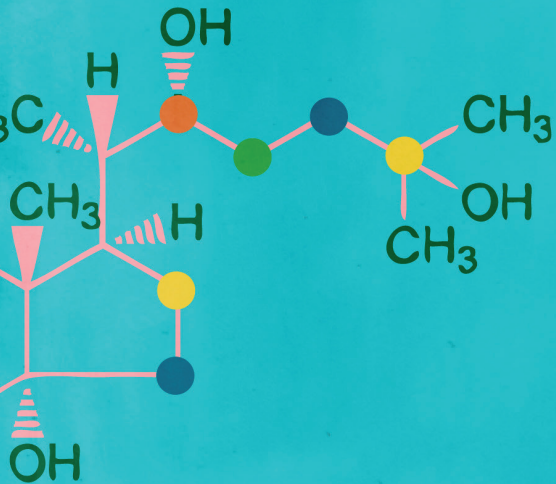


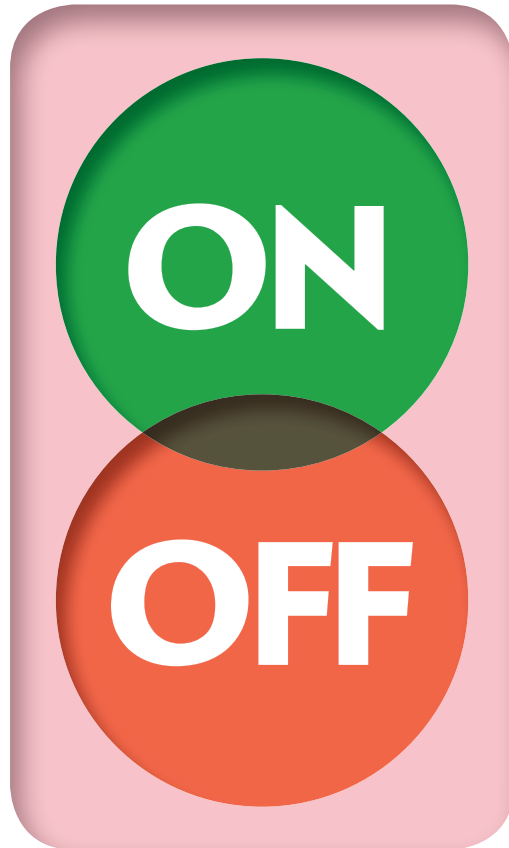
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SWITCH FOR GENES

Researchers are developing molecular switches that can inactivate transplanted genes, paving the way for safer gene therapies. First up—better treatments for cancer

By Jim Kozubek

Jim Kozubek is a computational scientist and writer who lives in Cambridge, Mass.



HUMANS DON'T MOLT," R.J. KIRK TELLS ME. KIRK IS A BILLIONAIRE GEEK WHO runs his offices out of West Palm Beach, Fla., a balmy land of pelicans and tangled mangroves. He built his fortune on conventional medications that can be taken as a pill, and I had phoned to talk about his newest endeavors in biotech. I wasn't expecting to hear about bugs. But the molting process, in which a growing insect builds a new exoskeleton to replace an old one that no longer fits, turns out to have some very important properties that can be adapted to make gene therapy, still a largely experimental procedure, safer.

Doctors would like to deliver copies of working genes to people to treat a variety of hereditary ills. Genes provide cells with the instructions for manufacturing proteins, among other things, and so inserting a functional gene into the body can, in theory, provide a lasting supply of whatever missing proteins a patient might need. But gene therapy has had a troubled history, in part because scientists cannot precisely control where a new gene inserts into a cell's DNA and how active it is once there (which determines how much protein is produced). These problems can lead to unwanted side effects—including the development of malignant tumors.

A logical solution to the problem of having proteins made in undesirable places and amounts would be to combine a therapeutic gene with a switch that could reliably turn it on or off as needed. As it happens, says Kirk—who is chairman and chief executive officer of Intrexon, a company that is developing new genetic engineering techniques—insects routinely use just such a switch to control molting.

Here's the thing. Insects do not just sort of molt, starting and then stopping partway; they either do it, or they don't. The genetic pathway that drives the process must remain completely

turned off until the time is right. The gene that interests Kirk serves as the master switch for all this activity. It codes for a hormone called ecdysone. As ecdysone surges through the insect, it turns on a raft of other genes to start building the new exoskeleton. After the new exoskeleton is ready, the insect discards the old one. Once molting is nearing completion, the levels of ecdysone fall to zero—at which point the genetic pathway turns off. More important, from Intrexon's point of view, the switch is airtight when turned off—molting does not happen in the absence of ecdysone. The switch does not allow this group of genes to act together again until molting is set to begin.

