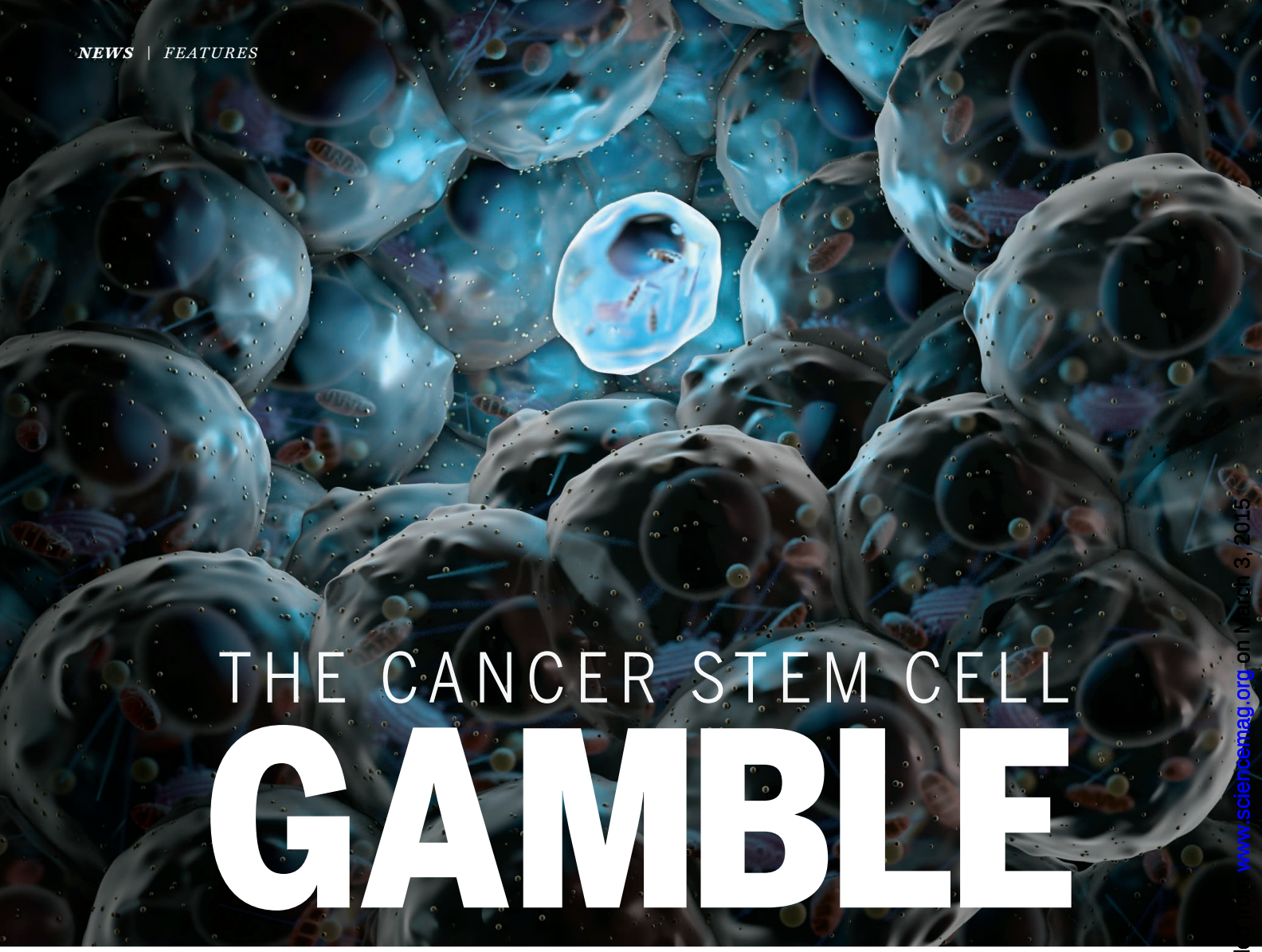


THE CANCER STEM CELL GAMBLE

Researchers are betting that a round of clinical trials will prove a controversial cancer theory and deliver new treatments

