

New apoptosis drugs face critical test

Surging numbers of therapeutic proteins and small molecules, rationally designed to trigger cell death, are entering the clinic against cancer. In theory, they should provide an entirely new kind of targeted therapy, but troubling questions of basic biology remain. Ken Garber investigates.

In late February, startup company Joyant Pharmaceuticals in Dallas, Texas, licensed some anticancer compounds from the University of Texas-Southwestern (UTSW). There is already a buzz around these compounds. In a September 2004 paper in *Science*¹, UTSW investigators described how one of them killed cancer cell lines (in combination with another compound) by inducing apoptosis, or programmed cell death, at spectacularly low concentrations. Normal cells were unaffected. Former Pfizer cancer researcher Yi Sun, now at the University of Michigan in Ann Arbor, independently tested the UTSW agent on breast cancer cell lines, and was astounded. "It's the best compound I've ever used," he says. "Five nanomolar [concentration] to induce apoptosis as a single agent!"

Of course, such *in vitro* tests are anything but a guarantee of success in humans. And no drug designed to induce apoptosis has yet to be approved for cancer. The first attempt, by Genta's antisense drug Genasense, failed when a US Food and Drug Administration (FDA) advisory panel last May turned it down for metastatic melanoma. (The company, based in Berkeley Heights, New Jersey, saw its stock fall 87% the next day.) But Genasense's troubles aren't seen as target-related, due to antisense's perceived deficiencies as a delivery system, so Genasense hasn't shaken anyone's confidence in the newer apoptotic agents. Several such drugs, both proteins and small molecules, are already in clinical trials, targeting three key apoptosis proteins (Table 1). "There's a lot of

activity...and there's a lot of anticipation," says Gordon Shore, CSO of GeminX, in Montreal, one of the companies with an apoptosis drug in the clinic.

Suicide watch

There are compelling reasons for the excitement. Apoptosis is absolutely necessary for human development and survival, with millions of cells committing suicide daily as a way to prevent uncontrolled growth. Defects in apoptosis, together with amplified growth signals, often lead to cancer. Targeting apoptosis defects in cancer "has tremendous potential," says Avi Ashkenazi, a staff scientist in the department of molecular oncology at Genentech, in S. San Francisco, California, one of the companies most involved in the effort.

Embryologists, over a hundred years ago, were the first to recognize cell death as a necessary process, but the critical importance of apoptosis in eliminating unwanted adult cells only became accepted with the isolation of its molecular components beginning in the 1980s. The first human apoptotic protein identified was BCL-2, an inhibitor of apoptosis, in 1984. (Genasense targets BCL-2.) The role of caspases—proteases that act as the cell's direct executioners by cleaving other cellular proteins—was revealed in humans beginning in 1993. This followed Robert Horvitz's discovery at the Massachusetts Institute of Technology in Cambridge of apoptotic proteins in *Caenorhabditis elegans* in the 1980s. (Horvitz was awarded the 2002 Nobel Prize in physiology or medicine for his apoptosis work.) In cells ready to die, pro-apoptotic BCL-2 family members, like BAX, disrupt mitochondria, causing the release of other proteins that lead to caspase release and cell death (Fig. 1). Activation of this so-called 'intrinsic' apoptotic pathway is the goal of many of the new cancer drugs. Joyant's compounds featured in *Science* target XIAP (X-linked inhibitor of apoptosis), a key component of the intrinsic pathway first identified in humans a decade ago.

A second, 'extrinsic,' cell death pathway is also an important target. Around 1996 the first so-called 'death receptor,' DR4, was discovered independently at the University of Michigan and at biotech company Human Genome Sciences (HGS) in Rockville, Maryland. Bound by an endogenous ligand, dubbed TRAIL (TNF-related apoptosis-inducing ligand), DR4 and its sister receptor, DR5, activate a pathway that ultimately also leads to cell death, with remarkable—and still largely mysterious—specificity for cancer cells. HGS, over the last seven years, has mounted a major effort to find drugs activating the TRAIL death receptors.

