DISCOVERY REPORT

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The future of cancer immunotherapy

New moves to kick-start self-defense

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The future of cancer immunotherapy

It's wonderful to celebrate the slew of innovations in cancer treatment that we saw in the previous decade. Of all drug approvals between 2010 and 2018, 27% were cancer therapies, compared with 4% in the 1980s. Yet cancer remains the leading cause of death globally, accounting for nearly 10 million deaths in 2020. (In comparison, COVID-19 killed about 3 million people in 2020.) There's much work left to do.

Much of cancer treatment involves some combination of surgery, radiation, and chemotherapy. The excitement in the past decade centers on a different family of treatments called immunotherapies, which are designed to help the immune system ferret out and obliterate cancer. Antibody treatments for cancer have been available since 1997. But in the 2010s they were joined by customized cancer-destroying versions of the white blood cells known as T cells, a virus that bursts malignant cells open, and therapies that target T cells' so-called checkpoint proteins to unleash stalled immune systems on tumors. In fact, the 2018 Nobel Prize in Medicine went to a duo whose work led to checkpoint inhibitors.

With all these options, why is cancer still winning? Not all cancers respond to treatment, and the disease can recur. The drugs cost hundreds of thousands of dollars per patient, so they aren't accessible globally. This report examines how chemists and entrepreneurs are addressing those challenges. You'll encounter start-ups boosting therapies' activity with microbes, discovering new targets to broaden the universe of cancers that might respond to treatment, moving toward less costly off-the-shelf therapies that are not made from a patient's own cells, and much more.

Contributing editor Carmen Drahl, who has covered organic chemistry and green chemistry for C&EN, edited this report. It includes a reading list of papers and patents curated by our sources, as well as by information scientists at the CAS division of the American Chemical Society.

As an ACS member, you get exclusive access to the Discovery Report, a quarterly publication bringing you cutting-edge research defining the chemical sciences and our industry. Look for the next one in the second quarter of 2022.

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INSIDE

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CHEAT SHEET

5 questions and answers about cancer immunotherapy

Q. Why are researchers excited about cancer immunotherapy?

» **It recognizes, attacks, and kills cancer with** the human body's own immune system instead of the synthetic agents that characterize chemotherapy.

» **It is over 100 years in the making.** In the late 19th century, scientists attempted to unleash the immune system on cancer. Surgery, chemotherapy, and radiation yielded more reproducible results, so they dominated cancer treatment in the 20th century. The 21st century may be a different story, because scientists can now characterize—and target—the immune system's components.

» **It has the potential to keep one step ahead of cancer.** Unlike chemotherapeutics, the immune system can adapt to catch malignant cells that evade detection by mutating. It can also "remember" past assaults to be ready in case cancer recurs.

Q. What are some available types of immunotherapy?

» **Antibodies** bind to specific targets on the surfaces of cancer cells or immune cells.

» **Cancer vaccines** treat a malignancy or prevent specific cancers caused by viruses.

» **Checkpoint inhibitors** take the brakes off the immune system, freeing it to attack cancer.

» **Chimeric antigen receptor T-cell (CAR-T) therapies** equip a person's own T cells with a synthetic receptor, bestowing the ability to spot and eradicate tumors.

» **Cytokines** signal the immune system to mount an attack, and they control growth of other immune cells.

» **Oncolytic viruses** selectively infect and destroy cancer cells by bursting them open, releasing chemical signals that trigger the immune system.

Q. What are their limitations?

» **They don't work on every cancer.** A 2019 analysis suggests that less than 13% of people with cancer respond to checkpoint inhibitors. CAR-T therapies are so far approved only for blood cancers, not solid tumors like brain and ovarian tumors.

» **Their side effects can be life-threatening or lethal.** More than a dozen people taking CAR-Ts have died of brain swelling or systemic inflammation. Cytokines' side effects are severe enough that a hospital stay is required for people taking high doses (see page 9).

» **They don't come cheap.** The vast majority of immunotherapies are biologics, which are more expensive to manufacture than small molecules. Some, like CAR-T, must be tailor-made for each patient. Price tags can reach hundreds of thousands of dollars for a course of treatment.

Q. How are scientists developing better options?

» **Several start-ups aim to expand the population that responds to treatment** by finding and lifting additional checkpoints that blockade the immune system.

» **Combining two immunotherapies** or an immunotherapy and a chemotherapy could provide long-lasting cancer destruction, some scientists say. Trials studying combinations have mushroomed.

» **Modifying immunotherapies with control systems** such as kill switches, removable protein masks, or linkers that bring immune cells and tumor cells into proximity could tamp down side effects.

» **Moving away from tailoring a patient's own T cells** and toward off-the-shelf natural killer cells or therapies from donor cells could streamline manufacturing.

Q. What's next for cancer immunotherapy?

» **Increasing racial diversity in cancer clinical trials** is a priority. In the US, Black women are twice as likely as white women to be diagnosed with a certain aggressive breast cancer. Yet Black women made up just 7% of participants in a clinical trial that evaluated a therapy.

» **Clinical trials of immunotherapies in solid tumors** are underway, but if a halted trial of a CAR-T candidate is any indication (see page 16), the studies are as likely to provide information about obstacles to overcome as they are treatments.

» **Machine learning and other techniques may be able to predict** who will benefit from immunotherapies.

» **Foods and supplements can influence immunotherapies' efficacy**, and researchers want to know why (see page 19).

8 experts identify opportunities for cancer immunotherapy

Sachin Bhagchandani

» Graduate student, Massachusetts Institute of Technology

Since the 1980s, drugs called imidazoquinolines have been known to spur immune cells to kill cancers. When Sachin Bhagchandani first read about them, he was struck by their potency—and their toxicity, which restricts their current use to ointments for skin cancers.

For his doctoral research at Massachusetts Institute of Technology, Bhagchandani studies ways to safely deliver drugs such as imidazoquinolines to tumors by transporting them through the bloodstream on inert, bottlebrush-shaped polymer scaffolds.

Binding the drug to the scaffold inactivates it. But cellular enzymes easily cleave that connection—and reactivate the drug—at the lower pH common in tumors, "so we see strong antitumor effects without systemic toxicity," Bhagchandani says.

In ongoing studies, he's refining the linkers that connect the drug to the bottlebrush polymer. The linker's position on the drug and its chemical structure are both crucial, Bhagchandani says. If they are not ideal, the linker may not fully inactivate the drug, which results in toxicity. Finding the sweet spot requires expertise in synthesizing new polymers and in testing the constructs' immune actions in animal models. That interdisciplinary feat earned Bhagchandani a prestigious National Cancer Institute Predoctoral to Postdoctoral Fellow Transition Award (F99/K00) to support his research. While imidazoquinolines' efficacy is well known, he says, ensuring the prodrug system is safe is crucial to success. "Finding biomarkers that indicate any signs of toxicity will be key."

Chris Bourne

» Graduate student, Memorial Sloan Kettering Cancer Center, and board member, #BlackinCancer

Some immunotherapy approaches rely on drugs and antibodies, others on living T cells. But they share a common challenge: tumor cells will eventually find a way to suppress or evade anticancer immune responses, according to Chris Bourne.

But Bourne and his colleagues have managed to kill those escapees in petri dishes and mice. They engineered T cells to deliver a bacterial enzyme that converts an inactive, nontoxic prodrug into a cancer-killing drug when at the tumor. And the drug in question doesn't stimulate the immune system; instead, it is a small molecule that spurs apoptotic cell death.

> **We're addressing the challenge of tumors being able to shut down whatever immunotherapy you throw at them by using something that's not immunotherapy.**

"We're addressing the challenge of tumors being able to shut down whatever immunotherapy you throw at them by using something that's not immunotherapy," Bourne says.

While traditional chimeric antigen receptor T-cell (CAR-T) therapies focus on priming immune cells to kill tumors, engineering T cells in this way offers the potential to add a multitude of therapeutic functions. In the long run, Bourne envisions adding more genetic circuitry to enhance the killing activity of these cells by affixing enzyme-prodrug combinations, cytokines, or other cargo.

Bourne's work was recently recognized with a $300,000 fellowship from Memorial Sloan Kettering Cancer Center to support his graduate and postdoctoral research. The award gives him essential career stability, he says. "It's a really good demonstration of how investing in underrepresented scientists can help them stay in science."

CREDIT: COURTESY OF INSTITUTION UNLESS OTHERWISE NOTED

Jean-Simon Diallo

» CEO and scientific founder, Virica Biotech, and associate scientist, University of Ottawa

Scientists' initial excitement around oncolytic viruses focused on their ability to enter and kill cancer cells. Over time, though, researchers came to appreciate the viruses' skill at awakening the immune system. Because the viruses can be loaded with a range of genetic machinery, "they're really a mix of gene therapy and immunotherapy," Jean-Simon Diallo says.

In 2009, Diallo was screening molecular libraries to identify drugs that could boost oncolytic viruses' tumor-killing activity. He discovered viral sensitizer molecules, which suppress cells' antiviral defenses and make it easier for viruses to enter cancer cells.

Coincidentally, his data revealed a solution to a public health crisis: the 2009 H1N1 flu pandemic had left vaccine makers struggling to keep up with increased demands for viral vaccines, and the sensitizers Diallo had discovered could also lower antiviral defenses to enhance virus production. He founded Virica Biotech, a firm focused on deploying viral sensitizer molecules to scale up viral production for vaccines and other applications.

