effects of other cytotoxic and noncytotoxic drugs on cell death pathways. We have considerably less information about what kinds of toxicities to anticipate with agents capable of altering the death/survival balance in a direct and potent way. Thus, any effort involving experimental

therapeutics targeting these pathways will require a considerable amount of basic cellular and physiologic research directed at anticipating and controlling unforeseen toxicities.

Third, the complex pathways described in each of the four reviews are particularly heavily dependent on protein-protein interactions, the formation of multiprotein complexes, alterations in transmembrane transport, and perturbation of core metabolic pathways involved in basic cell maintenance. The complex interactions currently understood for intrinsic and extrinsic apoptotic pathways are shown in Fig. 1. As noted in the article by Walensky and Verdine, making these pathways druggable may well require a different kind of medicinal chemistry, one focused on agents that directly perturb protein-protein or protein-organelle interactions, rather than inhibiting or stimulating enzymatic activities (6).

Fourth, the system tilts toward the preservation of cell survival in most of the cell types relevant to the major forms of cancer. Given the intricacy of multiple redundant pathways, each balanced against multiple countervailing measures, one must wonder whether any single agent aimed at cell death pathways will display great efficacy in traditional clinical trials. Even if the agent is highly efficient and specific in hitting and interdicting its target, it is questionable whether impeding any one step in the pathway will have a sufficient effect to tip the balance in favor of the destruction of the cancer cell. Exceptions are likely to be found if the main driver of the sustained neoplastic state is a lesion in a single pathway, such as the monolithic role of BCR-ABL in establishing and maintaining chronic myelogenous leukemia (7). In this case, the cell did not need to escape the stress induced by oncogenesis.

These considerations suggest that demonstrating efficacy in single agent trials may be difficult, making the introduction of the drug into a practical setting more daunting. A good example of this challenge is the excitement generated by early phase clinical trials of the antisense Bcl-2 molecule oblimersen. Although promising phase I/II results prompted multiple randomized phase III clinical trials in chronic lymphocytic leukemia, metastatic melanoma, multiple myeloma, and acute myelogenous leukemia, oblimersen as a single agent did not improve survival in any randomized phase III trial (8). A randomized trial with oblimersen in combination with dacarbazine in melanoma also failed to improve survival (9), but in recently reported results, oblimersen, in combination with cyclophosphamide and fludarabine, met its primary efficacy end point of improving complete response (10). Seven years after its entry into human trials, trials of oblimersen are ongoing and this experience underscores the difficulty in establishing clinical roles for agents that target cell death pathways. Finally, because these agents will likely be incorporated quickly into multiagent regimens, an additional challenge is assigning praise and blame to the novel prodeath agent or the existing cancer therapy for antitumor benefits and toxicities observed in the patient.