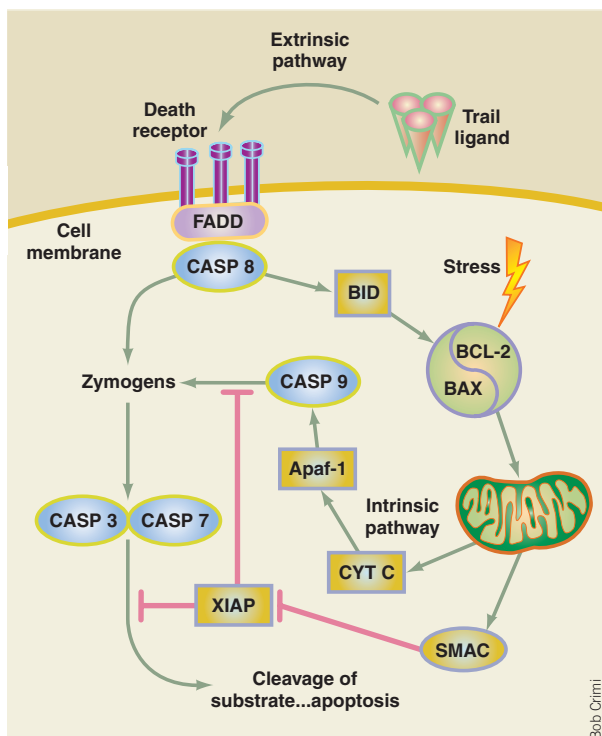


Figure 1 Pathways to death.

Table 1 Selected companies targeting apoptosis in cancer

Target	Company	Agent	Clinical stage
XIAP	Burnham Institute (La Jolla, California)	Small molecule Smac mimetics	Preclinical
	Idun Pharmaceuticals (San Diego, California)	Small molecule Smac mimetics	Preclinical
	Joyant Pharmaceuticals (Dallas, Texas)	Small molecule Smac mimetics	Preclinical
	Gentara (Malvern, Pennsylvania)	Small molecule Smac mimetics	Preclinical
	Novartis (Basel)	Small molecule Smac mimetics	Undisclosed
	Aegera (Montreal)	Antisense XIAP inhibitor	Phase 1
	Avi BioPharma (Portland, Oregon)	Antisense XIAP inhibitor	Phase 1
TRAIL receptors	Human Genome Sciences (Rockville, Maryland)	Agonist monoclonal antibodies for TRAIL receptors	Phase 1 and 2
	Sankyo (Tokyo)	Agonist monoclonal antibody for TRAIL receptor	Preclinical
	Novartis (Basel)	Agonist monoclonal antibody for TRAIL receptor	Undisclosed
	Genentech (S. San Francisco, California) and Amgen (Thousand Oaks, California)	Recombinant TRAIL ligand	Phase 1
BCL-2	Abbott Labs (Abbott Park, Illinois)	Small molecule BCL-2 inhibitor	Preclinical
	Genta (Berkeley Heights, New Jersey)	Genasense, anti-BCL-2 antisense	Phase 3
	GeminX (Montreal)	Small molecule BCL-2 inhibitor	Phase 1
	Ascenta (San Diego, California)	Small molecule BCL-2 inhibitor	Phase 1
	Infinity Pharmaceuticals (Cambridge, Massachusetts)	Small molecule BCL-2 inhibitor	Preclinical
	Burnham Institute (La Jolla, California)	Small molecule BCL-2 inhibitor	Preclinical

Three are now in clinical trials, with the most advanced candidate in phase 2 (Table 1).

HGS's drugs are agonist, or activating, monoclonal antibodies—they engage and activate the TRAIL receptors, unlike all monoclonal antibody therapeutics on the market, which block their targets. According to HGS vice president and COO, Craig Rosen, straightforward assays to look for TRAIL receptor binding and cell death made finding such unlikely antibodies possible, as did massive phage display libraries. It helped that the activated TRAIL receptor is a trimer that, presumably, only needs to come together to turn on. "The biology suggests that crosslinking is what causes activation," says Rosen—hence the success of agonist antibodies. "Single receptors... probably would be much more difficult."

Agonist antibodies targeting DR4 and DR5 have proven safe in phase 1 clinical trials, according to HGS, and "stable disease was observed... in multiple patients in both studies," says HGS vice president for drug development David Stump. Phase 2 trials for HGS's anti-DR4 antibody are ongoing in lung cancer, colorectal cancer and non-Hodgkin's lymphoma, and results should be announced by the end of the year. Sankyo of Tokyo and Novartis of Basel also are developing agonist monoclonal antibodies against TRAIL receptors.

Meanwhile Genentech, in collaboration with Amgen of Thousand Oaks, California, are targeting the same pathway using recombinant TRAIL ligand instead of monoclonal antibodies. Scientists at Genentech and at Immunex (now part of Amgen) in Seattle, Washington, separately cloned TRAIL in the mid-90s, and they're moving forward with

Genentech's recombinant version, consisting of the functional part of the protein's extracellular domain. It's in phase 1. Possible advantages of Genentech's approach include the ability to target both death receptors with a single drug; on the other hand, HGS's antibodies have a much longer half-life in serum. At this early stage, "it's really impossible to tell which molecules are going to be more effective, and if there will be any winners here," says Genentech's Ashkenazi.

