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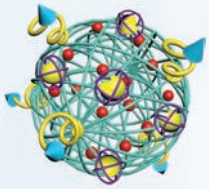
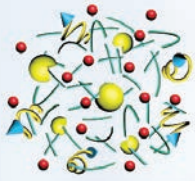
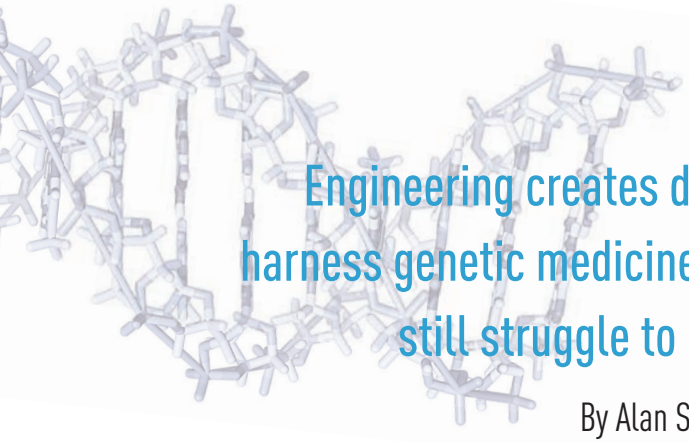
NEAT LITTLE PACKAGES







Engineering creates delivery systems that harness genetic medicine—even as researchers still struggle to understand it.

By Alan S. Brown

When Layla Richards was only 14 weeks old, she was diagnosed with acute lymphoblastic leukemia, a type of cancer that kills three quarters of the infants who contract it. Her doctors at Great Ormond Street Hospital in London immediately started the standard treatment—chemotherapy to kill the cancer and a bone marrow transplant so her body could replace her damaged blood cells—and hoped for the best.

Seven weeks later, the cancer returned.

The doctors then tried an experimental treatment. That also failed.

The doctors told her parents that Layla faced certain death and gently suggested palliative care.

In another part of Great Ormond Street Hospital, Waseem Qasim, a professor of cell and gene therapy at University College London, was treating mice by genetically modifying their immune cells. Immune cells are nature's first line of defense against disease. They are designed to attack any foreign body. Unless they are a very, very close match, immune cells transplanted from one animal into another will also attack their new host.

Working with mice, Qasim had shown he could genetically modify immune cells from donors so they would ignore their new host. He did that through use of an artificial enzyme called TALEN (for "transcription activator-like effector nuclease"). Like a pair of robotic scissors, TALEN hunts down and cuts DNA at a pre-programmed location. Once the DNA is cut, Qasim can add or subtract genetic material or even an entire gene. That enables him to alter the DNA's code, and therefore the cell's behavior.

Instead of going after the host, Qasim's altered immune cells targeted only leukemia cells. Qasim also found a way to boost the immune cell's resistance to a powerful drug used in the treatment.

When Layla's parents were told about Qasim's work, they were adamant about going ahead with what was a treatment that had never been tested on humans.

"We didn't want to accept palliative care, and so we asked the doctors to try anything for our daughter, even if it hadn't been tried before," her mother, Lisa Foley, said.

At that point, however, Qasim had just begun the laborious process of treating enough immune cells to test for safety on humans. He had only enough to fill one vial. Layla was injected with 1 ml of the experimental cells.

Within a few weeks, Layla's health improved. After two months she was without cancer and had received a bone marrow transplant so that she could begin making her own immune cells. One month later, in the fall of 2015, Layla returned home.

Stories of genetics-based cures like this make it seem as if we live in an age of medical wonders. And to a certain degree, we do. But for all the advanced proposals for treating intractable disease, says Matthew Porteus, associate professor of pediatrics at Stanford Medical Center, there is still one pressing problem. He and his colleagues need better delivery systems.

"You can have the fanciest ideas and molecules," said Porteus, who himself was the first researcher to modify genes in human cells at rates high enough to cure diseases. "But if you can't get them into the cell, they are no use," he said.