The technology could also help boost oncolytic viruses' potential as immunotherapies by enhancing tumor penetration and reducing off-target effects on normal cells. If developed as immunotherapies, larger viruses could also be loaded with therapeutic genes and targeted to cancer cells, expanding the ways they could act to kill tumors. "There's so much you can do with these vectors," Diallo says. "The real power of these viruses is in their capacity to encode therapeutic programs."

Zhen Gu

» Dean, College of Pharmaceutical Sciences, Zhejiang University, and cofounder, ZCapsule

The biggest challenge for cancer immunotherapies is delivery—getting them where they're needed without triggering runaway immune responses, Zhen Gu says.

Solving drug delivery for checkpoint inhibitors, antibodies, and T-cell-based treatments alike will be crucial to extend immunotherapy's benefits to more patients, Gu says. His research is focused on targeted delivery systems that reduce cancer therapies' side effects.

Gu is focused on three distinct approaches that are currently in laboratory studies. First is a spray gel containing antibody-coated nanobeads that, when spritzed on sites where a tumor was surgically removed, kills stray cells left behind. Another project conscripts platelets to deliver tumor-targeting antibodies, which can be either tethered to the platelets' surface or loaded inside the platelets by genetically engineering their cellular precursors. Because platelets naturally target sites of inflammation or bleeding, "we use them as Trojan horses to deliver drugs," Gu says. A third strategy relies on a patch of hollow microneedles loaded with CAR T cells. If delivered to solid tumors via minimally invasive surgery, the needles inject their payload into the tumors in high doses with minimal immune side effects.

All three approaches aim to overcome the physical hurdles that immune therapies face: the extracellular scaffolding that surrounds and feeds tumors also blocks immune cells from recognizing and destroying cancer cells. "Overcoming these physical barriers is crucial to the success of immunotherapy," Gu says.

"Even with extremely robust data, we don't know what we don't know. But once we do these studies, there might be a real breakthrough."

Karin Jooss

» Head of research and development, Gritstone Bio

Decades of research have focused on vaccines that can prime the immune system to spot and kill cancer cells. But disappointment has been the norm, likely because vaccines lacked the right combination of tumor-targeting antigens to properly steer immune reactions and a delivery system that would yield long-lasting protective effects, Karin Jooss says. "If you miss out on either one, you have an ineffective vaccine."

Gritstone Bio's method tackles both challenges. The team identifies tumor antigens with artificial intelligence trained on millions of data sets, then delivers those antigens with a self-amplifying messenger RNA (mRNA)–based delivery platform.

When injected into muscles, the mRNA delivery system ensures the antigens are expressed in high amounts for a prolonged period, giving the immune system plenty of opportunities to respond. Ongoing early and midstage clinical trials are evaluating dosing, safety, and efficacy against colorectal, gastroesophageal, and other solid tumors.

The vaccines work best when combined with checkpoint inhibitory drugs, Jooss says. Without them, vaccine-stimulated T cells and immune responses are quickly shut down in peripheral lymph nodes and within tumors themselves. But when dosed with other immunotherapy, cancer vaccines have the potential to "reset the immune system to monitor what's going on over the next few decades of a patient's life," she says. "Even if we don't cure it, we can make cancer a chronic disease, so patients have a good quality of life."

Louis Kayitalire

» Chief medical officer, F-Star Therapeutics

Watching his patients suffer chemotherapy's toxic side effects drove Louis Kayitalire to research better alternatives. Immunotherapy's relatively mild side effects make it a promising alternative for many people—but only if their tumors have high levels of the checkpoint protein PD-L1, which indicates that they will respond well to checkpoint inhibitors.

To create drugs for patients who don't fit this profile, F-star Therapeutics' researchers have focused on bispecific antibodies that target two checkpoint proteins at once—for example, PD1 and LAG3. They make a naturally occurring, Y-shaped antibody bind four sites instead of the usual two by adding two more binding sites on its stem, which typically has a relatively constant amino acid sequence.

The tetravalent antibodies cross-link immune cells and cancer cells, so the former are only activated when they're close to tumor targets. "It's a good safety feature, because if they're not near each other, there's no biologic action taking place," Kayitalire says.

The company has three such antibodies in early- and midstage clinical trials to evaluate their safety, dosing, and efficacy. The drugs aim to improve outcomes in tumors with low PD-L1 levels and act on tumors that have failed to respond to checkpoint inhibitors. While it's still early days for this type of therapy, Kayitalire says,"what I see in clinical studies of patients confirms the benefits of the approach."

Li Peng

» Chief scientific officer, Palleon Pharmaceuticals

In the quest for more immune therapeutics, no drugs have proved quite as promising as the current group of checkpoint inhibitors, which take the brakes off the immune system. This made researchers realize that "there might be another dimension of biology we haven't appreciated," Li Peng says. The trail led Palleon and others to glycobiology.

Palleon aims to thwart another brake—Siglecs, which are proteins that bind to sugars known as sialic acids (see page 16). Homing in on cells' sugary coats is tricky: the immunesignaling pathway includes at least 15 Siglecs, only some of them immunosuppressive, and hundreds of possible cell-surface glycoproteins for Siglecs to bind to. Despite being a "brilliant academic concept," Peng says, finding the right protein to target with a drug is difficult and risky.

> **"Even if we don't cure it, we can make cancer a chronic disease, so patients have a good quality of life."**

Instead of focusing on Siglec proteins, Palleon pivoted to the sugars. The company develops therapeutics by fusing the enzyme sialidase with monoclonal antibodies. The enzyme cleaves sialic acid—a common motif recognized by all Siglecs—off cell surfaces to activate immune cells. "It's the Achilles' heel" for any Siglec, Peng says. Siglec-targeting drugs could work as monotherapies or in conjunction with existing checkpoint inhibitors since they target parallel pathways, she adds.

Palleon announced March 8 that it had dosed the first patient in a clinical trial of its lead compound, a bisialidase. Because the drugs target a novel aspect of cancer biology, there are many unknowns, Peng says. "It's the first time we're putting a drug with this mechanism of action into humans," she says. "Even with extremely robust data, we don't know what we don't know. But once we do these studies, there might be a real breakthrough."

Nicki Vasquez

» Chief portfolio strategy and alliance officer, Sutro Biopharma

Antibody-drug conjugates (ADCs) that harness the immune system to deliver tumor-killing medications have been in scientists' imaginations for more than a century, Nicki Vasquez says. But early versions of ADCs, developed in the 1980s, were often problematic. Weak antibody-drug linkers broke in the bloodstream, leading to severe side effects, or the drugs attached to different spots on antibodies, resulting in a molecular mélange of varying efficacy.

Sutro Biopharma's strategy solves these problems by using stronger linker chemistry and linking drugs to nonnatural amino acids inserted into precise positions within antibodies.

And because Sutro links drugs to immune-stimulating antibodies, its ADCs have a dual effect: drugs kill tumor cells, and resident immune cells gain the ability to recognize and target cancers. "We call it an in-situ vaccine," Vasquez says. "This is really the third generation of ADCs—site specific, more stable, and less toxic."

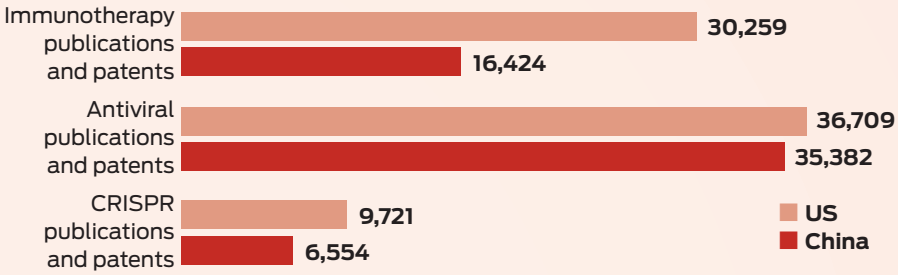
The company's lead candidate, which targets ovarian cancer, is now in clinical trials to evaluate its safety, dosing, and preliminary efficacy. Most ADCs cause ocular side effects such as blurred vision or corneal issues, but Sutro has yet to observe any in ongoing studies, Vasquez says. She sees ADCs as having a key place in the broader armament of cancer therapeutics. "Combination therapies are emerging as really critical for cancer," she says, and unlike CAR T cells, ADCs are "an off-the-shelf product that can be used as an alternative or in conjunction with immunotherapy."■

BY THE NUMBERS

Discover trends in cancer immunotherapy

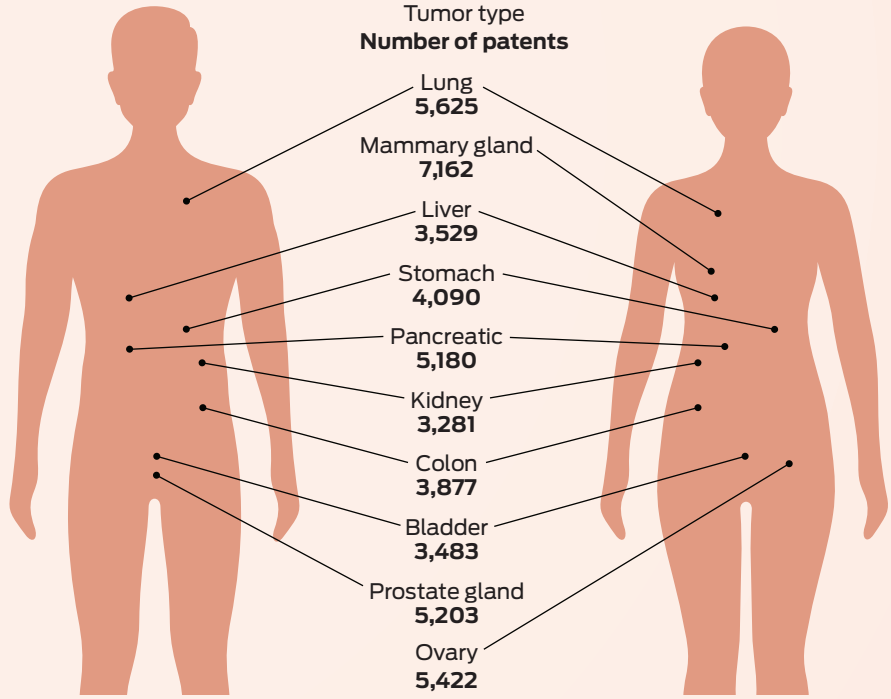
Margins by field

The US and China commonly dominate patents and publishing for topics covered in Discovery Reports. The US has a substantial lead over China in immunotherapy, whereas the countries have more similar activity on other medicinal topics.



