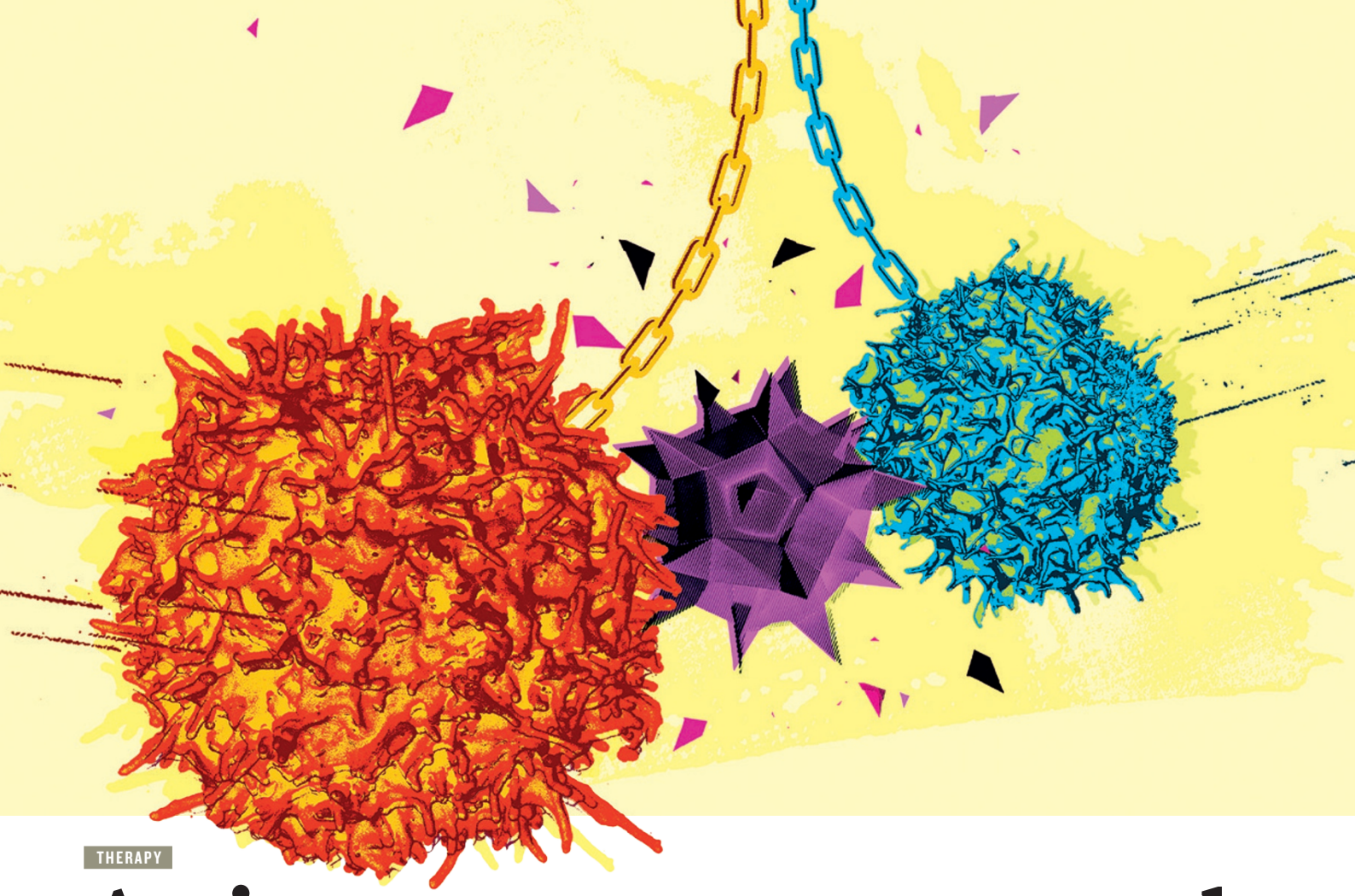

GARY NEILL

THERAPY

An immune one–two punch

Combination therapies that activate the immune system in complementary ways could help more men with prostate cancer to contain their disease long term.

