

A Better Immunotoxin

Through inspired engineering, proteins that enter and kill cancer cells are finding their place among cancer treatments.

(Photo: R. Baer)



Ira Pastan, M.D.

Just as computer makers continue to devise smaller, faster, and more powerful laptops, the scientists in CCR's Laboratory of Molecular Biology (LMB) will not stop re-engineering their cancer-killing immunotoxins until they have created the optimum life-saving treatment.

Immunotoxins are proteins designed to deliver a lethal blow directly to cancer cells. Each has two parts: a custom-designed antibody that can home in on a specific target on the surface of the cancer cells and a toxin, which delivers the fatal blow. When the antibody binds to its target, the whole complex is pulled inside of the cancer cell, where the toxin can do its work.

The team of CCR researchers, led by Ira Pastan, M.D., the Laboratory's Chief, shares a drive to do more for people with cancer. They have already begun to see their determination to devise better immunotoxins pay off, but they are continuing to dig deeply into the workings of the cell to learn why immunotoxins work in some patients and not in others. And their ability to quickly translate these insights from the labs at CCR to the infusion rooms at the NIH Clinical Center is helping them move deftly toward new anti-cancer therapies.

After spending most of his career studying cell surface receptors and their signaling pathways, Pastan shifted gears. "Since I was a physician trained to do research," he recalled, "I wanted to use what I knew to do something relevant to the treatment of cancer." He began working with *Pseudomonas* exotoxin A (PE), a bacterial protein with potent cell-killing activity, and became intent on finding a way to deliver the lethal toxin to tumor cells.

Proof of Concept

When Pastan first started working with immunotoxins in the early 1980s, existing PE-based immunotoxins had several limitations. They were complex and costly to produce, and their large size prevented them from efficiently penetrating bulky tumors.

Pastan was able to make several important improvements by using recombinant DNA techniques to engineer smaller, more nimble immunotoxins that could be

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produced inexpensively in large quantities. "That conversion," said Pastan, "from coupling toxins to antibodies using chemistry to using genetic engineering to make these molecules, was really a big breakthrough."

His team's first target was a receptor, CD25, found on the surface of many B- and T-cell cancers as well as some normal B and T cells. Using an immunotoxin called LMB-2, Pastan and CCR colleague Robert J. Kreitman, M.D., Head of the LMB Clinical Immunotherapy Section, conducted a Phase

I clinical trial in patients with CD25-expressing hematological malignancies that had failed previous treatment.

LMB-2 was well tolerated by the study's patients. More importantly, several patients on the trial experienced a disease response within one week of treatment with LMB-2. In particular, four trial participants with a rare cancer called hairy cell leukemia (HCL) all demonstrated major responses to the immunotoxin. In one patient, the count of malignant cells dropped to an undetectable level. The LMB-2 trial clearly illustrated that powerful, targeted cell-killing agents could be designed and produced using genetic engineering.

Though the results were encouraging, Pastan and his colleagues wanted to develop immunotoxins to treat more common cancers (only 900 to 1,000 people are diagnosed with HCL in the United States each year). They decided to target CD22, a membrane protein commonly expressed on B-cell lymphomas and leukemias. While CD22 is also expressed on normal B cells, it is not present on the stem cells that generate B cells, allowing the body to readily regenerate its B cells and making CD22 a potentially good target for immunotoxin therapy.

Pastan teamed up with David J. FitzGerald, Ph.D., Head of the LMB Biotherapy Section, and Kreitman to design a new immunotoxin—dubbed BL22—to deliver PE to CD22-expressing cells and test it in a Phase I clinical trial in patients with chemotherapy-resistant non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), and HCL.

Even at low doses, many HCL patients responded to BL22. With increased doses, their responses grew more frequent and more dramatic. The cancer cells of many HCL patients completely disappeared from the blood, bone marrow, and spleen, while normal blood cells—which are commonly at dangerously low levels in patients with advanced blood malignancies—returned to normal levels.

More than half of the patients with advanced HCL were able to return to a normal life, and many remain in complete remission more than three years after the initiation of treatment. In a 2005 review article, Pastan noted that “This clinical experiment, in which more than half of the patients with an advanced malignancy were able to resume a normal life, was certainly the most rewarding experiment in my research career.”