Tackling tough targets

An ongoing goal for immunotherapy is to address solid tumors. We determined which solid tumor types are mentioned most frequently in patents overall.



Total number of solid tumor patents, all countries
26,477

Sources: American Cancer Society, CAS Content Collection, Clinicaltrials.gov, *JAMA Netw. Open*, US Food & Drug Administration.
Notes: CAS information scientists searched patents and publications containing the concept of cancer immunotherapy from 2000 to 2021, the concept of antivirals from 2000 to 2020, and the concept of CRISPR from 2010 to 2021. "Publications" include journal publications, conference reports, books, dissertations, reports, and preprints. Preprints were excluded from antivirals data because of the large number of COVID-19 preprints. Antivirals data do not include vaccines. To find patents related to various cancers, CAS information scientists searched for patents containing the concept of "neoplasm." Patents may mention more than one type of cancer.

Immunotherapy stats

Boost your knowledge with our selection of facts and figures.

2011
Year the first cancer immunotherapy was approved.

0.14%
Estimated proportion of cancer cases to respond to checkpoint inhibitor drugs in 2011.

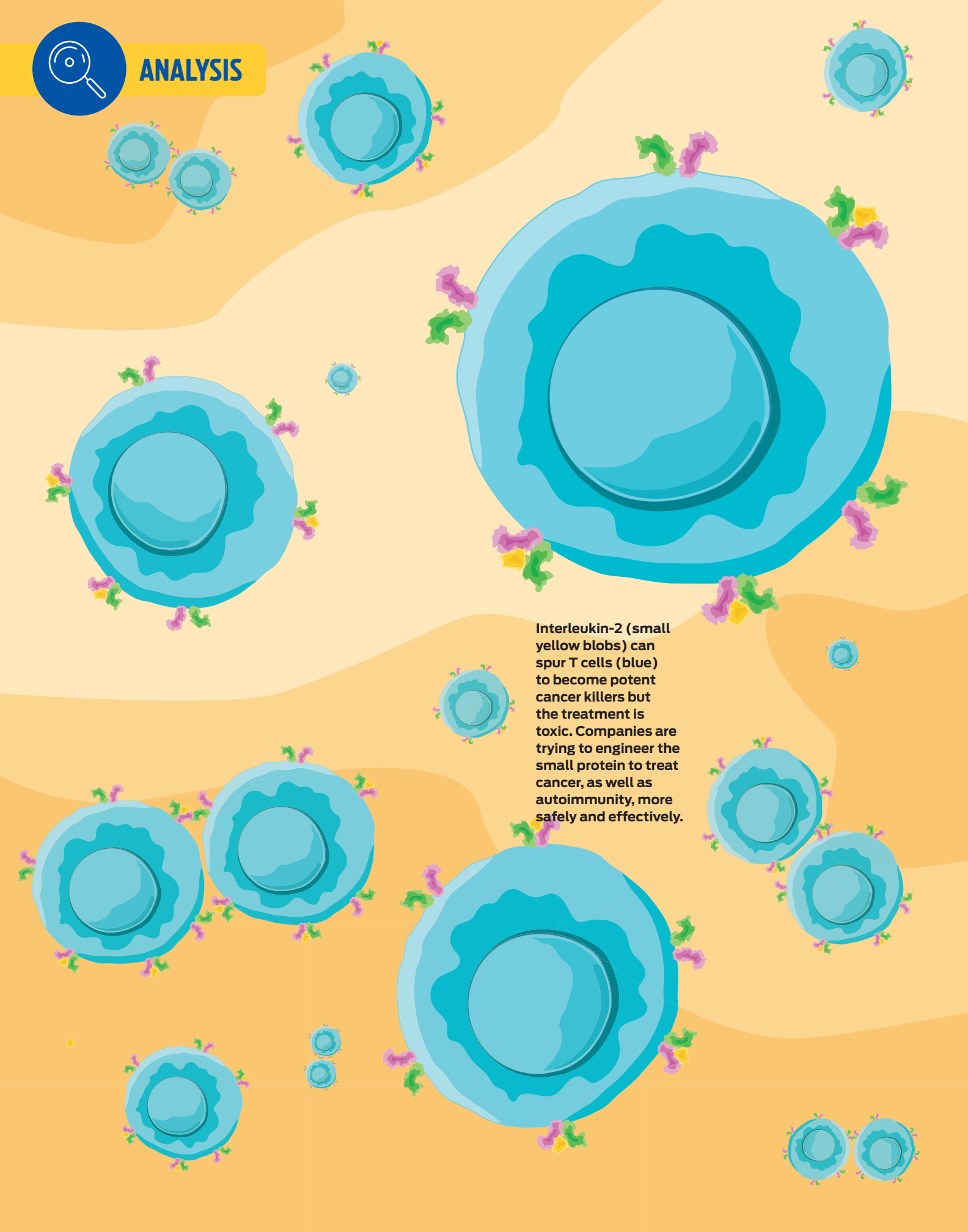
12.46%
Estimated proportion of cancer cases to respond to checkpoint inhibitor drugs in 2018.

1.9 million
People in the US expected to be diagnosed with cancer in 2022.

609,000
People in the US expected to die of cancer in 2022.

2,327
Active or recruiting clinical trials of immunotherapies globally as of Feb. 25, 2022.

71%
Proportion of all publications and patents related to cancer immunotherapy originating from the five most active countries (in descending order): the US, China, Germany, Japan, and Italy.



Interleukin-2 (small yellow blobs) can spur T cells (blue) to become potent cancer killers but the treatment is toxic. Companies are trying to engineer the small protein to treat cancer, as well as autoimmunity, more safely and effectively.

A smarter, safer IL-2

MEGHA SATYANARAYANA, C&EN STAFF

In his first years as an oncologist, Jonathan Drachman treated people with kidney cancer and melanoma. There were few treatment options for them in the 1990s, he says, and cancer immunotherapy—the manipulation of a person's immune system to kill off cancer cells—was still new.

But there was a drug called Proleukin that, for better or worse, gripped his attention. Proleukin, also called aldesleukin, was a therapeutic version of interleukin-2 (IL-2), a small human protein called a cytokine that could attach to specific kinds of immune cells and stimulate them to grow and divide. Some of these immune cells could kill foreign invaders, including cancer cells.

Proleukin was an early cancer immunotherapy, and when it worked, Drachman says, it worked well. About 15% of people treated went into remission, he says, and of those, up to 8% survived for years, even decades. Drachman, now the CEO of Neoleukin Therapeutics, a company developing an IL-2-like therapy, says he couldn't have been more excited.

"It was one of the first immunotherapies that actually worked in anyone," he says.

But there was a catch. Proleukin could be dangerous. At a time when many cancer treatments could be taken without checking into the hospital, Drachman says, a person on Proleukin had to be admitted to the cancer ward and, almost always, the intensive care unit. With a half-life so short that it started disappearing almost as soon as the infusion was over, the drug had to be given at high doses every few hours, for days to be effective.

For many people, that high dose induced shakes and chills as their bodies responded to the cytokine, Drachman says. Many developed vascular leak syndrome: their blood vessels leaked fluid, their bodies swelled, their blood pressures dropped quickly, and they became confused or lost consciousness altogether. Nearly 75% of patients treated with Proleukin eventually developed antibodies to the drug, raising fears that the antibodies would decrease efficacy. Many people couldn't tolerate the treatment.

For every person seemingly cured, Drachman says, there were as many who died of their cancers.

But Drachman kept thinking about those 15% of people who went into remission. He and others wondered how many more people IL-2 could successfully treat if researchers could figure out how to engineer out the toxicity. It would be years before technology would catch up—before biology, computing, and protein chemistry were advanced enough to build a better IL-2.

These new IL-2s have been attracting the drug industry's attention, and companies have inked a flurry of six- and seven-figure deals over the past few years to develop the molecules for the clinic. Many of these molecules aim to rev up the immune system's cancer response. Others aim to calm the overactive response at the root of autoimmune diseases. But none of these tweaks is a sure shot. A few clinical trials of IL-2 drug candidates have failed or been canceled.

The IL-2 drug candidates under investigation are new proteins that don't exist in nature. Some are molecules that resemble IL-2 but have amino acid changes that strengthen some actions and weaken others. Others are IL-2s with antibodies and other chemical compounds attached that thwart some immune interactions and facilitate others.