BY KATHERINE BOURZAC

When cancer immunologist Michael Curran was a postdoc, he made a discovery of the magnitude that scientists only dream about. He showed that two antibodies that unleash the immune system had a synergistic effect, bringing about the eradication of melanoma tumours in mice. What is more, this effect also worked in people. Curran and his colleagues published their mouse results in 2010 (ref. 1); subsequent clinical trials showed that the combination therapy is so effective at treating people with melanoma that some patients are "durably cured" of their cancer, he says.

Immunotherapy works well for people with melanoma, and researchers such as Curran, now an immunologist at the University of Texas MD Anderson Cancer Center in Houston, are trying to create similarly dramatic effects in other cancers. But Curran's therapy does not work for prostate cancer — not even in mice. Immunotherapies are new, and researchers are still figuring out how they work, says Curran. There is one immunotherapy for prostate cancer approved for use, and only in the United States. Sipuleucel-T adds, on average, a few months to a man's life. But anecdotally, oncologists report men who have undergone the therapy living for years without needing further treatment.

To make prostate-cancer-immunotherapy success stories more common, physicians and immunologists need to understand why some men respond to the treatment, and some do not. Such insights will help them to predict which patients are most likely to benefit from these expensive treatments, and could guide the design of new versions that work better for more people. Several clinical trials are testing cancer vaccines (see 'Immunotherapy on trial'), as well as therapies that combat a tumour's tendency to muffle immune responses. Many of these trials are exploring combinations of therapies that act on different immune-system or cancer pathways, to make sure that tumour-killing T cells are fully equipped to do their work.

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CHASING THE LONG TAIL

For a patient whose prostate cancer has spread to the lungs, bone or elsewhere, the prognosis is bleak. Chemotherapy and radiation shrink tumours and extend life by a few months, but then they stop working — either because the tumour mutates to get around a targeted therapy or because patients are taken off the treatments because of the side effects. Immunotherapy drugs can have longer term effects when they work well, but so far that is rare for prostate cancer.

Sipuleucel-T is controversial. The median survival benefit is only four months[2] — about the same as conventional therapies — and it costs US$93,000. That kind of limited benefit and high cost is not unheard of for cancer drugs in the United States, but it is unusual. And sipuleucel-T is more complicated to administer than a conventional drug. Unlike most drugs, which come premade and can be sold off-the-shelf, sipuleucel-T is

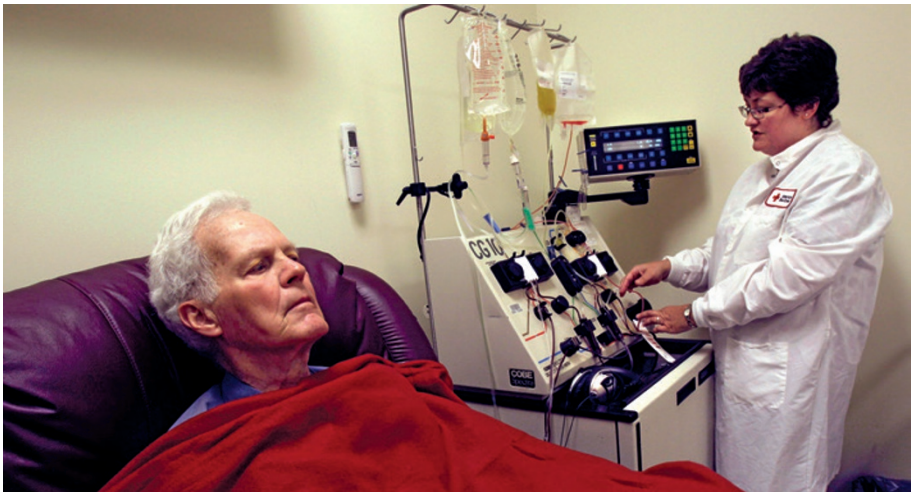
personalized. The patient's white blood cells are separated from their blood and sent to a central processing facility. There, these cells are incubated with the enzyme prostatic acid phosphatase — to train them to seek out cancer cells that produce this protein. The cells are then returned to a local clinic and infused back into the patient. This process is done three times. And although the cell harvesting can be performed at any Red Cross blood bank in the United States, it is still much more complicated than writing a prescription for a pill or sending a patient to an infusion clinic for conventional chemotherapy, says Lawrence Fong, an immunologist who treats men with prostate cancer in his clinic at the University of California, San Francisco.