The scientists at the company decided to take advantage of this foolproof characteristic to tightly control any genes transplanted into people. Imagine equipping each replacement gene with a biological switch that turns on—and thus activates the therapeutic gene—only in the presence of ecdysone molecules that have been adapted to work with human physiology. Patients given low doses of this activating drug (technically known as a ligand) could turn on only a few copies of the new gene, thus producing low amounts of whatever compounds were encoded. Patients given high doses of this activating drug could

IN BRIEF

Early experiments in gene therapy ran into problems, in part because researchers could not control how active

newly inserted genes would become. **Researchers are addressing** the issue by developing molecular switches that

can reliably adjust the function of a transplanted gene by remote control. **Some of these gene switches** are al-

ready being combined in human trials with experimental immunotherapies to treat cancer.

Two Strategies for Controlling Gene Activity with Pills

A major challenge to the development of successful gene therapies is making sure that the newly inserted genes are not too active—which can cause cancer, among other things. All genes, which are made up of DNA, instruct cells to make another molecule, called RNA, which in turn often directs the manufacture of proteins. Researchers are studying various approaches (*two of which are pictured below*) for creating biological switches that can shift a gene's operation (and thus the production of proteins) into gear—or shut it down altogether.

turn on many copies of the gene and thus manufacture large quantities of the related compound. In an unexpected emergency, however, withdrawing the ligand would shut the entire process down. No ecdysone, no activity by the introduced gene. As an added bonus, the ecdysone would not interfere with any other genes, which biologists call “cross talk,” because the human body does not otherwise use, or need, the hormone to regulate genetic activity. Or, as Kirk puts it, “Humans don't molt.”

Over the past eight years Intrexon has “wired” its ecdysone switches to thousands of human genes, demonstrating in laboratory tests that virtually any gene in the body can be put under the hormone's control. In addition, Kirk's group has added a second layer of checks by stitching in so-called cell-specific promoters to their switches. Cell-specific promoters are swatches of genetic material that cause genes to turn on (be “expressed”) only in specific tissues, such as neurons or blood or liver cells, or only in certain conditions, such as the low-oxygen environment of a tumor. The addition of these molecular gatekeepers further reduces the chances of unwanted side effects occurring in parts of the body that had not been targeted for treatment.

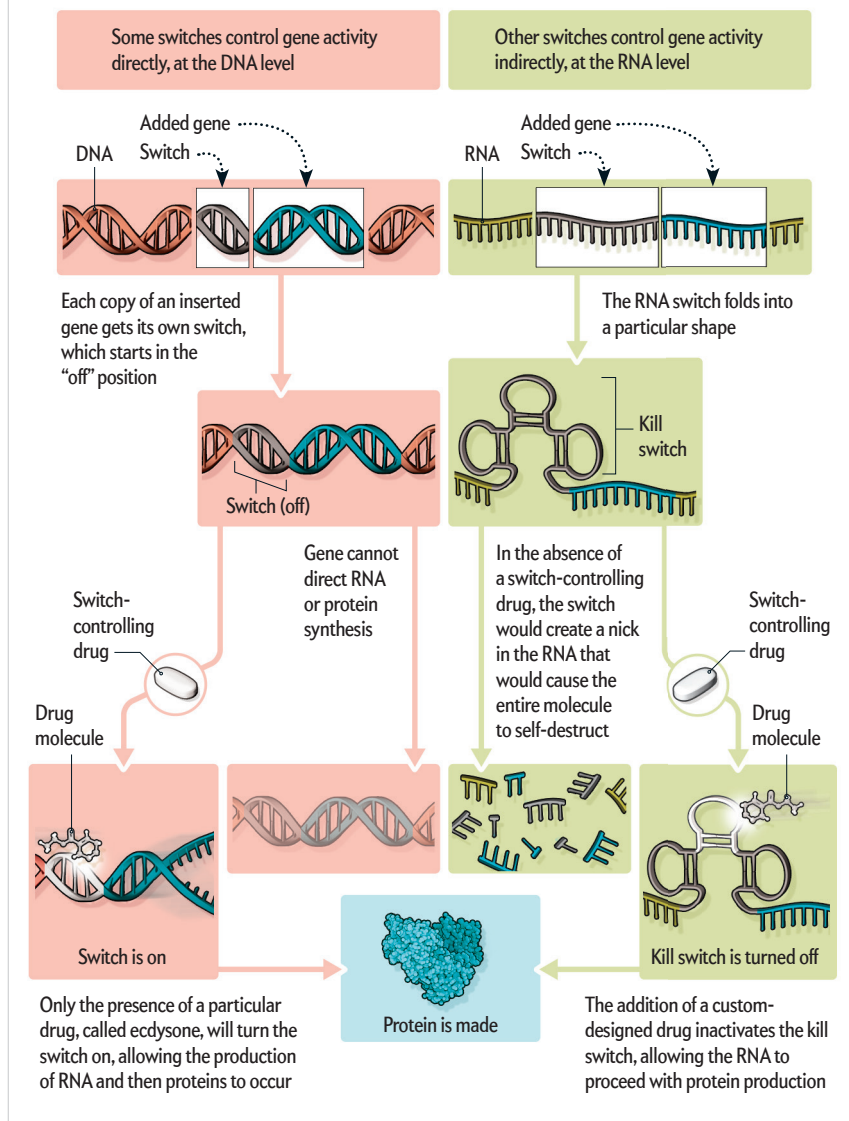
Meanwhile other groups are borrowing from different biological processes to develop their own genetic switches and added control mechanisms. Eventually the ability to deliver several switch-controlled genes—each one able to be dialed up or down as needed—should make gene therapy safe and effective enough that it can become part of mainstream medicine. Or at least that is the idea. Preliminary tests in humans suggest that the switch approach could work as intended. So far it has been studied mostly in cancer and will likely make its biggest mark there first.