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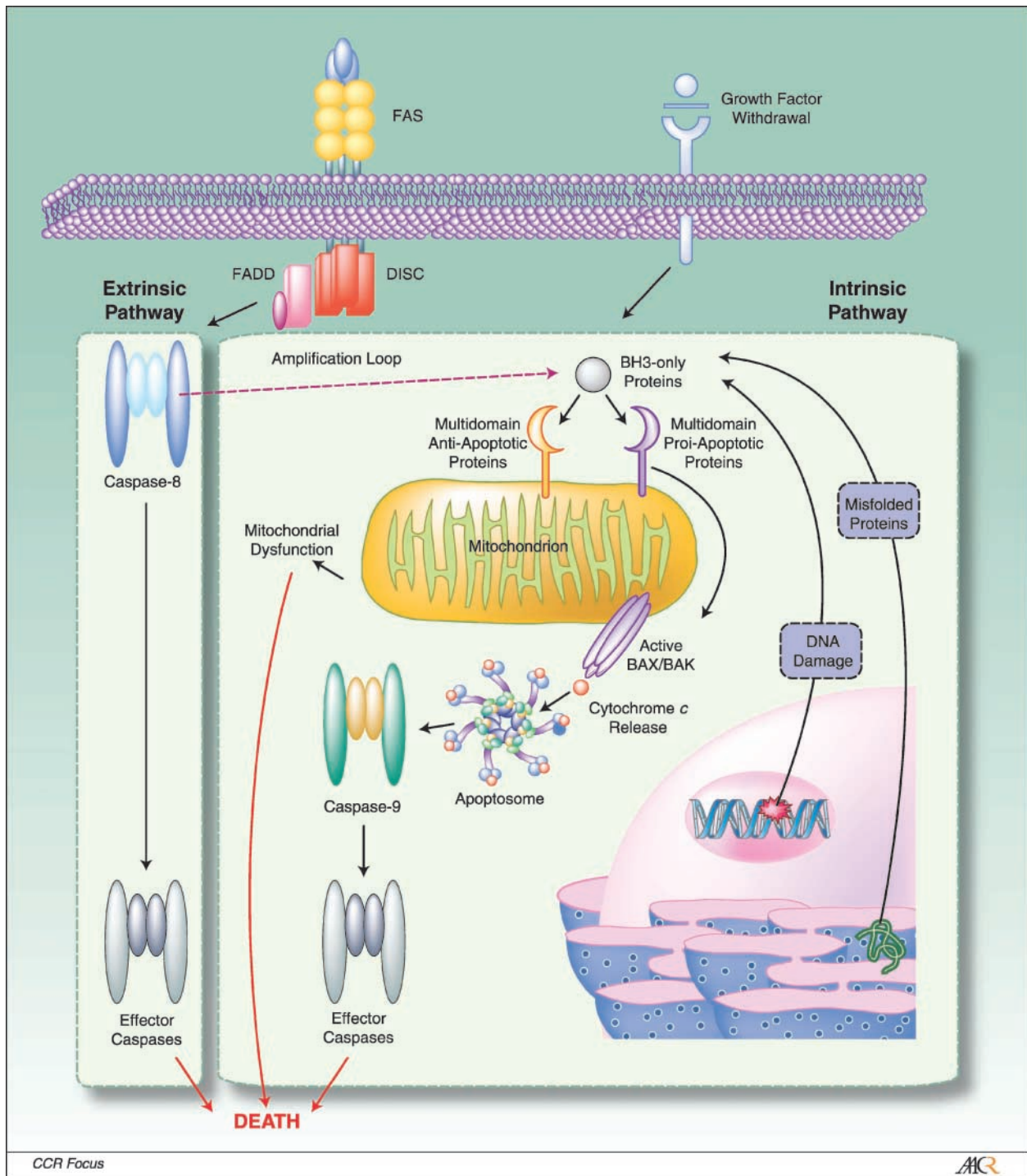


Fig. 1. Apoptotic pathways. The execution of apoptosis downstream of the death stimuli is governed by two molecular programs known as the intrinsic and the extrinsic apoptotic pathways that ultimately terminate in caspase activation. The intrinsic pathway is activated by multiple cellular stresses, including growth factor deprivation, DNA damage, and misfolded proteins. Mitochondria participate in the intrinsic pathway by releasing apoptogenic factors such as cytochrome *c*. Upon release, cytochrome *c* assembles into a molecular platform known as the apoptosome leading to the activation of initiator caspase-9. The BCL-2 protein family consists of antiapoptotic and proapoptotic molecules that function immediately upstream of mitochondria and control the release of apoptogenic factors. The BH3-only subclass of proapoptotic BCL-2 proteins respond to cellular stress and activate the mitochondrial apoptotic program by neutralizing the antiapoptotic members of the family and activating proapoptotic BAX and BAK. Upon activation, BAX and BAK undergo allosteric activation to permeabilize the mitochondrial outer membrane leading to cytochrome *c* release. The second apoptotic program, the extrinsic pathway, operates downstream of death receptors, including FAS. Activated death receptors then signal to recruit specialized protein complexes to activate initiator caspase-8. For example, a death-inducing signaling complex (*DISC*) is formed upon the recruitment of adaptor proteins, such as FADD, to activated FAS and the subsequent recruitment and activation of caspase-8. In certain cell types, the extrinsic and intrinsic pathways are linked through a mitochondrial amplification loop, involving caspase-8-mediated cleavage and activation of the BH3-only protein BID, which triggers the intrinsic pathway. Activation of effector caspases by initiator caspase-8 and caspase-9 leads to the cleavage of key cellular substrates and cell demise.

