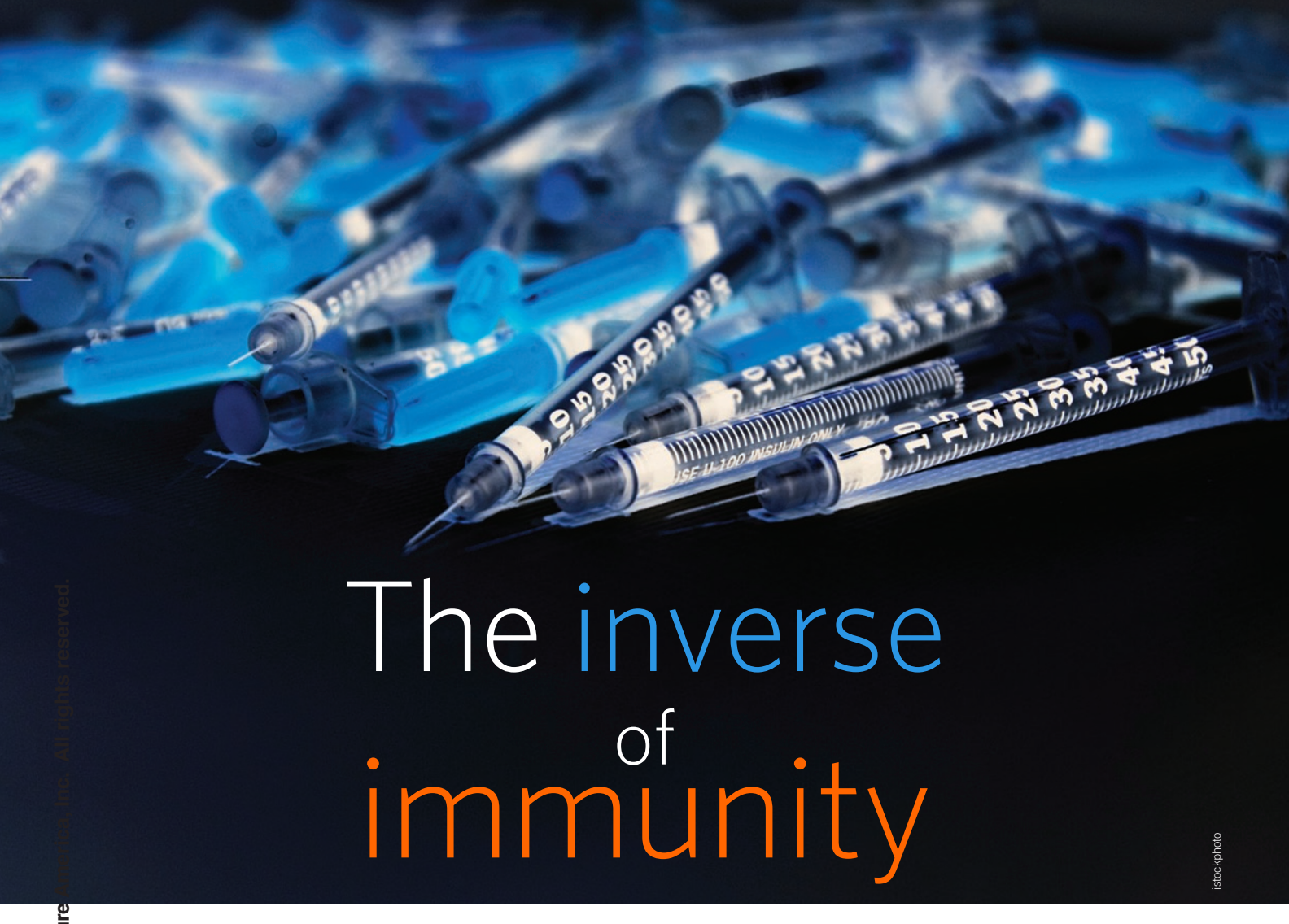

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The inverse of immunity

Ever since Edward Jenner discovered the smallpox vaccine two centuries ago, immunization efforts have almost exclusively focused on activating the immune system. But when it comes to multiple sclerosis and other autoimmune disorders, researchers hope to switch off—rather than ramp up—the body's defenses. Even an automotive service mogul has taken the idea on board. **Elie Dolgin** investigates how the idea of vaccination is being turned on its head.