FIRST TRIALS

IN PARTICULAR, investigators are studying the switch approach as a way of making cancer immunotherapy less of a harrowing ordeal for patients. Cancer immunotherapies, which have made a lot of headlines of late, aim either to reawaken an immune response that has been lulled to sleep by chemical signals from a malignancy or to jump-start an entirely new and more powerful

anticancer response than a patient's immune system can achieve on its own. Trouble is, a rebooted immune system can easily slip into overdrive, triggering life-threatening fevers and the potentially lethal buildup of fluids throughout the body.

Gene switches are now being evaluated, for example, in



small trials of carefully chosen patients with recurrent melanoma (a type of skin cancer) and breast cancer. Doctors inject one or two tumors in these individuals with genes designed to boost production of cytokines—signaling molecules (such as interferon and various interleukins) that the immune system uses to monitor and adjust the fight against tumors. Investigators believe that they may not need to treat all the malignant lesions in each patient, because once the immune system is properly primed to tackle one nest of cancerous cells, it should start searching for others elsewhere in the body without the need for further prompting.

Cytokines trigger a wide range of physiological reactions— from opening up blood vessels so that immune cells can rush to the scene of an infection to activating ruthless killer T cells, which, among other things, specialize in destroying cancerous cells. But to date, doctors have not been able to treat patients successfully with one of the most powerful cytokines, known as interleukin-12, or IL-12.

to develop, they can skip the next scheduled dose, thereby averting the worst of the damage.

Ziopharm, which is working with Intrexon to develop switch-enabled cytokine treatment in people, reports encouraging results so far. Kirk acknowledges that they might have chosen to test their approach on something less potent than the IL-12 gene—where the slightest misstep could prove fatal. But he says, “We chose one of the hardest genes because we wanted to pressure-test the switch.” In other words, when it counts most, does a switch that has been turned off remain completely off?

Results from two safety studies conducted at several medical centers (and totaling fewer than 40 patients) suggest that the answer is yes. Although no one was cured, the switch-controlled regimen appeared to be reasonably safe. As anticipated, a few patients did begin showing signs of a dangerous overreaction, but it dissipated soon after they stopped taking the ecdysone pills.

The researchers also found hints that the therapy could be helpful. In one of the studies, they injected the gene-plus-switch



INSPIRED BY NATURE: The hormone ecdysone regulates the process of molting, which transforms many insects, such as this butterfly, into their adult form. Now a biotech firm has reworked ecdysone into a much needed safety switch for gene therapy.

This failure stems in part from IL-12's propensity for unleashing a “cytokine storm,” in which the immune system seemingly goes on a rampage against the body. In the bloodstream, IL-12 can cause a sharp drop in blood pressure, difficulties with lung function, and heart problems, which together can easily lead to organ failure and death. And yet, says Laurence Cooper, a physician-scientist at the University of Texas M.D. Anderson Cancer Center and CEO of biotech company Ziopharm Oncology, “there is a ton of literature on its effectiveness in the tumor microenvironment. IL-12 is the holy grail of immunotherapy.” The idea, then, is to deliver as much IL-12 as possible to a single tumor but not so much that a cytokine storm occurs. Here is where the switch technology could prove revolutionary.

Researchers insert the switch-enabled IL-12 genes into an individual's tumor, where they take up residence in many cells, including the immune cells that are already there, giving the latter a boost. Because the switch can be activated only by the presence of the ligand, physicians can increase the levels of cytokine in the tumor very deliberately by slowly increasing the amount of drug they give their patient. If a cytokine storm starts

combination into 12 people with metastatic breast cancer. Each of them had already endured an average of eight previous cancer treatments, with diminishing hopes of survival. For various reasons, investigators were able to evaluate the new therapy's effect in only seven patients, however. The IL-12 treatment shrank some of their tumors, and in three people, the disease appeared to remain stable, at least for the short duration of the trial. The second safety study, in 26 patients who had been treated an average of six different times for metastatic melanoma, showed an uptick in cytokine levels and other cancer-fighting activity. In May 2015 Ziopharm initiated another study using switch-enabled IL-12 as an experimental treatment for glioblastoma multiforme, a particularly aggressive type of brain cancer.