CREDIT: YANG H. KU/C&EN/SHUTTERSTOCK

All are trying to solve therapeutic IL-2's fundamental problems—a short half-life that requires toxic dosing levels, nonspecific binding that stalls efficacy and causes side effects, and a tendency to spur the production of antibodies that could affect the efficacy of the drug or native IL-2.

Some scientists say the clinical trials are as much a test of the technologies that have gone into these engineered IL-2s as they are of the candidates' abilities to successfully treat cancer or autoimmunity.

Traditional IL-2 works, says Giovanni Abbadessa, an oncologist who leads early clinical development of cancer immunotherapies at Sanofi, which paid $2.5 billion for Synthorx, a biotechnology firm developing an engineered IL-2. But the cytokine could work better, he says. The question: Which of the many biochemical tweaks or chemical additions will overcome IL-2's limitations—without creating new ones?

"I think it's really fascinating to see how different companies have taken different technological strategies and approaches to find different ways to do the same thing," Abbadessa says. "And of course, I'm afraid only clinical trials—larger clinical trials—will tell us which one is the better way."

One molecule, two cell fates

About 45 years ago, while studying immune cells called T cells, researchers working with Robert Gallo at the US National Cancer Institute discovered a molecule that made T cells grow and divide. It was originally called T-cell growth factor and was eventually renamed IL-2.

A few years later, while trying to understand how IL-2 stimulates T cells to multiply, a team at the National Cancer Institute that included Warren Leonard, as well as a team led by eventual Nobel Prize winner Tasuku Honjo, discovered a molecule on the surface of T cells to which IL-2 binds.

But when researchers tried to re-create the effect of that binding, they couldn't. IL-2, bound to that molecule (eventually called α), wasn't enough to get T cells to multiply. It would take years and dozens of scientists to figure out that IL-2 stimulates immune cells in a complicated way, using a receptor that can have two prongs, β and γ, or three, α, β, and γ. It was eye opening that IL-2 uses a multiprong receptor, Leonard says, but it was even more revelatory when researchers later discovered that the way IL-2 attaches to the receptor is the source of its therapeutic potential.

"It was always in the back of our minds that this could be something of down-the-road therapeutic utility," Leonard says of understanding how IL-2 works. "But, at least at the time, we were driven by more basic-science considerations."

IL-2 stimulates many kinds of immune cells, including T cells and natural killer (NK) cells, says Jamie Spangler, a bioengineer at Johns Hopkins University who is developing antibody-coupled IL-2s as possible therapies. T cells can take on special tasks, including becoming effectors, which drive the immune response, or regulatory cells, restraining the immune response. Natural IL-2 stimulates both effector and regulatory T cells to multiply, providing an army that allows the immune system to do its job, but with checks and balances.

> **"It was always in the back of our minds that this could be something of down-the-road therapeutic utility."**

Indeed, providing checks and balances seems to be IL-2's most fundamental task, Spangler says. During the years of unraveling how the cytokine works came a surprising finding: mice missing the IL-2 gene can still mount an immune response; IL-2, it appears, has backups that can take the cytokine's place. But Spangler says, these mice have serious autoimmune problems.

It turns out that IL-2 binds very tightly to the α piece of the receptor, which is primarily found on the regulatory T cells, which dampen the immune response. And IL-2 binds less tightly to the β and γ pieces, which are found on both effector and regulatory T cells. So binding to all three parts of the receptor allows IL-2 to stimulate cells that reduce the immune response. Binding to only β and γ allows IL-2 to ramp up the response.

In those mice that can't make IL-2, other cytokines spur the multiplying of effector cells and the other immune cells which fight infection or cancer. But those same experiments suggest that IL-2 is critical in keeping the immune response in check.

What this means for IL-2 as a therapy is that building an IL-2-like compound that stimulates mainly effector cells to grow could spur a potent anticancer immune response, Spangler says. And building an artificial IL-2 to preferentially stimulate regulatory cells could create something that mainly fights autoimmunity.

"It's a fascinating molecule," she says of IL-2, "because it's sort of got this two-faced function."

But only in the past few years has the technology developed to allow scientists to take advantage of IL-2's two faces, Spangler says. "Up until maybe 10–15 years ago, we didn't have the tools and the wherewithal to actually hone the activities of IL-2 in a targeted way," she says. "I think that's really what's been a part of this whole renaissance."

Bulking up a skinny cytokine

Most of the industry effort to modify IL-2 focuses on developing it for cancer immunotherapy, says Nikolaos Sgourakis, a researcher at the University of Pennsylvania who studies the structure of cytokines. The scientists building IL-2 as a cancer agent are trying to make something that won't bind to the α piece of the receptor or are trying to boost IL-2's interaction with the β and γ pieces.

But in addition to building something that is specific to effector T cells and other cancer-killing immune cells, these scientists have to build something that is long lasting, has few side effects, and doesn't inadvertently prompt the antibody-making arm of the immune system to attack it.

Among the scientists working on ways to prevent IL-2 from binding to the α part of the receptor is Spangler. Her lab is developing therapeutic IL-2s that are attached to antibodies. One of these constructs blocks the ability of IL-2 to connect with

the α part of the receptor, keeping IL-2 from preferentially stimulating regulatory T cells to multiply and potentially spurring cancer-killing effector cells to multiply instead.

For several years, Roche has likewise sought to turn IL-2 into a therapeutic that can't bind to the α part of the IL-2 receptor. The company is doing this through muteins—versions of the IL-2 protein with altered, or mutated, amino acid sequences that change how the molecule interacts with its receptor. Roche calls its IL-2 mutein IL-2v, for IL-2 variant.

Pablo Umaña, who leads cancer immunotherapy projects at Roche, says the firm's scientists also tried coupling IL-2v to antibodies that attach to cancer cells that have small immune beacons called tumor antigens on their surfaces. The idea, he says, was to keep the company's IL-2 near the tumor so IL-2 could stimulate effector T cells.

The researchers ran into problems. One of their IL-2-antibody constructs became immunogenic—in trials, people given the drug developed antibodies against it. Another was well tolerated but didn't have the cancer-killing effect the company was looking for. The trials were stopped.

Now, Umaña says, Roche is working on an IL-2-antibody construct that attaches to two points on the same T cell: the β and γ parts of the IL-2 receptor as one point, and PD-l, an immune-regulating protein that many cancer immunotherapies attempt to override to make T cells more effective at killing, as the other. Umaña hopes the dual-action molecule, now in Phase 1 trials, will strengthen IL-2's ability to stimulate effector cells. The idea is that the antibody's binding to PD-l will create a strong interaction that helps solidify the weaker IL-2 interaction with its receptor on effector cells, urging those cells to divide.

"We call this our third-generation IL-2 variant," he says.

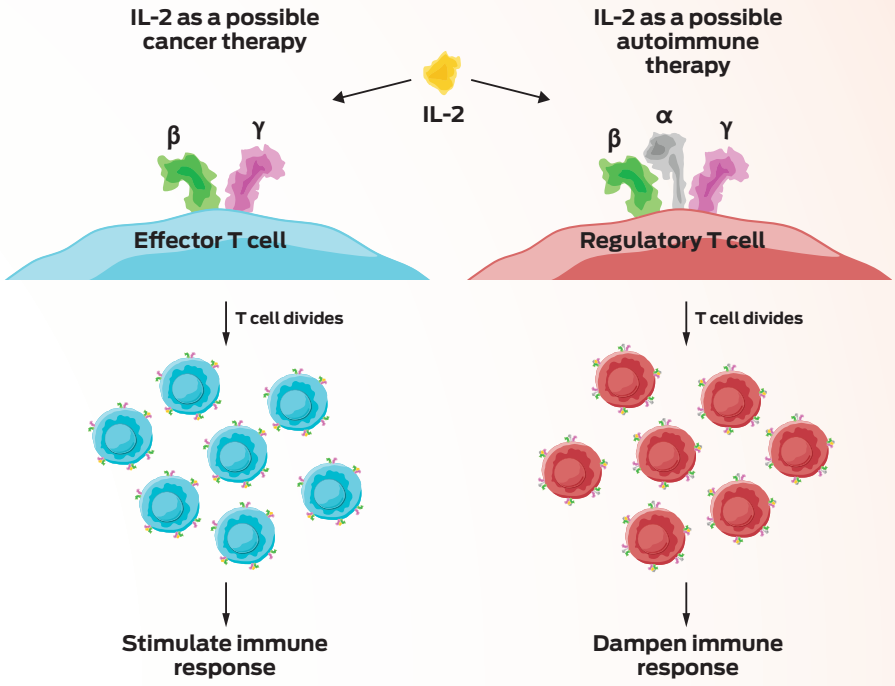
Companies are also trying ways to get IL-2 to stick around longer in the bloodstream so they can reduce the high doses that lead to toxicity and side effects. One strategy is to pegylate the molecule. Attaching polyethylene glycol (PEG) to specific points in the amino acid chain of IL-2 makes the construct bigger, lengthening its half-life, says Willem Overwijk, vice president of oncology research at Nektar Therapeutics.

Nektar's lead IL-2 candidate, bempegaldesleukin, or bempeg, features six strands of PEG attached to lysines on aldesleukin, the active ingredient in Proleukin.

Overwijk says the therapy is injectable and doesn't become active until some of the PEG chains fall off, freeing up the part of the protein that binds preferentially to the β and γ pieces on cancer-killing effector T cells and NK cells.