Table 1. Therapeutics that target apoptosis, autophagy, and necrosis

Class	Examples	Current status of drug development	Reference
Apoptosis			
BH3 BAD mimetic	ABT 737	Phase I	(1, 6)
Pan-BCL2 family inhibitor	GX15-070	Phase I	(1)
	TW-37		
Stapled peptide	BID SAHB _A	Preclinical	(6)
Death receptor ligand	Mapatumumab	Phase II	(19)
Caspase inhibitor	IDN-6556	Phase II	(20)
PARP inhibitor (activates apoptosis)	AG014699	Phase I	(21)
	KU59436	Phase II	
	ABT-888		
	BSI-201		
	INO-1001		
	GPI-21016		
	AZD-2281		
BCL2 antisense	Oblimersen	Phase III	(22)
	SPC-2996	Phase I/II	
MDM2 inhibitor	Nutlin-3	Preclinical	(23)
Autophagy			
Aminoquinoline	Chloroquine	Phase III	(2, 24)
	Hydroxychloroquine	Phase I	
Necrosis			
PARP inhibitor (inhibits necrosis)	AG014699	Phase I	(21)
	KU59436	Phase II	
	ABT-888		
	BSI-201		
	INO-1001		
	GPI-21016		
	AZD 2281		
Death receptor ligand	Mapatumumab	Phase II	(19)

To overcome some of these challenges, it will be important to incorporate biomarker studies into clinical trials to determine why this promising new class of drugs does not reproduce the results obtained in laboratory studies. Is the target present in the patient's tumor? Is the drug hitting its target? Is there measurable cell death? Is there evidence of activated resistance mechanisms? Should the primary efficacy end point of early trials be response rate or progression-free survival? Unless these questions are tackled head-on in the clinic, we risk rejecting life-saving therapeutics and turning our backs on the promising preclinical research that supports these agents.

Each of these reviews also makes it clear that a far more detailed understanding of the derangements of the cell survival/death balance will be critical if we are to understand the clinical behavior of individual forms of cancer in patients. The notion that the most important difference between tumor cells and their normal counterparts is their accelerated rate of cell division has long since been discarded in favor of more sophisticated notions of malregulation of cell growth and proliferation. Nonetheless, despite the knowledge gained by studies of the specific abnormalities in tumor cell genomes, much of our therapeutic approach to individual forms of cancer is still focused around the issue of cell proliferation. It is true that we now target specific steps in cell cycle regulation, signal transduction, receptor-mediated signaling, and hormone-driven proliferation, more precisely and selectively than in the past. Even in the advanced era of targeted drug development, many of the targets we select for drug

development continue to be those that, directly or indirectly, are thought to drive inappropriate proliferation or to fail to stop it.

It is very clear, as pointed out in these reviews, that the efficacy of many existing drugs is at least partly dependent on the cooperation of the targeted cell. If cell death pathways remain sufficiently intact or unblocked, the stimulus for apoptosis caused by drug-induced damage will lead to the death of the tumor cell. If not, the drugs are largely or completely ineffective. To improve the efficacy of targeted agents, it might be necessary to match the choice of a proliferation target with agents that target the specific aberrancy in the cell survival equation within the targeted cancer cells.

Another strategy that might identify the patient population that could most benefit from cell death modulators is to test these agents in molecularly defined tumors rather than traditional disease-specific clinical trials. In 1985, in the same year that the discovery of the association between B cell lymphomas and chronically overexpressed Bcl2 was reported (11), lymphomas were generated in transgenic mice chronically expressing the *c-Myc* oncogene (12). It was quickly appreciated that acute expression of *Myc* is a powerful stimulus for apoptosis (13) yet *Myc* is one of the most widely dysregulated oncogenes in cancer (14). Cancer cells that express *Myc* must have simultaneous defects in the pathways that control apoptosis to survive the *Myc*-induced oncogenic stress. Since then, nearly 18,000 articles have been published about *Myc*, but therapeutics have not yet been successfully developed to exploit the fact that unopposed *Myc* may be one of the most

powerful anticancer therapies nature will create. A central role for dysregulated Bcl-2 family proteins (15–17) has been established in allowing tumor cells to escape Myc-induced apoptosis. With the novel strategies to target apoptosis described in this issue of *CCR Focus*, a new light could be shed on exploiting the double-edged sword of oncogenesis for the benefit of our patients (18).

Taken together, all of these considerations make it clear that Korsmeyer's insight into the role played by impaired apoptosis has uncovered a complexity that we must understand and deal with if we are to develop effective and safe therapies. The work

inspired by Korsmeyer and his contemporaries has shown the extraordinary intricacy of the governing biochemical pathways. To date, most of what we have learned has been instructive but daunting. Finding ways to manipulate these pathways will not be a simple task. It is thus somewhat surprising and heartening that a number of agents have been identified and are advancing to clinical trials (Table 1). Remarkably, these agents have shown considerable promise in preclinical studies. We can only hope that at least a few of them will be chaperoned effectively through clinical trials and eventually promote a step change for cancer treatment.

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