Late-stage drug trials fail all the time. But last summer, when a small Canadian biotech company reported that its leading therapeutic candidate, a peptide-based vaccine for multiple sclerosis, did not halt disease progression in its pivotal trial, the announcement came as a particularly harsh blow to the scientists who had first designed the treatment.

After the disappointing result, BioMS, the Edmonton, Alberta–based startup behind the trial, abandoned development of the vaccine. Meanwhile, the industry remains without a targeted therapeutic vaccine to treat chronic autoimmune diseases. Instead, individuals suffering from such diseases continue to rely on broad-acting agents that cut down large swathes of the immune system in a nonspecific fashion to halt the body from attacking itself.

The approach is not without its flaws. "Any student taking 'immunology 101' will tell you that wiping out a large part of the immune system will lead to opportunistic infections," says Lawrence Steinman of Stanford University School of Medicine in California.

Steinman is one of a small number of immunologists working to tackle autoimmunity in a more targeted fashion through vaccines. However, these 'vaccines' are not prophylactic shots against disease; rather, they act as therapeutic treatments. And, unlike most conventional vaccines, which typically stimulate the immune system to react against foreign pathogens, Steinman and his colleagues hope to reduce the response of an overactive immune system to the body's own triggers while leaving the rest of the system intact. "If we can throw the 'on' switch, shouldn't we be able to throw the 'off' switch?" asks Steinman.

In May, Steinman coined a new name for this approach: 'inverse vaccination'[1]. With this approach, "rather than giving a broad-spectrum immune suppressive agent, you're really only targeting the T cell population that's responsible for that disease," says Robert Anderson, an autoimmune disease researcher at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia.

Steinman's new term for these unconventional vaccines has yet to catch on, but the technique of antigen-specific tolerance is beginning to take off. And despite the setback of the BioMS trial, researchers are not turning their backs on the goal of sensitizing the immune system to particular triggers. "One trial that doesn't hit its primary end point is not really indicative of what's going to happen in the future," says Mark Larché, a clinical immunologist at McMaster University in Hamilton, Ontario. "I still think that that approach will work."

Vacillating vaccine hopes

Kenneth Warren launched the Multiple Sclerosis Patient Care and Research Clinic

at the University of Alberta in Edmonton in the late 1970s with the goal of finding the molecular culprit behind the mysterious disease. At the time, his lab was performing hundreds of spinal taps on people with multiple sclerosis. "We were looking for something that we could link into multiple sclerosis disease activity," recalls Ingrid Catz, a research scientist who has worked with Warren for almost three decades.

After several years, Warren and Catz amassed an impressive collection of cerebrospinal fluid from people with multiple sclerosis and healthy individuals and showed that those with clinically active multiple sclerosis had elevated levels of antibodies against myelin basic protein (MBP), a key factor involved in maintaining the myelin sheath that protects and insulates neurons[2]. The researchers then purified the antibody and broke the 170–amino acid MBP protein into smaller synthetic peptides to find the particular site where the antibody bound.

> "If we can throw the 'on' switch, shouldn't we be able to throw the 'off' switch?"

After testing more than 50 small peptides for reactivity, the researchers narrowed in on the region recognized by affected individuals' immune cells. The region situated between amino acids 85 and 96 in the protein set off B cells in particular, they found[3]. Independently, David Hafler and his colleagues at the Brigham and Women's Hospital in Boston narrowed the binding region of T cells to residues 84 to 102, as they reported a few years earlier[4]. In the end, to develop a soluble protein, Warren and Catz expanded their peptide to 17 amino acids long, spanning residues 82 through 98, and *MBP8298* was born.