That complexity, and the expense that accompanies it, have brought about a backlash against sipuleucel-T. The therapy's creator Dendreon, based in Seattle, Washington, received US Food and Drug Administration approval to market sipuleucel-T in 2010. But when the drug's poor sales figures were revealed just a year later, the company's stock plunged 67% in a single day. In November 2014, Dendreon filed for bankruptcy; its assets were sold off the following February.

Montreal-based Valeant Pharmaceuticals, who picked up the drug, withdrew an application to market it in the European Union in May 2015. Questions were raised about the expense of the treatment and about the clinical trials, says Hardev Pandha, an oncologist at the University of Surrey, UK. "There were a few infusions and then that was it," he says. "It wasn't seen as a sustained treatment."

One factor weighing against broader acceptance is that the mechanism underpinning how sipuleucel-T works in patients was not established in the initial clinical trials. It is thought to work with dendritic cells in the blood. These cells have receptors that recognize the chemical signatures of microbes, cancer cells and other antigens, and when they spot one, the dendritic cells attach it to a protein on their surface like a red flag. These warnings kick-start T cells into action, spurring them to hunt down and kill foreign cells that display the antigen. But the clinical trials did not look for activated T cells or their markers in patient samples, says urologist Martin Sanda at Emory University School of Medicine in Atlanta, Georgia, and for that reason it is difficult to know why it works for some men and not for others. Researchers are now investigating the activity of specific cells in men who have had the treatment.

That the complex therapy works very well in some men is reason enough for many physicians to offer it. "I have to advocate for my patients," says Fong. He and other oncologists know that some patients respond well to immunotherapy — something that is not reflected in the average survival numbers. Their tumours do not shrink, but they stop growing, and some men are stable for years.



ELISE AMENDOLA/AP/PRESS ASSOCIATION IMAGES

Administering sipuleucel-T to patients is much more complicated than providing conventional therapies.

Survival rates for patients with late-stage disease who are given conventional treatment plunge to zero after a year or two. By contrast, the graph of survival over time for those given immunotherapy has a 'long tail' — never reaching zero in clinical trials. For Fong, one patient in particular illustrates the hope for the therapy. The patient's recurrent metastatic prostate cancer had become resistant to hormone therapy. "He got the usual treatment and responded as most patients do," Fong says. The treatments work for a while, shrinking tumours for a few months, after which they grew anew. Then Fong treated him with a course of sipuleucel-T. Five years later, his cancer has not grown, nor has he needed further treatment.

CANCER VACCINES

Even those such as Fong who offer the treatment to their patients agree with Pandha, who says that immunotherapy needs to move away from "bespoke personalized medicine" like sipuleucel-T. To that end, researchers are working on off-the-shelf vaccines for prostate cancer. Furthest along in clinical trials is a vaccine developed at the US National Cancer Institute (NCI).

Called PROSTVAC, this therapy borrows from the playbook of infectious diseases, using two weakened viruses — vaccinia and fowlpox — engineered to carry prostate-specific antigen (PSA). The vaccine has been in the works since the late 1990s, starting in the lab of NCI immunologist Jeffrey Schlom. It showed promising results in phase II clinical trials, in which patients remained progression free for an average of 12 months[3], and it is now in phase III clinical trials for treating metastatic prostate cancer.