Better delivery systems will take engineers.

The Package

Although the cancer treatment that Layla Richards received was a first, the concept of genetically modifying cells in a Petri dish and injecting them into a patient, as Qasim did, is fairly standard. Ideally, though, physicians would like to deliver genetic medicine to cells inside the body. It is the only way to attack cancer and disease where they live. It would also make it easier to treat genetic diseases by changing the DNA in cells that continue to divide and multiply.

Doing that, however, increases the difficulty for delivery systems. In addition to convincing the targeted cells to open up and accept a gene-altering payload—no mean feat—in-body systems must first find the right cells and also protect their package from the body's immune system.

Fortunately, researchers have been learning how to do that for more than 20 years while developing nanoscale drug delivery systems. Those solutions have now moved into the mainstream, said Mark Saltzman, a Yale University professor of biomedical and chemical engineering and physiology. Saltzman has published more than 300 papers in the field.

"The pharmaceutical industry was built on the notion that if you find the right chemical or compound, everything is going to be okay. If it has dangerous side effects or lacks effectiveness, you just tune the drug's chemistry," Saltzman said. "What's different now is that we can achieve greater safety and effectiveness by changing the packaging instead."

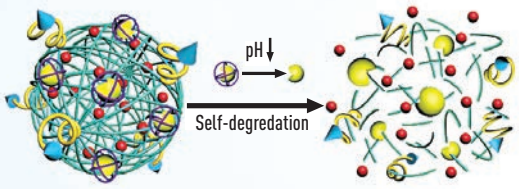


Image: Zhen Gu

Zhen Gu's DNA "nanoclew" has ligands on its surface that bind to receptors on the surface of cancer cells. It is then absorbed inside the cancer cell, whose acidic environment destroys the polymer sheath containing enzymes that slice through the DNA cocoon, spilling anticancer drugs into the cancer cell and killing it.

Pharmaceutical companies do that with doxorubicin, a cancer drug that also causes heart disease. Entangling the drug in liposomes, sac-like structures made from fatty acids, keeps it from interacting with heart cells or other tissues. And because the liposomes are smaller than 100 nanometers in diameter, the body's immune system ignores them. They are small enough to pass through the leaky blood vessels that surround tumors, and cancer cells have no mechanism to remove them.

The fact that fatty acids are common molecules helped allay Food and Drug Administration concerns about the packaging, Saltzman said. Doxorubicin became the first FDA-approved nanomedicine in 1995.

Since then, packaging has grown a great deal more sophisticated. Saltzman's work is a case in point. He prefers to work with synthetic polymers, for example, because they offer a great deal of flexibility. The polymers enable him to package two or more medicines at a time and control precisely how fast the packages will release their payloads. Also, since artificial materials do not trigger immune responses, he can deliver very high doses of medication without a reaction.

Saltzman draws on decades of research to target specific types of tissues or cells.

"People have been studying cancer for a long time," Saltzman said, "and some characteristics of cancer cells are well known. For example, they reproduce rapidly, and need to accumulate folate molecules to make DNA. We put folate on the surface of our molecules, and cancer cells think

they are folate and ingest them. We also put cell-penetrating peptides on the surface to speed uptake once the cell recognizes the package."

Saltzman developed a range of approaches to deliver cancer drugs. Yet many of these techniques would adapt easily for genetic medicine. In fact, many researchers are already putting them to work.

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>
> — Matthew Porteus, associate professor of pediatrics at Stanford Medical Center

The Ball of Yarn

Arcturus Therapeutics in San Diego bills itself as an RNA medicines company. Cells use RNA to carry instructions encoded in the DNA to ribosomes, structures inside the cells that build proteins to spec; by interfering with that process, RNA medicines can disrupt the formation of proteins that cause disease and tumor growth.

In June, Arcturus signed an agreement to commercialize RNA medicines with Janssen Pharmaceuticals, a Johnson & Johnson company.

