Whatever Happened to Immunotoxins? Research, and Hope, Are Still Alive

By David C. Holzman

At the start of the 1980s, researchers began developing a new idea for cancer therapy. The logic was simple: Attach a toxin to an antibody that targets proteins present only on cancer cells. The early examples, chemically conjugated, are called immunotoxins, whereas the more advanced versions, genetically combined, are called fusion protein toxins. The first successful class member began development in the mid-1980s and gained U.S. Food and Drug Administration approval in 1999 for cutaneous T-cell lymphoma.

Yet the field is 30 years old and this drug, denileukin diftitox (Ontak), is still the only FDA-approved biologic in its class with just one more about to enter a phase III trial. Some researchers blame technological hurdles for the dearth of approved products. Others trace interruptions in research and development to the complex world of drug company mergers and intellectual property issues. Despite these obstacles, the field has survived lean times and even seen a bit of a renaissance of late, according to experts who hope that recent advances will lead to fusion protein toxins aimed at a variety of solid tumors as well as leukemias.

Many of the technical hurdles in developing these agents relate to their adverse effects. In native form, the toxins used—primarily diphtheria and Pseudomonas exotoxin—are highly immunogenic, which can limit the numbers of treatments to one or two cycles, especially for solid tumors.

And despite precision targeting, some toxin molecules inevitably wander the body until they are filtered by the kidneys, subjecting these organs, as well as the liver and vascular endothelium, to a bolus of poison, said Daniel Vallera, Ph.D., an immunotoxin researcher at the University of Minnesota Cancer Center in Minneapolis. Also, the early investigators faced challenges in joining toxin to antibody. Chemical conjugation resulted in heterogeneous mixtures of desired product with unwanted compounds. Using genetic techniques, denileukin’s main inventor, John R. Murphy, Ph.D., a professor at Boston University, built a first-generation fusion protein toxin in a year and a half of 12-hour days, with weekly round trips between Boston and a BSL-4 laboratory (one that meets the most stringent biosafety standards) at the National Institutes of Health. A decade later, a week in a BSL-2 laboratory would have sufficed.

Business-related issues have also derailed promising drugs. In the course of drug development, companies have been bought, been sold, or gone out of business, and buyer companies have acquired portfolios of intellectual property related to the technology, parts of which are of no interest to them.

Three Generations

Arthur Frankel, M.D., a professor at the Texas A&M Health Science Center College of Medicine in College Station, loosely divides the history of the technology into three generations. The first generation faced several hurdles. The first was the unwanted heterogeneity of chemical conjugation products. “You had to try to find the one species that gives activity,” said Bob Harrison, Ph.D., co-founder and chief scientist at Anjin Group, a small biologics firm in Cockeysville, Md., that is developing a fusion protein toxin.

Also, these compounds’ binding affinities were often only modest. The drug molecules were large, hindering their ability to escape from the bloodstream to find their targets. That resulted in toxicity, because they “were seen at high concentrations by endothelial and liver cells,” said Frankel, who also is director of the Cancer Research Institute of Scott and White Memorial Hospital in Temple, Texas.

Another problem was that the toxins, once parked on the surface of the cancer cells, did not necessarily gain entry—a prerequisite for the kill.

Frankel dates the start of the second generation to the early 1990s, when researchers learned how to readily fuse toxin and antibody at the genetic level, creating a single protein that could be cranked out in bacterial factories. That boosted binding affinities and reduced toxicities while simplifying production.

A predecessor of denileukin was a fairly typical second-generation product. It was still large. The diphtheria toxin retained all 486 amino acids. But though it was hard to work with, “the first clinical trials were extremely promising in that one of the first patients achieved a durable complete clinical remission from his refractory chronic T-cell lymphoma,” said Murphy.

The diphtheria toxin moiety later was pruned to 389 amino acids, resulting in easier purification and greater stability. And instead of an antibody, it was linked, like many other current, mostly experimental fusion toxins, to a cytokine. As with antibodies, the cytokines used for targeting may have copious receptors on the cancer cells but have few or none on normal tissue. Their big advantage, Murphy said, is that their receptors almost always trigger import into the cell, in contrast to antibodies, which may not be internalized.

Generation three dates roughly to the last 6 years, as investigators have become far more adroit at engineering the drugs. The remodeled agents of this generation...
Mitigating Toxicity

Ellen Vitetta, M.D., Ph.D., for example, has been working on vascular leak, a common toxicity with this class of drugs that can cause problems ranging from mild weight gain to life-threatening pulmonary edema. A pioneer in the field three decades ago, Vitetta then chose a plant toxin, ricin, to serve as the lethal moiety because humans do not normally make antibodies to plant toxins.