Vaccines target specific antigens — and in the case of PROSTVAC, PSA is the molecule of choice. PSA is a self-antigen: it is made by healthy, as well as cancerous, prostate tissue. But PROSTVAC is a therapeutic vaccine, which is intended to be given only to men who have already had their cancerous prostate gland removed. In these men, the only cells producing PSA — and therefore the only cells that the vaccine will target — are cancer cells. The vaccines also seem to have an effect called antigen spreading, says James Gulley, a tumour immunologist at NCI. Once the immune system identifies and attacks the tumour, it recognizes and goes after other tumour antigens that it finds on its own.

Researchers have discovered additional targets for treating prostate cancer, and some believe that the immune system may be able to mount a better response to vaccines that target an antigen that is unique to the tumour, rather than a self-antigen such as PSA — or to one that targets multiple antigens.

Many transcription factors — regulatory proteins that promote or block gene expression — are overexpressed in tumour cells and so are a good target for cancer therapy. Sanda is testing, in animal models, whether the transcription factors ERG and SIM2 can be used as antigens.

Charles Drake, an oncologist and immunologist at the Johns Hopkins School of Medicine in Baltimore, Maryland, is taking a different approach: a quadruple-antigen vaccine akin to a Swiss Army knife. This experimental vaccine uses prostate acid phosphatase — the same antigen used in sipuleucel-T cell therapy — along with another protein called prostate-specific membrane antigen. Both are found in normal prostate tissue, but a third antigen is specific to prostate cancer. And a fourth is a protein that is overexpressed in cells left behind after prostate removal, and is considered a prostate-cancer precursor gene product.

Instead of a virus, Drake's vaccine uses attenuated *Listeria* bacteria as the carrier. These weakened microbes have been used in other vaccines, including one for pancreatic cancer, which Drake says elicited a strong immune response in a phase II trial led by another group at Johns Hopkins. He hopes to see the

THERAPY

Immunotherapy on trial

After disappointing results from the first approved immunotherapy for prostate cancer, researchers are developing a host of alternatives that could deliver better results for more patients.

PROSTVAC (phase III). Developed at the National Cancer Institute, this multicourse viral vaccine activates the immune system against prostate-specific antigen.

Hormone and checkpoint therapy (phase II). The high levels of testosterone in prostate tumours inhibit the activity of cancer-killing T cells. Combining hormone therapy with the checkpoint therapy ipilimumab could combat this.

PROSTVAC and ipilimumab (phase II). By combining a viral vaccine and a checkpoint therapy, it is hoped that one will activate T cells and the other will keep the tumour from suppressing them.

Sipuleucel-T with checkpoint therapy and chemotherapy (phase II). This trial is looking for synergy between the cell therapy sipuleucel-T and checkpoint therapy, along with the conventional chemotherapy drug cyclophosphamide.

Sipuleucel-T with ipilimumab (phase II). By combining sipuleucel-T with checkpoint therapy researchers hope to determine what order they should be given in — block tumour suppression first then provide cell therapy, or the other way around?

DNA vaccine (phase II). Instead of a microbial carrier, this vaccine uses a naked DNA plasmid that codes for prostate acid phosphatase. K.B.

same in the first clinical trials of the prostate-cancer vaccine, set to begin by early 2016.

Drake says *Listeria* is easier to grow in culture than vaccinia and fowlpox. And the *Listeria* vaccine can be given multiple times without the need for different carriers like PROSTVAC. Other researchers are experimenting with vaccines that use DNA, with no carrier at all. A phase II trial of a DNA vaccine now under way will indicate whether this method elicits as strong an immune response as the attenuated pathogens — a result that will be of keen interest to researchers such as Sanda who have not yet chosen a carrier for their novel antigen targets.

Trials of these vaccines depend on better monitoring of biomarkers, which are the key to finding out why some patients respond very well and others not at all. "We're learning how to collect patient samples and not just look at everything in a mouse," says MD Anderson oncologist Padmanee Sharma. Although much can be learned from mouse studies, she says, the interconnected co-evolution of tumour and immune system needs to be studied in people.