Good reception

Interleukin-2 (IL-2) is an immune system molecule called a cytokine that interacts with the α-, β-, and γ-receptor subunits on the surface of effector and regulatory T cells, causing the cells to multiply. Engineering IL-2 to interact with only effector T cells could make the cytokine a more potent cancer immunotherapy. Engineering IL-2 to interact with only regulatory T cells is a strategy some companies are taking to develop the cytokine as an autoimmune therapy.



Overwijk says people with tumors who have been treated with bempeg have many effector T cells around their tumors and few regulatory cells.

And unlike with Proleukin, people treated with bempeg are not likely to require hospitalization.

"That's a huge difference for the patient. They can go home basically right after; they don't have to stay in the hospital," Overwijk says. "It's a totally different drug now."

Bempeg is in testing as a stand-alone therapy and as a combination with other cancer immunotherapies. In general, the trials have been small, and Nektar has had to grapple with investors concerned about trial results as well as manufacturing issues that may have affected some early results. Nektar reports that in Phase 2 trials of bempeg combined with Opdivo, a checkpoint inhibitor from Bristol Myers Squibb, about one-third of the people in its 38-person cohort saw all their cancerous lesions disappear after treatment. Many of the trial participants lived 30 months after treatment started before their cancers started progressing again.

Unearthing unearthly IL-2s

Many researchers trying to bring modified IL-2s to the cancer clinic use aldesleukin, which only slightly varies from natural IL-2, as a starting point for mutations, or they couple natural IL-2 to another molecule.

Targeting IL-2

Several companies are trying to engineer the interleukin-2 (IL-2) protein to steer its activity toward T cells that kill cancer or T cells that block autoimmune responses. Here are some examples.

» **Technology:** IL-2 with mutations
» **Goal:** Change amino acids to strengthen or weaken interactions .between IL-2 and various combinations of its receptors
» **Companies:** Pandion Therapeutics (Merck & Co.), Roche

» **Technology:** IL-2 with nonnatural amino acids
» **Goal:** Strengthen or weaken interactions between IL-2 and various combinations of its receptors; provide anchors to add molecules to influence immune response
» **Companies:** Synthorx, Bright Peak Therapeutics

» **Technology:** IL-2 or IL-2 mutants coupled to antibodies
» **Goal:** Improve IL-2's half-life and steer immune response
» **Companies:** Pandion Therapeutics (Merck & Co.), Roche

» **Technology:** IL-2 or IL-2 mutants bound to polyethylene glycol (PEG)
» **Goal:** Improve IL-2's half-life by making the molecule inactive until PEG chains fall off
» **Company:** Nektar Therapeutics

» **Technology:** IL-2 mimics
» **Goal:** Re-create IL-2's function but without half-life and toxicity issues
» **Company:** Neoleukin Therapeutics

Sources: Companies. **Note:** This list is not exhaustive.

But several companies are exploring molecules that act like IL-2 but are completely artificial. Neoleukin's lead compound does what IL-2 is supposed to do to stimulate effector T cells, but it's smaller and more compact, Drachman says.

NL-201, Neoleukin's IL-2-mimicking compound, came out of the Institute for Protein Design at the University of Washington. Carl Walkey, an executive at Neoleukin who was previously at the institute, says the team used computational biology to envision what an ideal IL-2 would look like as a cancer treatment. Researchers sorted through an astronomical combination of amino acid sequences to understand how altering amino acids in the IL-2 molecule might affect its structure and function, Walkey says.

They then made versions of the best candidates and chose NL-201 for its activity. The molecule barely looks like natural IL-2, yet it binds to the β and γ parts of the IL-2 receptor with enough affinity to potentially stimulate effector T cells to divide and kill.

"IL-2 has these funny geometries. It bends and twists in funny ways" because it has so many different jobs across many cell types, Walkey says. NL-201 "is much more ideal. It's straighter. It's more compact."

NL-201 is also stabler than the native cytokine, he says; it has a rich hydrophobic center and a hydrophilic exterior that makes it soluble. It's an "idealized protein structure," Walkey says.

The US Food and Drug Administration seems to be moving carefully with what Walkey says is the first completely engineered, made-from-scratch protein to approach the clinic. In January 2021, FDA officials asked Neoleukin to make some changes to its clinical trial protocols to better measure dosages. The company dosed the first patient in its Phase 1 trial of NL-201 in May 2021.

For Sanofi, IL-2 immunotherapy dreams rest on the unnatural amino acid N_6-[(2-azidoethoxy)carbonyl]-L-lysine, or azidolysine. The novel amino acid was developed by Synthorx cofounder Floyd Romesberg and engineered into an IL-2 candidate called THOR-707. Sanofi now owns Synthorx and THOR-707. This unnatural lysine has a PEG attached to it that blocks the molecule from interacting with the α part of the IL-2 receptor. THOR-707 is in early trials in people with a wide range of tumors.

"I see it as a cleaner approach to try and have a selective drug," Abbadessa says, comparing it with other modes of engineering IL-2. "We hope that it will work."

Then there's Bright Peak Therapeutics, which has taken protein building completely out of cells. Rather than coaxing bacterial or animal cells to spit out modified IL-2s, which is the norm, Bright Peak's IL-2-like molecule is made by chemically stitching together small peptides.

Taking the protein-assembly process out of cells allows the company's scientists to better understand the growing amino acid chain as it's assembling and folding, says CEO Sef Kurstjens. Company scientists can more easily incorporate things like noncanonical amino acids.

Vijaya Pattabiraman, Bright Peak's cofounder and an organic chemist, says the company's chemists can stitch together a peptide and modify an amino acid on that peptide to improve its half-life. Researchers can build the next section of the protein and, if needed, modify it.

The scientists can then join these pieces with all the different elements they want to build their perfect molecule, and once it's folded, the scientists can add molecules like PEG, if that's what their design calls for. This process doesn't require microbes or cells that have to determine how to add an artificial amino acid.

"It can be anything that chemists and biologists can think about and dream of," Pattabiraman says of a molecule built using Bright Peak's technology. "There's no other way you can really introduce multiple noncanonical amino acids within the protein and get the biology that we want."

CREDIT: YANG H. KU/C&EN

Steering IL-2 toward autoimmunity

For all of IL-2's potential as a cancer therapeutic, Hopkins's Spangler says we shouldn't forget its potential as a strong suppressor of the immune response. Because IL-2 binds so strongly to the α part of the receptor, low doses can trigger the multiplication of regulatory T cells, which dampen the immune response, making the cytokine an enticing drug candidate for autoimmune disorders. She is developing an antibody-coupled IL-2 to skew toward activating regulatory T cells.

But modulating IL-2 to activate regulatory T cells is a more nuanced task than treating cancer, Penn's Sgourakis says. T cells aren't the only cells that express the α part of the IL-2 receptor. Any effort to boost the activity of regulatory T cells could also stimulate nontarget cells. And, he says, effector cells can start producing that α segment, scooping up a therapeutic IL-2 meant to tamp down the immune system.

As IL-2 and its receptor move in space, he says, there are fleeting conformations—shapes that last just an instant but could mediate some of the most important interactions IL-2 might have with a molecule that affects the immune response. Using nuclear magnetic resonance, Sgourakis's team is trying to capture the fleeting moments to design mutants to mimic those interactions, including ones that could lead to IL-2-based autoimmune therapies.

"You really have a much more narrow set of conformations, or of IL-2 forms that could be potentially useful for an autoimmune disease treatment," he says. "Trying to make an IL-2 that targets cancer immunotherapies is like hitting a free throw. Trying to make a molecule that targets autoimmunity or dampens a certain set of immunity is like trying to hit a half-court shot."

Still, a few companies are trying. This includes Amgen, which is developing its own IL-2 products, and Merck & Co., which in 2021 bought Pandion Therapeutics for $1.85 billion. Pandion's pipeline includes two IL-2-based molecules, plus the technology to deliver those molecules and others to specific tissues.

Pandion cofounder Jo Viney says turning therapeutic IL-2 toward autoimmunity should require lower doses than used in cancer treatment and should thus lower the risk of side effects. But using existing IL-2 is still daunting, she says. While a person with cancer would take IL-2 therapy for about a week, autoimmune diseases are chronic, and IL-2 might have to be given over long periods. IL-2 could activate other immune cells, which could counteract the autoimmune effort. And with a short half-life, IL-2 would be an almost daily treatment, she says.

On the plus side, the use of IL-2 therapies for autoimmunity could unleash a more comprehensive shutdown signal on an overreacting immune system, she says; in contrast, current autoimmune therapies tend to be molecules and antibodies that target only one component of an autoimmune response.

> **"The clinical trials are as much a test of the technologies that have gone into these engineered IL-2s as they are of the candidates' abilities to successfully treat cancer or autoimmunity."**

Pandion and Amgen are using similar technology to build autoimmune-specific IL-2: coupling IL-2 muteins to antibody fragments that extend IL-2's circulation time.

Making Pandion's candidate, PT101, involved testing many amino acid mutations in the native IL-2 protein, Viney says. The firm's scientists found that acidifying a critical asparagine destabilized IL-2's binding to the β piece of the receptor. Adding four other amino acids to the IL-2 mutein created a molecule that would preferentially bind to the α piece of the IL-2 receptor, favoring the growth of regulatory T cells.

Viney says that because Pandion's IL-2 is coupled to the antibody fragment, the therapy could be given to people every few weeks instead of every day. In a small Phase 1 trial, people who received PT101 produced many more regulatory T cells than people who were untreated. The experimental biologic seemed to produce few side effects and no antidrug antibodies. The candidate, renamed MK-6194, is in a Phase 1 trial for ulcerative colitis.