Warren and Catz thought that, much like allergy injections, the oldest and only kind of inverse vaccines on the market, whopping doses of the peptide in the absence of an adjuvant would train the immune system not to react to the body's own harmless proteins in the brain. "It would teach the immune system that the myelin basic protein is not a foreign substance," says Catz.

In 1997, the researchers launched a two-year, placebo-controlled phase 2 clinical trial in 32 subjects with multiple sclerosis injected intravenously every six months with *MBP8298*. Reporting close to a decade later, after tracking the subjects for seven years, they showed that the drug delayed the progression of multiple sclerosis by around five years compared to placebo treatment in a subset of subjects whose immune cells shared a reactivity characteristic (more specifically, they shared what's known as a 'major histocompatibility complex signature')[5].

These promising results prompted BioMS—formed in 2000 by entrepreneur Clifford Giese, founder of the Mr. Lube chain of automotive service centers—to acquire the rights to the compound and launch a series of larger phase 3 trials. In January 2007, after securing funding and regulatory approval, the company completed recruitment for a 600-person trial conducted in Canada and nine Western European countries; by June 2007, subjects were starting to be enrolled for a similar US-based study. Six months later, BioMS netted an $87 million licensing agreement with the Indiana-based pharma giant Eli Lilly with the prospects of hundreds of millions more in milestone payments and royalties.

Everything looked rosy until the data started to roll in, in July 2009. Although the drug was safe, *MBP8298* (renamed as dirucotide by the company) did not delay disease progression after two years, as measured by the Expanded Disability Status Scale, the gold standard for quantifying disability in multiple sclerosis. Two months later, Lilly severed ties with BioMS, and by the end of the year, BioMS announced that it was halting further clinical trials with dirucotide and shifting its operations toward investing and consulting.

Warren was deeply disappointed by the clinical failure. "It was really an awful big mountain of work," he says. "I would sure like to see this research continue with bigger and better specificity per patient."

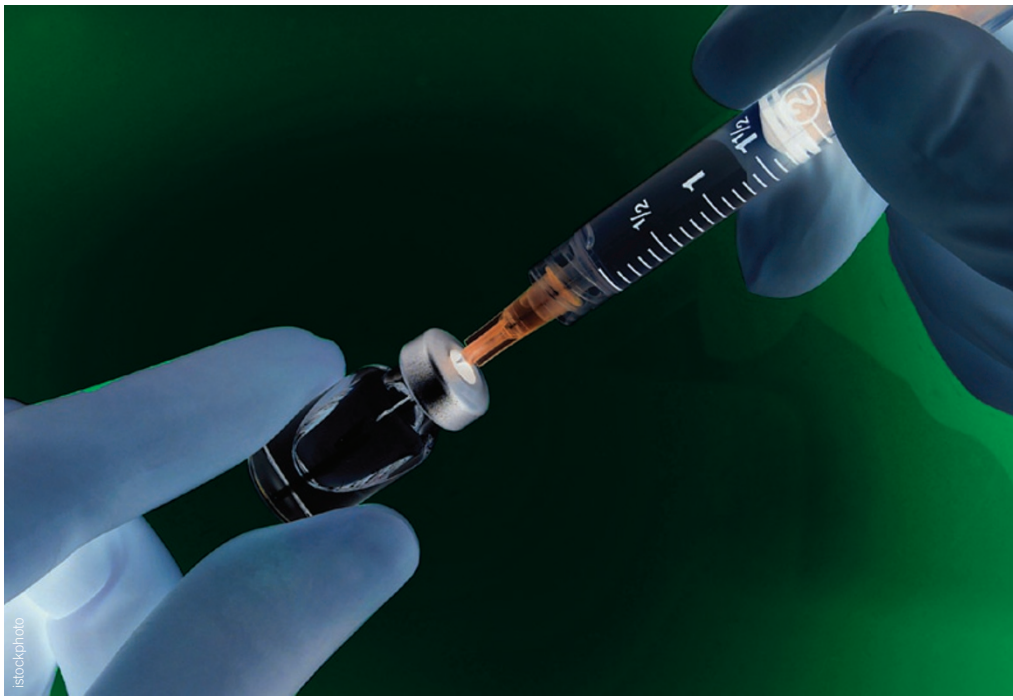
Warren pauses. "But we're old and bent and tired out a little bit."