RIBOSWITCHES

RICHARD MULLIGAN of Harvard Medical School has been working on a different kind of switch. His approach features small naturally occurring RNA molecules called ribozymes. First described in the 1980s, ribozymes are like enzymes in that they catalyze chemical reactions in the body, but most enzymes are proteins, and ribozymes consist of RNA. In a feature useful for switches, some ri-

STEPHEN J. KRASEMANN/Science Source

bozymes also have the ability to cut themselves up and induce genetic molecules to which they have been attached to self-destruct.

Mulligan's constructs give rise to a ribozyme linked not to a classic gene but to a messenger RNA (mRNA) molecule. When cells make proteins, they first copy the DNA in a gene into messenger RNA (a mobile, single-stranded transcript), after which the mRNA gets translated into the protein. From the cell's point of view, the addition of a stretch of DNA or its corresponding mRNA should result in the same outcome—production of a specific protein.

As a first step, researchers assemble and inject a strand of DNA that codes for a “self-cleaving” ribozyme plus the selected therapeutic protein. If a human cell transcribed this synthetic DNA into mRNA, the ribozyme portion would cut itself, causing the rest of the mRNA molecule to appear defective; the sur-

swallowed the appropriate pill. Take the pill, and you have, in effect, turned on a gene. Stop the pill, and the gene stays off.

MULTIPLE SWITCHES

ALTHOUGH SINGLE GENE SWITCHES are not yet perfected, investigators can envision a not too distant future in which multiple switches become the norm, allowing increasingly precise control of gene therapy. Combining switch-enabled gene therapy with other anticancer regimens may also bear tremendous fruit.

Already M.D. Anderson's Cooper, for example, is combining a couple of switch-enabled genes with a cell-based cancer treatment. The genes will contribute interleukin-12 and another cytokine, interleukin-15; lab tests suggest that IL-15 makes IL-12 even more effective at rallying immune cells. The third part of this experimental treatment—the cells—is a group of genetically engineered immune cells called CAR T cells that are better able to direct their firepower on cancerous tissue than naturally occurring immune cells can. Adding switch-bearing IL-12 and IL-15 genes to the CAR T cells should allow Cooper to boost the cells' potency and effectiveness. Because the gene switches and their respective activators will allow him to adjust the levels of IL-12 and IL-15 independently, he should be able to fine-tune the treatment to produce the best results with the least amount of IL-12, thereby further reducing the risk of unleashing a cytokine storm. With a touch of whimsy, Cooper calls this new suite of engineered cells “remote-control CARs.”

Many technical hurdles must still be overcome, but the potential arc of progress is beginning to take shape. If inserting new genes into our bodies in the 1990s was Genetic Engineering 1.0, then the switch-based control of our genes is Genetic Engineering 2.0. Someday many of the pills that doctors give patients may be used to switch on various transferred genes

at precisely the right place and time in the body instead of flooding every organ and tissue with the powerful pharmaceutical agents that act both where they are needed and elsewhere, causing side effects. Many drugs will no longer be manufactured in giant vats and so-called bioreactors in pharmaceutical facilities. Instead new gene treatments will allow patients to churn out a molecule exactly where and when it is needed most in the body. ■

The ability to deliver several switch-controlled genes—each one able to be dialed up or down as needed—should make gene therapy safe and effective enough that it can become part of mainstream medicine.

rounding cellular machinery would then break the mRNA apart and cause the entire process of building a protein to grind to a halt. It would be as if the gene had been turned off.

Starting in 2000, Ronald R. Breaker and his colleagues at Yale University showed how to protect the mRNA but also to turn off protein synthesis when desired. The trick was to link the ribozyme to an additional molecule called an aptamer, which is a kind of sensor that is designed to be activated by a drug. In the presence of the drug (and only in the presence of the drug), the sensor changes shape in a way that prevents the ribozyme from destroying the mRNA. With the full length of mRNA intact, the cell makes the protein. When the drug that acts on the sensor is withdrawn, the ribozyme and the mRNA self-destruct.

By 2004 Mulligan and his colleagues were regularly equipping his ribozyme switches with carefully customized drug-sensitive sensors, and he continues to hone the technology today. The sensors can be designed with great specificity, Mulligan says, further reducing the chances of unwanted side effects. As with ecdysone, the mRNA that is connected to the ribozyme would allow production of the protein only if the patient

MORE TO EXPLORE

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