Amgen's lead IL-2 construct, efavaleukin alfa, is in testing for lupus and graft-versus-host disease. The company ended a trial of the drug candidate—a fusion of a mutein IL-2 and an antibody—as a "business decision," according to ClinicalTrials.gov. Nektar is also exploring pegylated IL-2 as an autoimmune therapy.

While people wait to see which, if any, of these novel IL-2s will become the next generation of IL-2 immunotherapy, Neoleukin's Drachman says Proleukin is still in occasional use as a late-stage therapy for people who have not responded to other treatments. He says he recently spoke to a man with melanoma whose doctors pulled him off Proleukin after his heart stopped during treatment. He went on to receive checkpoint inhibitors, immunotherapies that block some of the regulatory functions of the immune system, and has survived for several years.

He is one of the lucky ones. Even with checkpoint inhibitors, Drachman says, fewer than half of people with melanoma go into stable remission. Melanoma is a tough cancer to treat. It's resistant to many drugs, Drachman says, but not IL-2. This is why developing a safer version is so important, he says.

Sgourakis and others believe that the technologies in play in the effort to create a better IL-2 may also lead to other, more tolerable and potent cytokines and immune modulators to treat diseases beyond cancer and autoimmunity. What structural and computational biology reveal could guide scientists to molecules that affect a host of immune functions.

It's an enticing possibility, Sgourakis says. "This field is coming of age."

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We choose 20 promising companies generating innovative immunotherapies

4D pharma plc

- **4D Pharma**
- 4dpharmaplc.com
- **Based:** Leeds, England
- **Launched:** 2014
- **Money raised in start-up funding rounds:** $53.9 million
- **Publicly traded:** Yes, IPO 2021
- **Key partnerships:** MD Anderson Cancer Center, Merck & Co., Merck KGaA, Pfizer
- **Strategy:** 4D Pharma is testing microbiome therapies in patients in combination with checkpoint inhibitors, which take the brakes off the immune response. The working hypothesis is that the gut bugs will help more people respond to the immunotherapy.
- **Why watch:** The firm published preclinical studies in cells in 2021 suggesting that a microbial therapy improves activity of chimeric antigen receptor (CAR) T-cell therapy.

affini-T

- **Affini-T Therapeutics**
- affinittx.com
- **Based:** Watertown, Massachusetts
- **Launched:** 2022
- **Money raised in start-up funding rounds:** Not disclosed
- **Publicly traded:** No
- **Key partnerships:** Not disclosed
- **Strategy:** Affini-T's platform discovers T-cell receptors that recognize tumor cells with certain cancer-associated mutations in key proteins KRAS or P53. Enhancing patients' own T cells with these receptors plus a coreceptor and then infusing the cells back into patients has the potential to shrink pancreatic, colorectal, and lung tumors.
- **Why watch:** Scientific cofounder Philip Greenberg previously cofounded Juno Therapeutics. Juno developed the CAR-T therapy lisocabtagene maraleucel (Breyanzi). Celgene acquired Juno for $9 billion in 2018.

ARCELLX

- **Arcellx**
- arcellx.com
- **Based:** Gaithersburg, Maryland
- **Launched:** 2014
- **Money raised in start-up funding rounds:** $200 million
- **Publicly traded:** Yes, IPO 2022
- **Key partnerships:** Oxford BioMedica
- **Strategy:** Arcellx aims to quell CAR T cells' toxicity and make them useful for a broader range of cancers by replacing the biological antigen-binding domain on the cells' surface with a synthetic binding scaffold.
- **Why watch:** Arcellx reported positive preliminary results in a Phase 1 clinical trial in people with multiple myeloma that has returned or stopped responding to treatments.

AVENGE BIO

- **Avenge Bio**
- avengebio.com
- **Based:** Natick, Massachusetts
- **Launched:** 2019
- **Money raised in start-up funding rounds:** $45 million
- **Publicly traded:** No
- **Key partnerships:** Not disclosed
- **Strategy:** Avenge engineers cells from human donors to produce immunotherapies such as interleukin-2 (IL-2). It then encapsulates the cells in a polymer that controls dosing. By implanting the cells close to ovarian tumors, the company aims to avoid the toxicity that occurs when IL-2 circulates throughout the body (see page 9).
- **Why watch:** The company has plans to take on lung and breast cancers as well.

CALVIRI

- **Calviri**
- calviri.com
- **Based:** Phoenix
- **Launched: 2018**
- **Money raised in start-up funding rounds:** $9.3 million
- **Publicly traded:** No
- **Key partnerships:** Arizona State University
- **Strategy:** Calviri is testing in 800 dogs its vaccine for preventing multiple cancers. The vaccine is designed to provoke an immune response to peptides that arise when tumors, in their rush to grow, mistranslate messenger RNA.

» **Why watch:** The company says that a successful dog vaccine could augur success for people and that its approach may also yield cancer diagnostics.

» **Candel Therapeutics**
» candeltx.com
» **Based:** Needham, Massachusetts
» **Launched:** 1999
» **Money raised in start-up funding rounds:** $139.9 million
» **Publicly traded:** Yes, IPO 2021
» **Key partnerships:** Bionaut Labs, Partnership for Accelerating Cancer Therapies
» **Strategy:** Candel engineers viruses to infect and break down cancer cells selectively. Its leading virus candidate encodes the enzyme thymidine kinase. Patients swallow an inexpensive prodrug pill and receive an injection of the virus at the site of their tumor. The virus co-opts tumor cells' systems to synthesize the enzyme, which converts the prodrug to a toxic nucleotide that kills the cells.
» **Why watch:** Candel's Phase 3 clinical trial in over 700 people with prostate cancer is expected to yield final data in 2024.

» **CARsgen Therapeutics**
» carsgen.com
» **Based:** Shanghai
» **Launched:** 2014
» **Money raised in start-up funding rounds:** $276 million
» **Publicly traded:** Yes, IPO 2021
» **Key partnerships:** Shanghai Cancer Institute
» **Strategy:** CARSgen's lead product candidate is an enhanced set of a person's own T cells that recognizes B-cell maturation antigen, a protein that is overexpressed in the malignant cells that cause multiple myeloma. The candidate is in clinical trials in China and North America.
» **Why watch:** CARSgen is developing next-generation CAR T cells expressing both a cytokine and a chemokine. It says the approach could address solid tumors, which have proved challenging to treat with CAR-T therapy.

» **Compugen**
» cgen.com
» **Based:** Holon, Israel
» **Launched:** 1993
» **Money raised in start-up funding rounds:** $67.4 million
» **Publicly traded:** Yes, IPO 2000
» **Key partnerships:** AstraZeneca, Bayer, Bristol Myers Squibb, Johns Hopkins University
» **Strategy:** Compugen analyzes vast data sets to discover new immune checkpoints, which suppress the body's ability to fight cancer. Its checkpoint-countering antibodies are in clinical trials both alone and in combination with other treatments.
» **Why watch:** In December 2021, the company released biopsy data from a handful of patients suggesting that its lead candidate increases levels of certain T cells and markers of immune activation in the normal cells that surround and feed tumors.

» **Dragonfly Therapeutics**
» dragonflytx.com
» **Based:** Waltham, Massachusetts
» **Launched:** 2015
» **Money raised in start-up funding rounds:** Over $300 million
» **Publicly traded:** No
» **Key partnerships:** AbbVie, Bristol Myers Squibb, MD Anderson Cancer Center, Merck & Co.
» **Strategy:** Dragonfly's antibodies bridge proteins on the surfaces of cancer cells and of white blood cells called natural killer (NK) cells. The proximity stimulates the NK cells to destroy the cancer and sound the alarm to the rest of the immune system.
» **Why watch:** In fall 2021, both Merck and BMS opted to license additional therapies from Dragonfly's portfolio.

» **Harpoon Therapeutics**
» harpoontx.com
» **Based:** South San Francisco, California
» **Launched: 2015**
» **Money raised in start-up funding rounds:** $130 million
» **Publicly traded:** Yes, IPO 2019
» **Key partnerships:** AbbVie, AGC Biologics, Werewolf Therapeutics
» **Strategy:** Harpoon is conducting clinical trials of its polypeptides, which link target cancer cells, T cells, and human serum albumin to induce tumor killing. The company says the constructs' small size relative to comparable therapies extends half-life, increases tumor penetration, and simplifies manufacturing.
» **Why watch:** The firm plans to seek permission to test a prodrug version of its technology in people by the end of 2022.

» **iTeos Therapeutics**
» iteostherapeutics.com
» **Based:** Cambridge, Massachusetts
» **Launched:** 2011
» **Money raised in start-up funding rounds:** $249.7 million
» **Publicly traded:** Yes, IPO 2020
» **Key partnerships:** GlaxoSmithKline, Merck & Co.
» **Strategy:** iTeos focuses on two obstacles to an effective immune response to cancer: adenosine, an immunosuppressive metabolite common just outside tumors, and T cell immunoreceptor with Ig and ITIM domains (TIGIT), a receptor that limits antitumor responses.
» **Why watch:** iTeos received $625 million from GSK, with the potential for up to $1.45 billion in milestone payments, to cocommercialize its anti-TIGIT antibody.