Multiple approaches

Immune systems are complicated beasts. As such, they can be tweaked and prodded in a number of intricate ways. So while Warren and Catz were experimenting with injecting their short, unaltered peptides, others have been moving forward with different—albeit somewhat more complicated—methods for manipulating immune responses to induce tolerance.

Instead of using truncated, but otherwise unchanged, myelin peptides, Roland Martin, a neuroimmunologist at the University Medical Center Hamburg-Eppendorf in Germany, has been working to rejigger some of the amino acids in the MBP protein. He started by trying to form modified peptides that could more radically modulate T cell activity. These engineered proteins, called altered peptide ligands, showed early promise, but a phase 2 trial had to be stopped prematurely in 2000 after a few subjects developed multiple sclerosis relapses soon after receiving the treatment[6].

Martin doesn't fully understand what went wrong, but, at the time, he knew he needed an alternative approach. So he teamed up



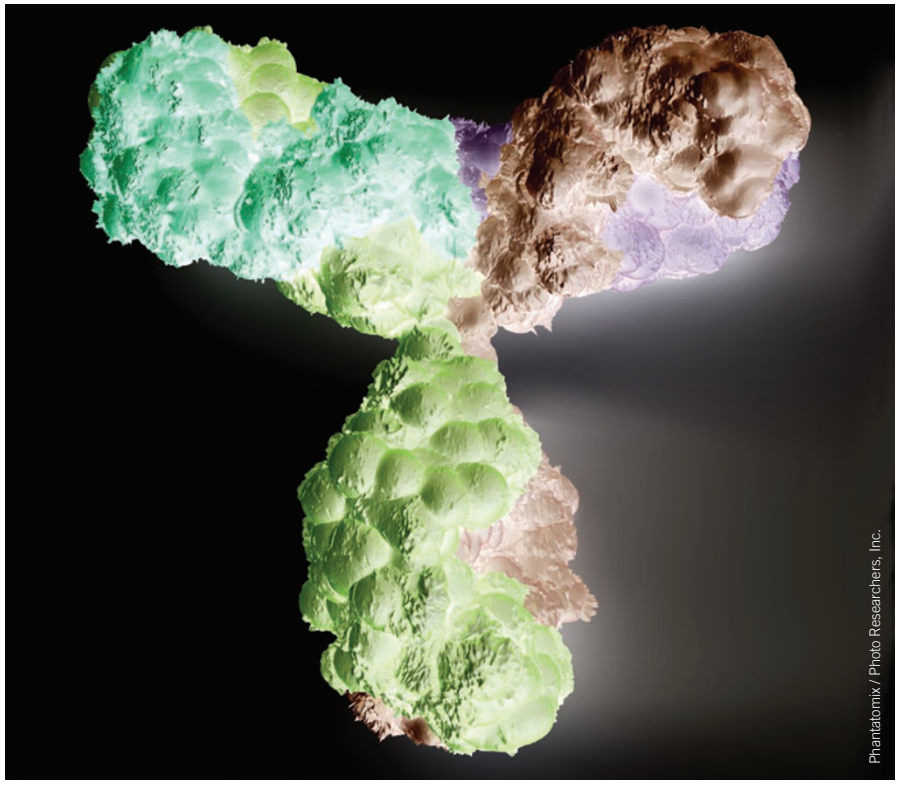
with Stephen Miller, a neuroimmunologist at Northwestern University Medical School in Chicago who had previously developed an elaborate procedure that involved extracting a person's own spleen cells and attaching several myelin peptides to the surface of the maturing white blood cells with the help of a chemical called ethylene carbodiimide (ECDI). Two decades ago, Miller showed that re-injecting these manipulated cells could prevent disease progression in a mouse model of multiple sclerosis[7].