Beyond Hairy Cell

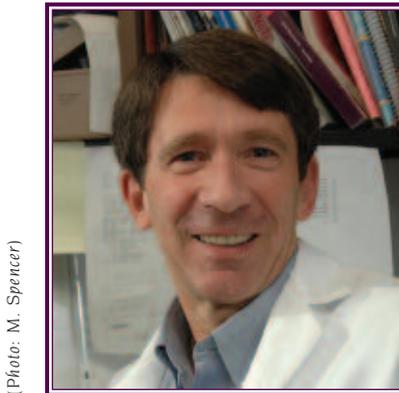
Although the responses in HCL patients to BL22 were profound, results in patients with other cancers were less encouraging. This information left the Pastan research team with a challenging decision. Should they continue to work on BL22 and try to get it approved for treatment of the relatively rare HCL? Or should they try to engineer an immunotoxin that could effectively treat more common CD22-expressing cancers like CLL, which afflicts more than 15,000 each year?

The group decided they could make an immunotoxin that did both. They knew that they had to find a way to deliver more immunotoxin to CLL cells. “CLL cells don’t have as much CD22 as HCL cells,” Kreitman explained. Indeed, a single HCL cell might have as many as 70,000 CD22 proteins on its surface, giving BL22 ample opportunity to latch onto the cell. CLL cells often have fewer than 1,000.

They needed an antibody that binds with higher affinity (more tightly) to CD22. This would make it less likely that the immunotoxin will fall off once it binds to the cell, increasing the probability that it will be ushered inside.

To optimize BL22’s affinity, the Pastan lab utilized a method called hot spot

mutagenesis, which mimics the way the immune system would do it. They altered specific regions of their anti-CD22 antibody and looked for mutants that bound more tightly to CD22. When they found what they were looking for, they built HA22—which is up to 20-fold more powerful than its predecessor. Pastan and his colleagues recently launched a Phase I/II trial to find out if HA22 can more effectively combat both cancers. Initial results have been promising, and the team hopes to know within the next few years whether the new immunotoxin will prove to be an improved treatment against CD22-expressing malignancies.



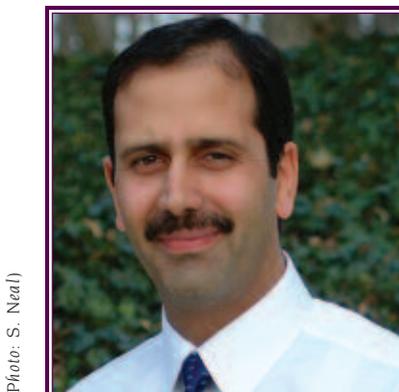
(Photo: M. Spencer)

David J. FitzGerald, Ph.D.



(Photo: M. Spencer)

Robert J. Kreitman, M.D.



(Photo: S. Neal)

Raffit Hassan, M.D.

Tackling Solid Tumors

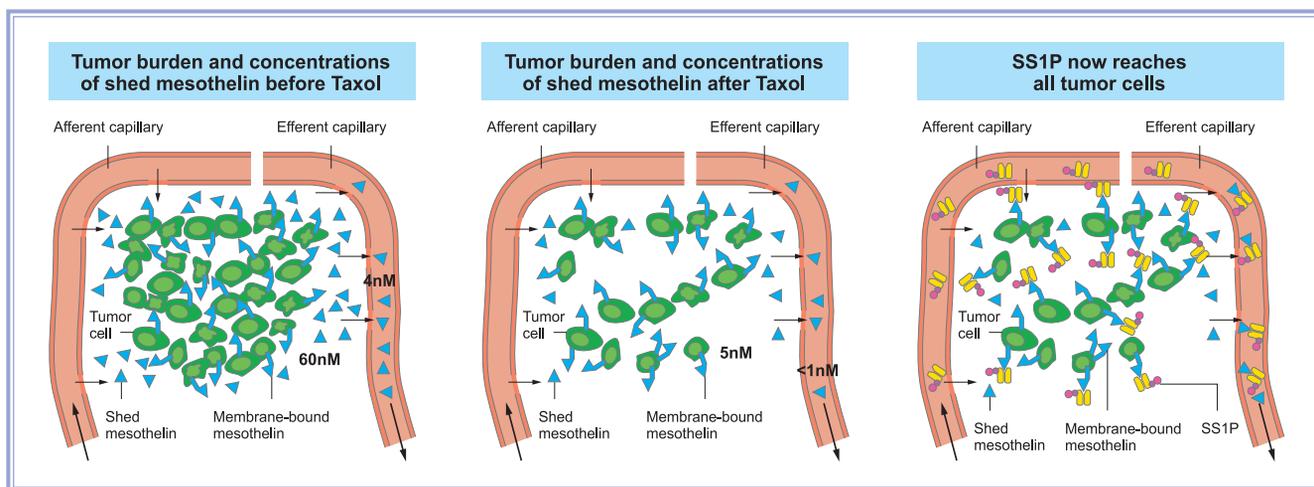
While hematological malignancies are well suited for immunotoxin treatment because they are easily accessible via the bloodstream, solid tumors are harder to reach and to penetrate. Pastan and his group, however, believe that these barriers are surmountable and have set out to identify a target to help them confirm their belief.