» **NextCure**
» nextcure.com
» **Based:** Beltsville, Maryland
» **Launched:** 2015
» **Money raised in start-up funding rounds:** $310 million
» **Publicly traded:** Yes, IPO 2019
» **Key partnerships:** Emory University, US National Cancer Institute, Vanderbilt University, Yale University
» **Strategy:** NextCure screens cell lines and donor cells to unearth cell-surface interactions that drive immunity. The company then manipulates the interactions with biologic drugs to enhance tumor killing.
» **Why watch:** NextCure is conducting clinical trials of an antibody that targets Siglec-15, a cell-surface protein that binds sugars rather than a protein partner and that is emblematic of a new wave of glycoscience drug discovery for cancer.

» **Palleon Pharmaceuticals**
» palleonpharma.com
» **Based:** Waltham, Massachusetts
» **Launched:** 2016
» **Money raised in start-up funding rounds:** $147.6 million
» **Publicly traded:** No
» **Key partnerships:** King's College London
» **Strategy:** Palleon unleashes the immune system on cancers by snipping away sugars called sialic acids that coat tumor cells and poorly functioning T cells (see page 6). Pairing a sugar-trimming enzyme with an antibody that binds key proteins on tumors, such as the HER2 protein in breast cancer, keeps the treatment from targeting normal cells.
» **Why watch:** On March 8, Palleon announced that it had dosed the first patient in a clinical trial of its lead compound, a bisialidase.

» **PDC Line Pharma**
» pdc-line-pharma.com
» **Based:** La Tronche, France
» **Launched:** 2014
» **Money raised in start-up funding rounds:** $47.1 million
» **Publicly traded:** No
» **Key partnerships:** French Blood Establishment, Grenoble Alpes University Hospital, LG Chem
» **Strategy:** PDC Line develops vaccines with the goal of revving up the immune system against existing cancers. The company derives a line of dendritic cells—which patrol the body for invaders—from human leukemia cells, then loads them with peptides from a target cancer, such as lung cancer.
» **Why watch:** The firm says its product is easier to manufacture than established cancer vaccine therapies that require harvesting a patient's own cells.

» **Pionyr Immunotherapeutics**
» pionyrtx.com
» **Based:** South San Francisco, California
» **Launched:** 2015
» **Money raised in start-up funding rounds:** $352 million
» **Publicly traded:** No
» **Key partnerships:** Abcam, Celonic, Gilead Sciences, Lonza, ProBioGen
» **Strategy:** Pionyr is conducting clinical trials of two antibodies: one that removes and one that reprograms immune-suppressive cells in the environment that surrounds and nourishes tumors. Both approaches enable the immune system to fight cancer.
» **Why watch:** Pionyr cofounder Max Krummel coinvented ipilimumab (Yervoy), the first checkpoint inhibitor approved by the FDA.

» **Tmunity Therapeutics**
» tmunity.com
» **Based:** Philadelphia
» **Launched:** 2015
» **Money raised in start-up funding rounds:** $230 million
» **Publicly traded:** No
» **Key partnerships:** Parker Institute for Cancer Immunotherapy; University of California, San Francisco; University of Pennsylvania
» **Strategy:** Tmunity's founders discovered tisagenlecleucel (Kymriah), the first CAR T-cell therapy to be approved by the FDA, but that therapy is approved only for blood-based cancers. The company's T cell pipeline is aimed at solid tumors such as lung or ovarian.
» **Why watch:** Tmunity stopped clinical trials of its leading candidate in 2021 because two patients died. It is warning the field about the neurotoxicity it encountered and working to develop a safer version of the therapy.

» **Turnstone Biologics**
» turnstonebio.com
» **Based:** New York City
» **Launched:** 2015
» **Money raised in start-up funding rounds:** $132.7 million
» **Publicly traded:** No
» **Key partnerships:** AbbVie, Moffitt Cancer Center, Takeda Pharmaceutical
» **Strategy:** Turnstone's lead candidate is a one-two-punch virus that not only infects and breaks down cancer cells but encodes a trio of immune-boosting proteins designed to make tumors' environs less hospitable.
» **Why watch:** In 2022, the company plans to test a complementary technology: harvesting immune cells called lymphocytes from tumors, enriching the most relevant cells for tumor killing, and delivering those back to the patients.

» **Werewolf Therapeutics**
» werewolftx.com
» **Based:** Cambridge, Massachusetts
» **Launched:** 2017
» **Money raised in start-up funding rounds:** $128.2 million
» **Publicly traded:** Yes, IPO 2021
» **Key partnerships:** Harpoon Therapeutics, Lonza, Merck & Co., Patheon by Thermo Fisher Scientific
» **Strategy:** Werewolf creates cloaked cytokines, proteins that stimulate the immune system. They transform to their active state only when they approach tumors. This tactic minimizes cytokines' side effects, the company says.
» **Why watch:** Werewolf plans to seek permission in the first half of 2022 to initiate clinical trials of its conditionally activated IL-2 (see page 9) and IL-12.

» **Wugen**
» wugen.com
» **Based:** St. Louis
» **Launched:** 2018
» **Money raised in start-up funding rounds:** $172 million
» **Publicly traded:** No
» **Key partnerships:** Alpha Biopharma, HCW Biologics, Washington University in St. Louis
» **Strategy:** Wugen aims to counteract the low cancer-killing activity typical of stand-alone NK-cell treatments. It engineers healthy donor cells that "remember" previous invaders to ignore inhibitory signals and release more tumor-killing molecules.
» **Why watch:** The company plans to recruit patients this year for clinical trials of a therapy based on CAR-T cells, its other main focus.

» **Xilio Therapeutics**
» xiliotx.com
» **Based:** Waltham, Massachusetts
» **Launched:** 2016
» **Money raised in start-up funding rounds:** $233 million
» **Publicly traded: Yes, IPO 2021**
» **Key partnerships:** Applied BioMath, Merck & Co.
» **Strategy:** Xilio masks immunotherapies with proteins to keep them in check until they reach the normal cells that surround and feed a tumor. Once there, enzymes called matrix metalloproteinases release the drugs in active form.
» **Why watch:** As of January 2022, the company has both a checkpoint inhibitor and an IL-2 cytokine (see page 9) in human clinical trials.

Note: Companies were included because of the novelty and potential of their methods, amount of capital raised, number of partnerships, and number and identity of investors.

Sources: Crunchbase (accessed January 2022), company websites, news reports.

Lingyin Li: Sounding the immune system's alarm to fight cancer

LAURA HOWES, C&EN STAFF

When chemist Lingyin Li moved to Tim Mitchison's lab at Harvard Medical School in 2010 for her postdoctoral studies, she wanted to learn some biology. What she didn't want was to get stuck on a project that was going nowhere. So when Mitchison suggested she look at why a drug called DMXAA had cured cancer in mice but showed no effect in multiple studies in people with lung cancer, she pushed back.

DMXAA, which the big pharma firm Novartis had tested in many cancer trials, was purported to target the stimulator of interferon genes (STING) pathway. STING is part of the body's innate immune system—the body's generalist, first line of defense response to infections. When activated, STING ramps up inflammation. The inflammatory proteins activated by STING then start the more specialized adaptive immune system. Together, these two parts of the immune system work to eliminate pathogens, and drug companies hoped they could also be used to fight cancer.

Angarus Therapeutics cofounder Lingyin Li

Within a few months, Li found that DMXAA binds mouse, but not human, STING. It was the drug, not the target, that was the problem, and researchers went back to find new molecules.

In Mitchison's lab, Li began to piece together the complex workings of a signaling molecule called cyclic guanosine monophosphate–adenosine monophosphate (cGAMP). Although cGAMP is made inside cells for the express purpose of activating STING, it is broken apart by an enzyme found outside cells.

Scientists waved away that enzymatic mystery, but Li wondered if it was something more. Could this signaling molecule have a role outside of cells? She took that puzzle with her when she moved to Stanford in 2015 to set up her own lab.

Since then, Li's lab has focused on exploring cGAMP's relationship with STING. The lab has a growing body of research demonstrating that extracellular cGAMP is real.

In 2020, Li showed that while cGAMP is continually created inside stressed cells, it doesn't just stay there. A specific transporter pulls the signaling molecule out of cancer cells. The enzyme Li found during her postdoc, ENPP1, usually then breaks down cGAMP. But if that enzyme is blocked, levels of the signaling molecule rise and then spread to neighboring cells, in turn ringing the alarm for the immune system. Li thinks that inhibiting ENPP1 could be exploited as a cancer treatment. She is a cofounder of Angarus Therapeutics, a start-up aiming to commercialize that strategy.

Li says she would not have got where she is today without mentorship and support from her family back in China and from scientific mentors. "Now I'm where I am," she says, "and I realized, all these people paved the way."

Angarus Therapeutics at a glance

- **Launched**: 2018
- **Based:** Sunnyvale, California
- **Strategy:** Target ENPP1 (ecto-nucleotide pyrophosphatase I), a checkpoint of the innate immune STING pathway, to treat cancers
- **Funding:** $11.3 million

SOURCES: Crunchbase, Pitchbook, company website, news reports.

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CREDIT: LINGYIN LI/STANFORD

Cancer immunotherapy gets fiber boost

ALLA KATSNELSON, SPECIAL TO C&EN

A new study in people and mice reports that a high-fiber diet ratchets up the tumor-fighting power of immunotherapy drugs called checkpoint inhibitors in people undergoing treatment for melanoma (*Science* 2021, DOI: 10.1126/science.aaz7015). Common, commercially available probiotics do not help, the study found.

Checkpoint inhibitors bind to particular proteins and unleash the ability of T cells, a type of immune cell, to kill tumor cells. But these drugs work in only a few types of cancer, including melanoma, and even then for a minority of patients—and researchers don't know why.