COMBO DEAL

Tumours take advantage of naturally occurring checkpoints that prevent healthy immune reactions from becoming dangerous. Once a T cell is activated by an antigen and expands its numbers, it starts expressing a checkpoint receptor. "This puts an expiration on T cells of a day or three," says Curran. That is a good thing — you do not want billions of killer immune cells accumulating in your lungs after your cold clears up. But tumours turn this safety mechanism to their advantage, using that receptor as a target for its own suppressive signals, so that T cells never get going. Checkpoint therapies target and block these suppressive signals.

T cells have to be attracted to a tumour in the first place, however, otherwise checkpoint therapy has no effect. Treatments such as vaccines and cell therapies (sipuleucel-T) stimulate this process, and a combination of these treatments and checkpoint therapy maybe the best way forward. This is now being tested in patients.

The conventional wisdom is that checkpoint therapy works well in mutation-rich cancers such as melanoma because these tumours generate high levels of novel antigens that attract T cells to the tumour. The T cells then just need a little boost from checkpoint therapy. Prostate tumours are not as rich in T cells, a deficit that researchers suspect is because they have too few mutations to catch the immune system's attention.

> ***"We're learning how to collect patient samples and not just look at everything in a mouse."***

Curran thinks it is more complicated than that. After all, he says, prostate cancer is not uniquely low in mutations among cancers — it ranks somewhere in the middle. On average, prostate cancers have about 50 mutations — and each one of them should be read by the immune system as an antigen. That is almost five times as many potential antigen targets as the influenza A virus, which does provoke an immune response. It is not just about the numbers, Curran argues.

When Curran saw how much better checkpoint therapy worked against melanoma than prostate cancer, he came up with a new approach. He concentrated on the tumour microenvironment. Prostate tumours differ from healthy tissue in that they contain high levels of testosterone and low levels of oxygen. "That's everything T cells hate," he says. Besides which, the tumours are poorly vascularized — they are a backwater on the circulatory system that the T cells travel.

In 2011, Curran recalled something he had heard in graduate school about drugs that target tumour hypoxia, and wondered if that may be an avenue to improve the effectiveness of immunotherapy. To explore that possibility, he began a collaboration with Threshold Pharmaceuticals in South San Francisco, California, which makes a drug called evofosfamide. This compound circulates in a non-toxic form until it reaches a region of low oxygenation, which triggers the release of a DNA-damaging agent. Curran wondered what would happen after the drug had killed tumour cells in the hypoxic areas of tumours. Would those areas become a wasteland — or would T cells find a foothold? Curran found that, in a mouse model of prostate cancer, cancer-cell killing is followed by a wound-healing response and the growth of new blood vessels. That brings oxygenated blood and, it seems, a more T-cell friendly environment. "T cells can then enter the areas they were formerly blocked out of," says Curran. After introducing the evofosfamide, Curran and his colleagues in Houston administered checkpoint therapy to prevent the arriving T cells from being suppressed. Curran reported these results at The Inaugural International Cancer Immunotherapy Conference this year and is now designing a human trial of this combination.

Hypoxia is not the only environmental barrier to T cells. Another combination-therapy clinical trial addresses the high levels of immune-suppressing testosterone in prostate tumours by combining hormone therapy (to lower testosterone) and checkpoint therapy. And it is now becoming evident that some chemotherapies that were thought to work only by killing cancer cells are dependent on the immune system to work. They might also be fruitfully combined with checkpoint therapy to fight prostate cancer.

Combination therapy is the great hope of prostate-cancer immunologists. There is no guarantee that these therapies will avoid the cost problems associated with sipuleucel-T. But if combining treatments allows more men to go into remission — or perhaps even be cured — the high price tags may not raise as many eyebrows. For immunotherapy, says Pandha, combinations are "the final piece of the jigsaw." ■

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