Vitetta, who is director of the cancer immunobiology center at the University of Texas Southwestern Medical Center in Dallas, is developing a treatment for pre-B acute lymphocytic leukemia, a highly curable childhood cancer that becomes incurable if relapse occurs. She has developed a patented cocktail of immunotoxins called Combotox, consisting of the A chain of ricin chemically attached to two different antibodies, “in order to avoid escapee cells.” A small phase II trial has been completed, with promising results, she said. Vitetta has licensed the agent to Abiogen Biopharma.

Vascular leak has been the dose-limiting toxicity for Combotox. Vitetta identified the vascular leak–inducing sequences within the toxin and mutated them to ameliorate the problem. Investigators working on protein fusion toxins are taking similar approaches.

Toxicity also often occurs when affinity for targets is somewhat deficient. The University of Minnesota’s Valleria has tackled this issue, developing bispecific ligands, i.e., attaching the diphtheria toxin to two different cytokines, both of which target a different receptor on the cancer cell’s surface.

Reducing Immunogenicity

Researchers are also learning to attenuate immunogenicity. The immunogenic portions of proteins, said Ira Pastan, M.D., “are usually clusters of large, charged amino acids. Taking advantage of that, we have made toxins that no longer have these bulky hydrophilic amino acids on their surfaces.” Last year, Pastan’s group at the National Cancer Institute reported in Proceedings of the National Academy of Sciences that they had reduced immunogenicity of an immunotoxin for hairy cell leukemia by 85% in mice.

Reduced immunogenicity will make protein fusion toxins more practical for treatment of some solid tumors, where immune reaction is a much greater problem than in leukemias because more treatment cycles are needed. Besides glioblastoma, investigators are developing treatments for breast, prostate, ovarian, pancreatic, and lung cancers, as well as mesothelioma, Pastan said. The target antigen is mesothelin, which is expressed on these cancer cells but not on normal tissues except for epithelium (which is replaced so often that the resulting toxicity can be managed).

Another problem with solid tumors, though, is that large doses are needed to penetrate them, and the resulting side effects can limit efficacy. One solution may be to combine them with chemotherapy. In animal experiments, Pastan said he has found great synergy between a variety of fusion protein toxins and chemotherapeutic agents against three different tumor types, in agreement with other studies.

The reason for this synergy may have to do with tumor cells’ tendency to shed antigens. Various investigators have proposed that shed antigen within tumors represents a barrier to antibody-based therapies. In mouse studies, concurrent chemotherapy greatly reduced shedding, improving treatment efficacy, Pastan said.

Business and Cancer Therapy

The field has also faced obstacles related to the business of drug development. In 2007, a fusion protein aimed at glioblastoma was abandoned by Celtic Pharma Management LP, Hamilton, Bermuda, the fourth company to obtain the rights in the course of the clinical trials. The drug, transferrin-CRM107 (TransMID), was developed by Richard Youle, Ph.D., a senior investigator at NIH. Tumors need iron to grow, and so Youle linked the diphtheria toxin to human transferrin, a plasma protein that transports iron, which attaches to receptors on tumor cells. Because the compound is too big to cross the blood–brain barrier, it is catheter-injected into the tumor, under pressure so that it saturates the area beyond the tumor margin.

In a phase II trial whose results were published in 2000, Youle obtained a durable long-term complete response in about 10% of patients. The drug was then licensed to a company. A phase III study commenced around 2005, but a series of mergers and acquisitions followed, said Patrick Rossi, M.D., now senior medical director with Protox Therapeutics in Vancouver, British Columbia. Eventually, Celtic, Rossi’s previous employer, shelved TransMID, and a spokesman said the company had returned the rights to NIH.

Denileukin suffered delays for similar reasons. Seragen, the company that originally licensed the drug, closed its doors just weeks before the FDA issued approval, according to Boston University’s Murphy. Seragen’s intellectual property rights were sold to Ligand Pharmaceuticals. That company made no improvements in the drug, Murphy said, although about 40% of patients suffered vascular leak.

Here the intellectual property was actually a platform for diphtheria-based fusion protein toxins, but Ligand neither developed any others themselves nor licensed the technology, Murphy said. Novartis, which was working in the same area, challenged Ligand in court and lost.

Nonetheless, Anjin has patented modifications of the diphtheria toxin that mitigate vascular leak and that do not infringe on Ligand’s intellectual property rights, according to Murphy, who chairs the company’s board of directors.

Meanwhile, a Pseudomonas-based immunotoxin, HA22, from the NIH laboratories of Pastan and Robert Kreitman, M.D., aimed at hairy cell leukemia and chronic lymphogenous leukemia, is headed for more advanced trials. HA22 is an improved version of BL22 that in a phase II trial reported last year resulted in 47% complete remissions in chemotherapy-resistant hairy cell leukemia. The drug, patented by NIH and sponsored by AstraZeneca MedImmune, is scheduled to begin phase III trials this fall, Pastan said.

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