Arcturus wants to package these RNA medicines using a fatty acid-based nanoparticle system, a delivery technology that it calls "lipid-enabled and unlocked nucleic acid modified RNA" (so that its acronym can be LUNAR).

The company says this is an advance over previous lipid-based delivery systems. In those systems, lipids were made from permanently charged molecules called quaternary amines; their positive charge held negatively charged medicines and RNA molecules in place. Unfortunately, the charged particles accumulated in body tissues the way balloons with a static charge stick to a wall.

"That's not a problem if you deliver one or two doses," said Arcturus CEO Joe Payne. "But if you are dosing every day, every week, or even every month, it is a problem."

Arcturus's solution is a biodegradable lipid with a temporary charge, just enough to wrap medicines and RNA in a loose, yarn-like bundle. When the bundle reaches the targeted cell, the cell engulfs it, trapping it in a small sac that travels into the cell. By the time that sac breaks down, the lipid has fallen apart, releasing its medicines or RNA to go to work in the cell.

To target specific cells, Arcturus follows Saltzman's playbook. It decorates the LUNAR surface with different molecules, and also changes its size, shape, and charge.

This yields some surprisingly sophisticated systems. For example, Arcturus attaches small umbrella–like structures to LUNAR. They hook onto liver tissue, allowing the package to break free and enter the cells.

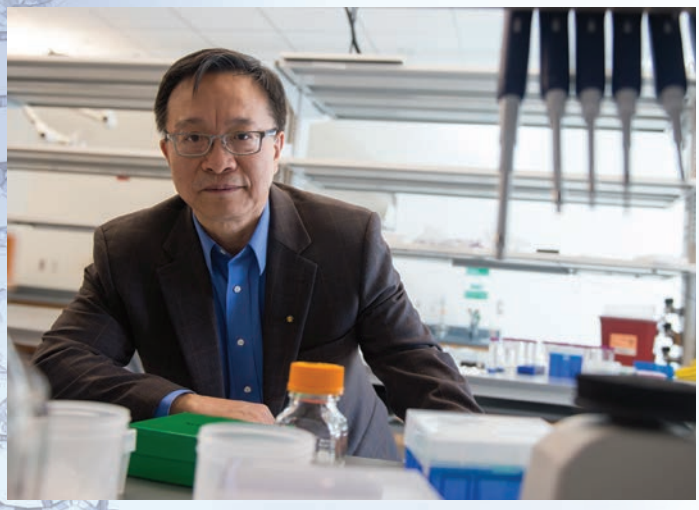
"A lot of this sounds like science fiction, but it's real," Payne said.

It must seem that way to Andre Watson, chief technology officer of Ligandal, a fledgling Silicon Valley startup building delivery systems for genetic medicines.

Three years ago, Watson was an undergraduate at Rensselaer Polytechnic Institute looking for a project he could work on in graduate school. A professor pointed him towards delivery systems.

Watson quickly hit upon the strategy of building a multilayer package. The outside layer would target specific types of cells. Once inside, it would disintegrate, leaving behind a second package containing scissor enzymes and genetic material.

Watson makes both shells from peptides. "Peptides are the way biology presents information. They dictate where things go inside the cell," he said.



"This is the most important question for genome editing, how to raise the percentage of template-based repairs."

Gang Bao, ASME Fellow, Nanomedicine Center for Nucleoprotein Machines, Rice University.

Watson leverages this by programming the peptide shell to carry its genetic payload into the cell nucleus, where DNA creates RNA. By targeting the nucleus and tuning his peptide package to disintegrate slowly, Watson can flood the DNA with active genetic material for weeks at a time. As a result, he claims he can achieve gene replacement rates that are much higher than standard processes.

Watson came to Silicon Valley with $1,500, but Ligandal has lined up $500,000 in angel investments. The company is working with several leading researchers and pharmaceutical firms, and plans to begin publishing results soon, he said.