Both approaches must overcome the problem of TRAIL resistance. "A majority of solid tumor lines we look at are fairly insensitive to TRAIL," says John Reed, a prominent apoptosis researcher at the Burnham Institute in La Jolla, California. Reasons for resistance are many, but combination treatment with chemotherapy provides one possible solution. In resistant cell lines, "when we add the chemotherapy with TRAIL, we overcome the resistance," says HGS's Rosen. Reed is working on drugs that target a protein called FLIP, an apoptosis-suppressing protein, which is implicated in TRAIL resistance.

Another concern about TRAIL therapy is that TRAIL's role in normal biology remains murky. Nobody knows why TRAIL kills cancer cells but not normal cells, or even what TRAIL may be doing to normal cells to begin with. TRAIL's cancer specificity "sounds too good to be true," acknowledges Ashkenazi. TRAIL's fellow family member, Fas ligand, triggers the identical death pathway in cancer cells, but also kills normal cells expressing its receptor. Not so TRAIL. "It's a real mystery," says Richard Siegel, an immunologist at the National Institutes of Health in Bethesda, Maryland. Some experiments hint

that TRAIL naturally protects against tumors or viruses. But a December paper in *Immunity* showed that TRAIL receptor knockout mice had enhanced innate immune function unrelated to apoptosis, suggesting that TRAIL functions to downregulate innate immunity². So might TRAIL therapy shut down patient's immune systems? "I don't think that there's a major risk," answers Ashkenazi. "Obviously the only way to rule this out is by actually testing it in patients."

Meanwhile, efforts to target the intrinsic pathway are also moving steadily forward, despite the travails of Genasense. Genasense itself still has a chance for FDA approval for treating chronic lymphocytic leukemia, based on phase 3 results showing more patients with major response to the drug in combination with chemotherapy than for chemotherapy alone. "We're excited to see the activity, and you know, we'll be anxious to see how the FDA views the results," says Reed, who invented Genasense in the late 1980s at the University of Pennsylvania in Philadelphia. But phase 3 Genasense benefits were "not as great as you might expect, using an antiapoptotic potentiating agent," says hematologist and leukemia researcher Beverly Mitchell of the University of North Carolina in Chapel Hill. The delivery system, not the target, may be the problem. "Antisense, while really appealing in concept, probably [is] not going to be as effective as small molecule inhibitors," Mitchell says.

Death wish

Many such small molecule BCL-2 family inhibitors are in the pipeline. In 1996 Steve Fesik of Abbott Pharmaceuticals, in Abbott Park,

Illinois, solved the crystal structure of BCL-XL, an antiapoptotic protein closely related to BCL-2. That structure intriguingly revealed a deep binding groove where BCL-2 proteins bind to proapoptotic family members (Bax and Bak, for example) inactivating them and preventing cell death. Some natural human proteins bind to the same pocket to inactivate BCL-2. If small molecule mimetics could be designed to do the same thing, reasoned many researchers, it should be possible to find an effective BCL-2 and BCL-XL inhibitor.

That theory is now being tested. Abbott has in advanced preclinical development such a compound—found together with Idun Pharmaceuticals, a San Diego, California, company that may soon become a part of Pfizer of New York. (In February, Pfizer announced its intent to purchase Idun.) Reed's group at the Burnham, and medicinal chemist Shaomeng Wang's group at the University of Michigan, have both found compounds fitting the BCL-2 pocket. They're based on gossypol, a natural product derived from a Chinese herbal medicine, with Wang's compound, licensed to San Diego-based biotech company Ascenta, already in phase 1.

All these compounds must block a protein-protein interaction, a formidable task for a small molecule. But, because endogenous peptides are known to block it, "BCL-2 is somewhat unique," says Gordon Shore of GeminX. "We really did believe that this protein-protein interaction was 'druggable.'" GeminX found a small molecule blocker through screening, and it's now in separate phase 1 trials in solid tumors and chronic lymphocytic leukemia. The drug hits all five antiapoptotic BCL-2 family members, which Shore believes is crucial for effectiveness. That's partly because new evidence points to BCL-2 alteration of cellular calcium levels as another way the protein blocks apoptosis, in addition to BCL-2's more familiar role as an inactivator of pro-apoptotic proteins. "A pan-BCL-2 inhibitor [has] a better chance to cover the full spectrum of regulation," says Shore.

The biggest question about targeting BCL-2 is side effects. "There is a problem with [cancer cell] specificity," says Emad Alnemri, a prominent apoptosis researcher at Thomas Jefferson University in Philadelphia. "A lot of cells, normal cells, are dependent on BCL-2 family members to maintain mitochondrial function." But cancer cells are uniquely vulnerable, Shore believes. Because of the stress imposed by transforming oncogenes, they're primed to self-destruct, he contends, and only overexpression of prosurvival proteins like BCL-2 is blocking apoptosis. Thus targeting BCL-2

should kill cancer cells but spare normal cells.