Recent studies suggest that their efficacy may be linked to the microbiome. A few years ago, Carrie Daniel-MacDougall, an epidemiologist at MD Anderson Cancer Center, and her colleagues found that people with higher numbers of the Ruminococcaceae family of bacteria in their guts responded better to these drugs when being treated for melanoma (*Science* 2017, DOI: 10.1126/science.aan4236). The researchers set out to track how diet and over-the-counter probiotics affect Ruminococcaceae levels and checkpoint inhibitor therapy in people with late-stage melanoma. Of the 128 study participants who provided information about their diets, people who consumed more than 20 g of fiber per day and who did not take probiotics "had a markedly better response rate than any other patient scenario," Daniel-MacDougall says. Probiotics didn't show a clear effect, she adds, but the wide range of different probiotics people consumed likely muddied the picture.

Parallel studies in mice showed that those fed a high-fiber diet had higher levels of T cells and fended off melanoma tumors with the help of these drugs significantly better than mice fed a low-fiber diet. Dietary fiber normally modulates *Ruminococcaceae* bacteria, and the results suggest it also does so in the context of checkpoint inhibitors, Daniel-MacDougall says.

The study shows a clear link between fiber intake and immune response in the tumor, according to Jason Locasale, a cancer biologist at Duke University who wasn't involved in the work. A better understanding of how exactly fiber boosts T-cell response may help extend the finding to other types of cancers, he says.

A high-fiber diet may improve performance of a checkpoint inhibitor against melanoma.

Alla Katsnelson is a freelance writer based in Massachusetts.

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Of the 128 study participants who provided information about their diets, people who consumed more than 20 g of fiber per day and who did not take probiotics "had a markedly better response rate than any other patient scenario."

CREDIT: SHUTTERSTOCK

Engineered bacteria reduce tumors in mice

MEGHA SATYANARAYANA, C&EN STAFF

A team of scientists has engineered bacteria to deliver cancer treatments that rev up immune cells directly to tumors.

In studies in mice, the team, led by Tal Danino and Nicholas Arpaia of Columbia University, found that the bacterial shuttles can shrink tumors with just one injection and that treating one tumor stimulates the immune system to kill distant tumors (*Sci. Transl. Med.* 2020, DOI: 10.1126/scitranslmed.aax0876).

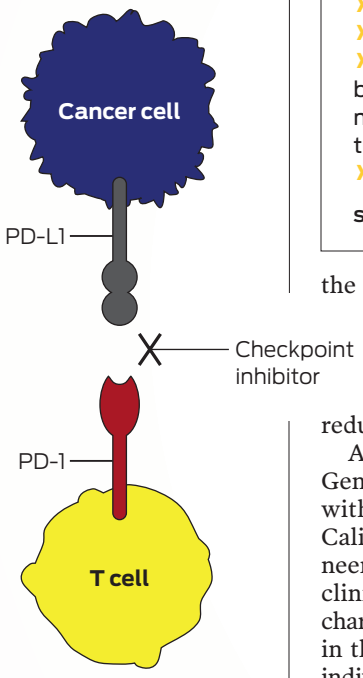
"The beauty of this approach is that the mechanism by which these agents work is not necessarily by directly killing the tumor," says Dmitriy Zamarin, an oncologist at Memorial Sloan Kettering Cancer Center who studies viruses as cancer therapeutics but was not involved in the this study. "It's really through the activation of the tumor-specific immune response." Zamarin thinks the systemic immune response makes the work promising.

To create the new anticancer system, the research team engineered probiotic *Escherichia coli* Nissle 1917 to produce nanobodies—segments of antibodies—that block a molecule called PD-L1 on cancer cells or CTLA-4 on immune cells. These are checkpoint molecules, and blocking them overrides a signal that cancer cells produce to tamp down the immune response. By blocking PD-L1 and CTLA-4, the nanobodies make immune cells better cancer killers.

To release the nanobodies, the researchers integrated a genetic circuit that prompts the bacteria to self-destruct when they reach a critical concentration inside a tumor, according to graduate student Candice Gurbatri, who led the development of the system. Surviving cells can grow and self-destruct again, continuing the treatment, she says.

The team injected bacteria with the PD-L1 or CTLA-4 blocker into mice with lymphoma. The tumors shrank, and the bacteria stayed within the tumor site. In mice with colorectal cancer, considered more resistant to immunotherapies, adding bacteria that released an immune stimulator called GM-CSF helped reduce tumor size.

Arpaia is on the advisory board of GenCirq, a company Danino cofounded with collaborators at the University of California, San Diego, to translate engineered bacterial cancer therapies to the clinic. He says the team needs to better characterize the self-destruct mechanism in the bacteria and determine whether individual bacteria can deliver more than one drug. He says the team is hopeful that some tweaks will make bacterial cargo ships a successful way to trigger the immune system to attack cancer.



Checkpoint inhibitors block the interactions between cancer cells and immune T cells that tell the immune cells to stand down. Checkpoint inhibitor targets include PD-L1 (shown) and CTLA-4 (not shown).

GenCirq at a glance

- **Launched:** 2015
- **Based:** San Diego
- **Strategy:** Self-destructing bacteria shuttle immune-modulating cancer treatments to tumors
- **Funding:** Over $10 million

SOURCES: GenCirq, LinkedIn, SBIR.gov.

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"The beauty of this approach is that the mechanism by which these agents work is not necessarily by directly killing the tumor."

READING LIST

Our picks of the patent and journal literature on cancer immunotherapy

2022

» Cook, Joselle, Kah Whye Peng, Thomas E. Witzig, Stephen M. Broski, Jose Caetano Villasboas, Jonas Paludo, Mrinal M. Patnaik, et al. **"Clinical Activity of Single Dose Systemic Oncolytic VSV Virotherapy in Patients with Relapsed Refractory T-Cell Lymphoma."** *Blood Adv.* (Feb. 17, 2022). https://doi.org/10.1182/bloodadvances.2021006631.

» Gardner, Thomas J., J. Peter Lee, Christopher M. Bourne, Dinali Wijewarnasuriya, Nihar Kinarivala, Keifer G. Kurtz, Broderick C. Corless, et al. **"Engineering CAR-T Cells to Activate Small-Molecule Drugs in Situ."** *Nat. Chem. Biol.* 18, no. 2 (February 2022): 216–25. https://doi.org/10.1038/s41589-021-00932-1.

» Palmer, Adam C., Benjamin Izar, Haeun Hwangbo, and Peter K. Sorger. **"Predictable Clinical Benefits without Evidence of Synergy in Trials of Combination Therapies with Immune-Checkpoint Inhibitors."** *Clin. Cancer Res.* 28, no. 2 (Jan. 15, 2022): 368–77. https://doi.org/10.1158/1078-0432.CCR-21-2275.

» Combes, Alexis J., Bushra Samad, Jessica Tsui, Nayvin W. Chew, Peter Yan, Gabriella C. Reeder, Divyashree Kushnoor, et al. **"Discovering Dominant Tumor Immune Archetypes in a Pan-Cancer Census."** *Cell* 185, no. 1 (Jan. 6, 2022): 184–203. https://doi.org/10.1016/j.cell.2021.12.004.

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» Hu, Quanyin, Hongjun Li, Edikan Archibong, Qian Chen, Huitong Ruan, Sarah Ahn, Elena Dukhovlinova, et al. **"Inhibition of Post-Surgery Tumour Recurrence via a Hydrogel Releasing CAR-T Cells and Anti-PDL1-Conjugated Platelets."** *Nat. Biomed. Eng.* 5, no. 9 (September 2021): 1038–47. https://doi.org/10.1038/s41551-021-00712-1.

» Shanghai Cancer Institute, CARsgen Therapeutics, and Cafa Therapeutics. **"Chimeric Antigen Receptor-Modified Immune Effector Cell Carrying PD-L1 Blocking Agent."** European Patent 3,382,009, filed Oct. 31, 2016, and issued July 7, 2021.

» Munshi, Nikhil C., Larry D. Anderson Jr., Nina Shah, Deepu Madduri, Jesús Berdeja, Sagar Lonial, Noopur Raje, et al. **"Idecabtagene Vicleucel in Relapsed and Refractory Multiple Myeloma."** *N. Engl. J. Med.* 384, no. 8 (Feb. 25, 2021): 705–16. https://doi.org/10.1056/nejmoa2024850.

» Henderson, Henry J., and Sigourney Bell. **"Black in Cancer: Championing Diversity in Cancer Research and Medicine."** *Cancer Discov.* 11, no. 2 (Feb. 1, 2021): 237–39. https://doi.org/10.1158/2159-8290.CD-20-1837.

» Paz-Ares, Luis, Tudor-Eliade Ciuleanu, Manuel Cobo, Michael Schenker, Bogdan Zurawski, Juliana Menezes, Eduardo Richardet, et al. **"First-Line Nivolumab Plus Ipilimumab Combined with Two Cycles of Chemotherapy in Patients with Non-Small-Cell Lung Cancer (CheckMate 9LA): An International, Randomised, Open-Label, Phase 3 Trial."** *Lancet Oncol.* 22, no. 2 (Feb. 1, 2021): 198–211. https://doi.org/10.1016/s1470-2045(20)30641-0.

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» Pionyr Immunotherapeutics. **"Anti-TREM1 Antibodies and Related Methods."** US Patent 10,836,828, filed April 17, 2020, and issued Nov. 17, 2020.

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Note: This list was chosen by experts who work in the field, CAS information scientists, and C&EN editorial staff.

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