More than ten years ago, Pastan and his LMB colleague Mark Willingham, M.D., now at Wake Forest University School of Medicine, identified mesothelin when they were searching for proteins expressed in ovarian cancer cells. Mesothelin is highly expressed in more than 70 percent of ovarian cancers, as well as a high percentage of mesotheliomas (cancers of the membrane that encapsulates most of the major organs), lung adenocarcinomas, and gastric and pancreatic cancers.

Though its function in cancer cells has not been well characterized, there is some evidence that mesothelin’s interactions with another protein, CA-125, may promote the spread of ovarian cancer. Mesothelin is normally expressed on the epithelial cells that line body cavities but not in essential organs such as the heart, brain, liver, and kidneys. “Since there is high expression in tumors, but very little expression in normal tissue, it makes a good target for antibody-based therapy,” said Raffit Hassan, M.D., Chief of LMB’s Solid Tumor Immunotherapy Section.

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(Image: J. Kelly)



To be effective against solid tumors like those of ovarian cancer, immunotoxins like SS1P (which targets a protein called mesothelin) need to be able to penetrate deeply into the tumor. In mice, treating tumors with a chemotherapy agent like Taxol® before administering SS1P greatly increases the immunotoxin’s activity, possibly in part by disrupting the tumor’s structure.

To target mesothelin, the Pastan lab engineered an immunotoxin called SS1P. In laboratory tests, SS1P had impressive activity against cells from ovarian cancer patients. It also made tumors shrink and prevented lung metastases in mouse models. Based on these preclinical data, Pastan, Hassan, and Kreitman launched two separate Phase I clinical trials to test SS1P in patients with recurrent mesothelioma, ovarian cancer, and pancreatic cancer.

SS1P did not cause toxicities in any essential tissues, such as heart and liver, but it did cause an uncomfortable but not life-threatening inflammation of the body cavity lining. Patient responses to treatment were modest, but they were considered encouraging when compared with results seen with other immunotoxins and antibody-based therapies used against solid tumors.

There currently are limitations to SS1P’s effectiveness. Like other immunotoxins and antibody-based therapies, SS1P has trouble penetrating solid tumors. Such tumors have poor vascular and lymphatic systems, impeding the delivery of treatment agents. They also have high internal pressures, which favor an outward rather than an inward flow of material. In addition, the Pastan lab has found that solid tumors shed large amounts of mesothelin from their cell surfaces, which may absorb the immunotoxin and keep it from interacting with and killing target cells.

Interestingly, Pastan’s group has found that tumor-bearing mice treated first with a chemotherapy agent called Taxol® and then with SS1P show dramatic tumor regressions due to a synergistic interaction of the two drugs. “We think the synergy is caused by two things,” Pastan explained. “We think the Taxol kills some tumor cells and disrupts the organization of the tumor, allowing the immunotoxins to penetrate better. And the shed mesothelin, which is very high in the tumor, falls to very low levels and doesn’t soak up the immunotoxin. Taken together, this strategy gives us fantastic synergy.”

Pastan and Hassan are preparing to test their theory in a Phase II clinical trial

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combining chemotherapy with SS1P. The researchers hope that this combination approach will be a newly effective strategy for targeting solid tumors with immunotoxins.

The other protection solid tumors have that hematologic malignancies do not is a strong immune system on the part of the

patient. “So far we can only give patients with solid tumors one or two cycles before their immune systems mount a very good antibody response to the toxin and neutralize it,” Pastan lamented. This issue is rarely a problem in patients with hematological malignancies because their immunity is much weaker, having been suppressed by both the disease and previous chemotherapies. As a result, these patients can receive multiple cycles of therapy.

Pastan’s team is pursuing several strategies to enable the immunotoxins to evade the immune system. For example, they are trying to identify regions of the toxin recognized by patients’ immune systems and then modify or delete them using protein engineering.