Now, backed by a €1.2 million ($1.5 million) grant from the German Federal Ministry of Education and Research, Martin and Miller have initiated a combined phase 1–phase 2a trial of a similar approach in people with early-relapsing multiple sclerosis. At the end of May, they injected the first of six subjects with manipulated peripheral blood leukocytes bound to an assortment of seven myelin peptides taken from three proteins: MBP, myelin proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG). After proving the approach's safety, the researchers plan to give a one-off treatment to another dozen patients and follow the subjects for one year, using monthly magnetic resonance imaging scans to track the inflammatory activity of the disease in their brains.

"Our expectations are very high," says Martin. "With the best outcome, it would make further therapy completely unnecessary."

Opexa Therapeutics, a biotech company out of The Woodlands, Texas, is also developing a patient-specific vaccine that involves collecting blood, harvesting and expanding disease-causing T cells acting against more than 100 specific peptides associated with MBP, MOG and PLP, and then returning the irradiated T cells back to the patient to incite the immune system to attack its own rogue T cells. "It's a personalized vaccine in that it's the patient's own cells, but we also assess the reactivity profile of the patient's own epitopes," says Opexa chief executive Neil Warma.

In a 150-person trial of people with early-relapsing multiple sclerosis, more than 70% of people given the vaccine, dubbed Tovaxin, stabilized or improved in their multiple sclerosis disability, compared to about half of the control individuals, Opexa officials announced last year. At the American Academy of Neurology meeting in Toronto in April, the company also reported that the vaccine increased the number of regulatory T cells, showing that the vaccine helped desensitize the immune system.



Miller admits that his approach of injecting a person's own manipulated cells back into the body involves an "extremely difficult manufacturing process." But, he stresses, "this method is much, much more effective at inducing tolerance in a much wider range of diseases," because his ECDI-fixed cells more efficiently home in on the cells that induce tolerance than any other known method of antigen delivery, he argues.

> "With the best outcome, it would make further therapy completely unnecessary."

Protein power

But extracting and manipulating an individual's own immune cells is far more complicated than necessary, maintains David Wraith from the University of Bristol, UK. "Arguably, if you're going to do that rather elaborate approach for delivering peptides, you might as well just give the peptide on its own," he says. "Effectively, that's what we do."

At first, Wraith took the approach of trying to deliver peptides through the nose. More than 15 years ago, he showed that mice with an experimental form of multiple sclerosis were completely protected from an induced form of the disease after sniffing a solution containing a fragment of the MBP peptide[8]. Hafler and his colleagues also had some success with a similar oral delivery of myelin peptides in people[9]. But "the regulatory authorities would not allow us to use the intranasal approach in man," Wraith says. So he started to develop other strategies.

Most recently, Wraith created a vaccine based on short peptides derived from MBP, much like Warren and Catz had done decades earlier. But unlike the Alberta researchers' approach, Wraith's vaccine contains not one but four peptide fragments. "I doubt whether one peptide is going to be enough to cure all of MS," Wraith says.

A preliminary trial in six individuals with progressive multiple sclerosis found that the vaccine was safe and reduced the level of MBP-induced T cells by up to 40% one month after treatment, Wraith's company Apitope reported in 2008. In May, Belgium-based Apitope also announced the start of a second phase 1 trial of the same vaccine in 40 subjects with relapse-remitting multiple sclerosis.

Instead of injecting peptides either in their native state or manipulated in some way, Stanford's Steinman has turned to yet another approach for tolerizing the body to certain myelin proteins: DNA vaccines. In 2003, he showed that injecting a mouse model of multiple sclerosis with a plasmid encoding a cocktail of four myelin proteins—MBP, PLP, MOG and myelin-associated glycoprotein—reduced the rate of relapse severity in the mice by about 40%, even more so with the addition of the cytokine interleukin-4 (ref. 10).