This explanation, so far, is mostly theoretical. "In normal cells, those kind of apoptosis pathways are not active," Shore says, so excess BCL-2 isn't present. And since the balance between pro- and antiapoptotic BCL-2 family members dictates the cell's fate, a drug might alter that skewed balance in cancer cells just enough to kill them, without bothering normal cells. It's an argument that can't be proven except in the clinic. GeminX expects to announce phase 1 results by year's end.

Freeing cells to die

The other apoptosis target generating great excitement is XIAP. Human XIAP binds to three key caspases, preventing them from activating and killing cancer cells. XIAP's allure as a target stems from the belief that it might be the only thing holding back cancer cells' inherent self-destructiveness. "What we hypothesize is that tumor cells, because of their aberrant behaviors, must be constantly activating caspases, including downstream effector caspases," says Reed. "The way [the cancer cells] tolerate that is by overproducing...proteins like XIAP." Hitting XIAP with a drug, the theory goes, frees caspases to finish off the cell. Normal cells, without activated caspases, would remain unaffected.

But, like BCL-2 inhibitors, XIAP inhibitors must block a protein-protein interaction. And, as with BCL-2, it was a crystal structure that provided the key to solving that challenge. In 2000, two groups independently discovered an endogenous protein, called Smac, that when released from mitochondria binds with XIAP and inactivates it, triggering apoptosis. That discovery raised the possibility of Smac mimetics to treat cancer. Later that year, Yigong Shi of Princeton University in New Jersey solved Smac's crystal structure, showing that a tiny part of Smac bound XIAP, immediately suggesting that a small molecule mimetic could work.

Several companies are developing small molecule XIAP inhibitors, including Gentara, a Malvern, Pennsylvania, startup cofounded by Shi. But, at least in terms of published results, the UTSW compounds featured in *Science* are the most potent. That's because they're dimeric compounds, mimicking Smac, which is a dimer. "It turns out that you need to touch the IAP protein at two surfaces," says UTSW medicinal chemist Patrick Harran. This became obvious only after the first dimeric compound was made "completely by accident," Harran says, while attempting a different chemical modification. When an alert chemist luckily tested it for activity, it turned out to be a 1,000-fold more potent than the best monomeric compound. Joyant

Pharmaceuticals plans to take optimized versions into preclinical development soon.

Most competing companies are designing drugs targeted to one of the two XIAP-caspase binding domains, but Alnemri agrees that both must be blocked to mimic Smac's full caspase activation function and ensure that apoptosis takes place. "If you only activate the upstream, or the initiator caspases, you may not actually be able to really induce cell death in a cancer cell," he says. "So you need the effector caspases to be activated as well." For his part, Michigan's Wang says he can target both domains with a single drug, "with extremely high affinity." No known small-molecule XIAP inhibitor, however, has yet made it to the clinic.

Meanwhile, basic biology is raising troubling questions. Work by University of Michigan at Ann Arbor molecular biologist Colin Duckett, who cloned human XIAP in 1995, suggests that XIAP promotes cancer as a signaling molecule, not by inactivating caspases. "To my mind, we really don't know what XIAP does, at the moment," he says. XIAP knockout mice show no obvious defects in apoptosis. To Reed, that just confirms that normal cells don't depend on XIAP in the way that cancer cells do. But it could also mean that XIAP's effect on apoptosis has been overestimated. "Our hypothesis [is] that XIAP is important to cancer, but it's not about caspases," says Duckett. He worries that drug development is moving forward without knowing enough about the target. "We got to this rational design for small molecules very quickly in the IAP field," he says. "The biology is kind of lagging a little bit behind."

All three approaches, TRAIL therapies, BCL-2 inhibitors and Smac mimetics, must be clinically validated. Human tumor cells may in fact be poised to die, and a nudging may be all it takes. On the other hand, tumors could develop resistance, or normal cells might also die in massive numbers.

"The issue is, how much toxicity are you going to have, in normal cells?" says Siegel. For an answer, "you then have to reach into your pocket for that sort of 'cancer cells are more susceptible' deal.... We'll have to see how all that pans out." After all, traditional cytotoxic chemotherapy also kills cancer cells by indirectly inducing apoptosis. Unfortunately, side effects are brutal, and most tumors become resistant. For all the elegant science behind the new rational apoptosis therapies, they must do better.

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1. Li, L. *et al.* A small molecule Smac mimic potentiates trail- and TNF α -mediated cell death. *Science* **305**, 1471-1474 (2004).
2. Diehl, G.E. *et al.*, TRAIL-R as a negative regulator of innate immune cell responses, *Immunity* **21**, 887-889 (2004).