If Watson is still trying to build his reputation, Zhen Gu has begun to establish one. He was named one of MIT Technology Review's 2015 list "35 Innovators Under 35," in part for an injectable nanoparticle package that breaks down and releases insulin when it encounters high levels of sugar in the bloodstream.

Gu, an assistant professor in University of North Carolina-North Carolina State University's Joint Department of Biomedical Engineering, is now seeking to do something similar by packaging genetic materials in DNA.

He calls his system a nanoclew, after a clew of yarn, because it looks like a tightly wound ball of DNA. The DNA is shaped to hold gene-cutting material as well as polyester groups that give it an electrical charge that promotes uptake in cells.

At the cellular level, Gu's DNA creations look good enough to eat, and cells do ingest them. The DNA, although biocompatible, is artificial and does not trigger an immune response.

To test the system, Gu's team gave mice tumors that had been modified to produce green fluorescent proteins. The researchers then programmed their nanoclews to cut out the DNA that made those proteins. According to Gu, about one third of the cancer cells stopped fluorescing after treatment.

The Template

The human body is made up of a couple of hundred different types of cells—blood, brain, muscle, skin, and so on, not even counting the microbial hitchhikers—and it is improbable that any one type of packaging will reach them all. As engineers test the design of delivery systems, they will find themselves working with scientists to understand how all the pieces fit together.

The gaps in science's understanding come across clearly when talking with Gang Bao, an ASME Fellow who heads the Nanomedicine Center for Nucleoprotein Machines at Rice University in Houston.

Bao has been working on genetic cures for sickle cell anemia. It is a promising application for genetic therapy, since a single mutation in a single gene in just one type of stem cell causes the disease. This gives Bao a very specific target to attack.

His game plan sounds simple: remove the stem cells that make blood cells from the bone marrow; splice in a gene segment to fix the mutation; and then inject the modified cells back into the bone marrow so they can produce healthy red blood cells. Executing the plan has proven difficult, which is one reason why Bao has been working on the problem since 2008.

For instance, Bao uses CRISPR—a powerful, low-cost genome-editing technology—to break DNA strands. Cells repair 95 percent of those breaks by rejoining the broken ends the way we might tie a broken shoelace. Often, this introduces small mutations into the gene, destroying its ability to produce RNA. This technique helps eliminate unwanted or dangerous proteins.

The other 5 percent of the time, however, cells use a molecular template to build and then insert a new gene into the missing gap. Bao hopes to take advantage of this mechanism to alter genes and reprogram anemic stem cells to make healthy red blood cells.

"This is the most important question for genome editing, how to raise the percentage of template-based repairs," Bao said. "We must learn to control which type of repair the cell selects, or how to separate the properly corrected cells from those with end-to-end repairs so they do not compete with each other when we replace them in the patient."

Bao is also tackling another template repair problem, when scissor enzymes sometimes make the wrong cut. CRISPR targets DNA by looking for a specific sequence of 20 pairs of molecular building blocks that form DNA. Human DNA contains 3 billion pairs, so that sequence is likely to appear in several different places. Scissor enzymes also may sometimes settle for getting 19 out of 20 pairs right.

Bao calls this off-target cleaving, and it could lead to unwanted mutations and unexpected side effects. He is developing a web-based tool that will rank DNA segments by their vulnerability to off-target cleaving.

"We can show researchers the problem, but we do not have a way to fix it yet," he said.

The Future

Bao's realism is bracing when set against the rise of venture capital-backed genetic medicine companies. The technology is risky. This is why Ligandal's Andre Watson describes the ideal test subject as someone in the late stages of a rare, lethal disease. Most other researchers would agree.

Yet the future is coming fast. Watson, for example, imagines that clinics 30 years from now will sequence patients' genomes and biopsy their conditions, and prescribe treatments that target the precise cause of disease.

That may seem like more fiction than science. But this time last year, so were the genetically modified immune cells that cured Layla Richards's cancer.

ALAN S. BROWN is associate editor at *Mechanical Engineering* magazine.