Immunotoxins of the Future

The CCR research team continues to learn how to make immunotoxins even more deadly to cancer cells and to identify new therapeutic targets. Scientists know that the PE toxin inhibits protein synthesis once inside the cell but not how this triggers cell death. FitzGerald is exploring where the toxin goes after gaining entry; perhaps, he said, “Different cell surface targets may shuttle the toxin along different intracellular paths. If one pathway offers greater benefit to patients, perhaps an immunotoxin could be engineered to follow that better path.”

Even with cutting-edge high-throughput techniques, finding the ideal immunotoxin target is a difficult task. Proteins must meet several stringent criteria to qualify as potential immunotoxin targets. They must appear on the cell surface, because immunotoxins are unable to cross cell membranes unassisted. And they must be expressed mainly on cancer cells and nonessential normal cells, not in essential tissues such as the kidneys, heart, and brain; any attack by an immunotoxin on these tissues could result in serious side effects.

Pastan and his colleagues found one new receptor by scouring the literature to identify proteins that are selectively expressed in certain types of cancers. FCRL1 is part of the Fc receptor-like (FCRL) family of proteins, which is expressed by different cells of the immune system. FCRL1 is selectively expressed on B cells and is present at high levels on the tumor cells of CLL patients. The scientists have generated two immunotoxins that recognize FCRL1, both of which have shown promise in the culture dish. These new immunotoxins are now ready for testing in animals.

The Pastan lab is also particularly interested in identifying new targets for breast and prostate cancers, since people can survive without the normal tissues associated with these organs. Searching through gene databases, Pastan lab Staff Scientist Tapan Bera, Ph.D., and colleagues in the lab have found a potential target for prostate cancer, a protein called NGEF, that is expressed in prostate cancer and normal prostate cells but not in essential tissues. The team is now developing antibodies to NGEF, which will be used as the basis for new immunotoxins that they hope will represent a new treatment option for prostate cancer patients.

Pastan's work has been over 20 years in the making, but his progress in turning his basic discoveries into effective tools has been accelerating quickly. Bera joined the Pastan lab in 1995, "to be involved in a research project where you can see the clinical benefit right away." He has not been disappointed.

From Dangerous Fatigue to an Energetic Retirement

(Photo: Courtesy of D. Brenneman)



As he neared his fiftieth birthday, Dave Brenneman, an engineer for the State of Pennsylvania, began to notice that his energy was flagging. He tried to convince himself that it was all in his head or the inevitable result of getting older, but when his fatigue persisted, he decided to get a check-up. His family doctor told

him that his blood counts were lower than normal. A visit to a local oncologist and a series of additional tests revealed the "odd-looking" cells of hairy cell leukemia (HCL) in his blood.

Like many HCL patients, Brenneman was treated with the drug cladribine. His blood counts climbed back to normal over the next few months. "I was feeling great," Brenneman related, "but after approximately three years, my counts started to drop again." Two years later, he received another round of cladribine, but this time the cancer began to come back after only a year.

A proactive patient, Brenneman turned to the Internet to find other potential treatment options for HCL. He stumbled across information on a clinical trial being conducted on a new drug called BL22 and contacted Robert Kreitman, M.D., at CCR to find out more.

Kreitman and his colleagues looked carefully at Brenneman's cancer and decided that he was a good candidate to receive BL22. "His bone marrow was 70 to 80 percent HCL cells," Kreitman recalled, "and his neutrophils, which help fight infection, had decreased to a count of 508. Less than 500 is considered life-threatening."

Brenneman had reservations about joining a clinical trial. "I was very skeptical, but as I learned more and talked with Kreitman and his staff, I became more comfortable with the idea." He traveled to the NIH Clinical Center to receive his first cycle of the immunotoxin in September 2005. That one cycle was enough to trigger a complete remission. "His neutrophil count four weeks later was normal and the hairy cells were gone from his bloodstream," Kreitman explained. "Repeat tests showed no hairy cells left in his bone marrow either." The benefits of that single cycle of BL22 have lasted; Brenneman's bone marrow biopsies and blood tests have remained clear for over two years.

Thanks to BL22 and the dedicated team of CCR researchers, Dave Brenneman is making the most of his recent retirement. He has plenty of energy to maintain the dairy farm that has been in his family for three generations, work on his classic car, and spend time with his wife, children, and grandchildren. His strong response also illustrates the potential of BL22 and next-generation immunotoxins for treating HCL and—with continued improvements—other cancers as well.