Steinman has since followed up with human trials in people with multiple sclerosis. Reporting two years ago, he showed that, after around one year of monthly intramuscular injections, the DNA vaccine cut the number of new brain lesions in half in a study of close to 300 people with relapsing-remitting multiple sclerosis. At the end of the year, the average volume of the brain lesions was also around half as high[11].

Participants stopped receiving the treatment after one year, but Steinman continued to track the study subjects for another year and says the unpublished results look even more promising. "Even though we weren't dosing anymore, the effect became more prominent," Steinman says.

Preparations for a phase 3 trial are now underway through Steinman's Palo Alto, California–based company, Bayhill Therapeutics. Bayhill also has other tolerizing DNA vaccines in the works, including one designed as an antigen-specific treatment for type 1 diabetes. At the American Diabetes Association meeting in June in Orlando, Florida, scientists affiliated with the company presented data from a phase 1–2 trial of 80 subjects, showing that the experimental vaccine was well tolerated and associated with improved glucose control and preservation of pancreatic beta cells, the cells that make insulin.

Diplomatic immunity

Despite the preclinical and early-phase promise of antigen-specific therapy, Hafler notes that the approach has never yet succeeded in any pivotal trials. "Taking self antigens and hoping to get tolerance clearly can work in experimental models, but as one starts to do this in live patients, it just concerns me," he says. That said, he adds, "I firmly believe in doing the clinical trials, and I've been humbled by nature so many times."

Richard Ransohoff, a multiple sclerosis researcher at the Cleveland Clinic in Ohio, is equally skeptical about the promise of inverse vaccines. "These types of antigen-specific approaches are delicious to think about, but it's like tilting at windmills," he says. "There's no doubt that the *in vivo* animal work has been first rate, but whether that will be an effective approach to the human disease—that's somewhat in the wind."

According to Catz, BioMS's clinical trial failed because of the company's poor trial design, which she claims was far too small and brief. "BioMS kind of—excuse my French—screwed up," she says. "For a disease like progressive MS, two years is a drop in the bucket."

> "These types of antigen-specific approaches are delicious to think about, but it's like tilting at windmills."

What's worse, Catz continues, the company recruited the wrong type of study subjects—only those with a fairly stable form of the disease. "The peptide is intended to stabilize disease progression; it is not intended to make people better," she says. "This is where BioMS got into trouble. They enrolled stable patients, so they didn't have anything to measure because the patients were fine from the beginning."

BioMS declined to comment for this article, but Mark Freedman, who led the company's phase 3 trial, refutes these criticisms. "We were probably more focused on progressive disease than anyone else to date," says Freedman, director of the multiple sclerosis research unit at the Ottawa Hospital Research Institute in Canada, noting that all participants were clinically diagnosed with a progressive form of the disease prior to enrollment. Yet, even with a solid trial design, "there was nothing, not a shred of a signal in this study," he says.

Notably, some aspects of Warren and Catz's studies have also proven to be irreproducible. For example, contrary to the Alberta researchers' previous reports, Hafler and his colleagues failed to detect autoantibodies capable of binding to MBP in the spinal fluid of peoples with multiple sclerosis[12].

Warren and Catz chalk up the discrepancy to differing assays, and they stand by their results. Now they are investigating why only some of the subjects in their phase 2 trial responded to drug, but they have no plans to take the molecule further commercially. "As long as BioMS has the license on this molecule, it's kind of shelved," says Catz. The company, meanwhile, continues to provide the drug for an ongoing compassionate trial of around 50 people who had taken part in earlier studies with the drug.

One of those patients, Colin Minor, a retired television executive from Edmonton, has been receiving semiannual injections of the drug for more than a decade. "You get to know your body, and I know the peptide is working," he says. "Whether that translates to everyone else, I don't know." Until the vaccine emerges from its commercial limbo, however, it might remain impossible to find out.

Elie Dolgin is **Nature Medicine***'s assistant news editor in New York.*

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