By **Jocelyn Kaiser**

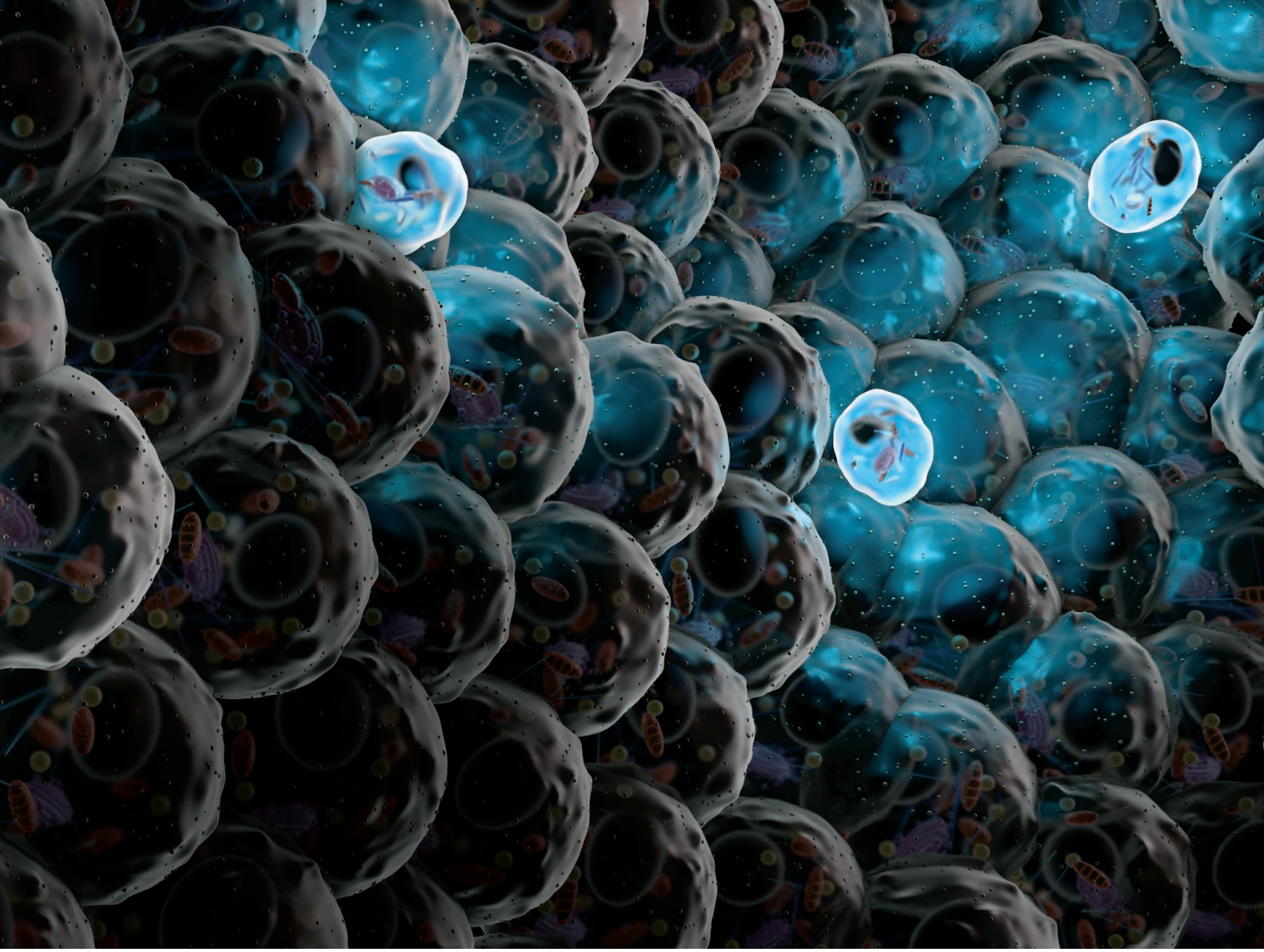
Robert Weinberg is one of the world's best known cancer biologists, thanks largely to his pioneering work identifying genes that underlie tumor development. He has seen hopes for cancer treatments come and go. "I've been in this business for better or worse for 40 years. Many of the things that we've worked on have proved to be relatively useless in the clinic." But at 72, he is optimistic again. "This is really the first time where I'm positioned to help effect the development of an agent or agents that actually will benefit cancer patients," he says.

The Massachusetts Institute of Technology researcher is now staking part of his considerable reputation, and nearly $200 million that investors have given to a company he co-founded, on a bold theory that has divided the cancer field. Weinberg and others contend that tumors contain a small number of cells that are distinctive because they resemble the stem cells that give rise to normal tissues. They believe that these cancer seeds, able to resist chemotherapy and spring back months or years after treatment, may explain the tragic relapses people often experience. Target these cancer stem cells specifically, the thinking goes, and the disease can be kept under control.

Verastem Inc., Weinberg's company in Needham, Massachusetts, is one of several that are launching a new round of clinical trials to find out whether the theory actually works. Beyond the promise of changing cancer care, the financial stakes are huge. OncoMed Pharmaceuticals Inc., another leader in this area, could win $5 billion in additional funding from major drug companies if its trials succeed.

But as Weinberg and others in the field acknowledge, it may be difficult to draw definitive conclusions from these trials. Unlike traditional chemotherapy, the drugs undergoing testing are not expected to quickly shrink tumors, because they are designed to kill just the tiny subset of cells that seed and resupply the main tumor. So detecting whether the drugs are working

ILLUSTRATION: C. BICKEL/*SCIENCE*



in the intended way is not straightforward. Indeed, for solid tumors, researchers lack simple, rigorous assays for measuring the number of cancer stem cells.

The efforts also face some fundamental skepticism: Many still don't believe cancer stem cells exist as a cell type distinct from other tumor cells, and some suggest that companies are hyping or at least oversimplifying the premise. A win in the clinic could resolve some of the controversy. "I think the onus is on all of us in the community that's developing cancer stem cell therapies to show beyond a doubt that these therapies really work," says Max Wicha of the University of Michigan, Ann Arbor.

>60
Ongoing or planned cancer stem cell clinical trials

200 million
Dollars invested so far in Verastem

THE CANCER STEM CELL model emerged in the mid-1990s, when stem cell biologist John Dick of the University of Toronto reported that his team had isolated rare cells in the blood of people with leukemia that seemed to play a key role in the cancer. Although such patients' blood teems with aberrant white blood cells, only a few of them were capable of growing into a new leukemia when injected into mice. Those cells appeared to be misguided versions of the normal adult blood stem cells that differentiate into mature blood cells. Like normal stem cells, the cancer stem cells carried distinctive surface proteins and were self-renewing: They could divide to produce both a regular cancer cell and a new stem cell.

Other teams subsequently reported finding apparent cancer stem cells in solid tumors such as breast, colon, brain, and pancreas. Dick and others suggested that these cells, making up perhaps 1% to 3% of most solid tumors, evade chemotherapy and radiation, partly because most treatments selectively kill rapidly dividing cells, and cancer stem cells grow more slowly than other malignant cells. After lying low, these cancer stem cells could later regenerate the original tumor or spawn metastases in other organs (*Science*, 24 August 2007, p. 1029).

There were problems with these studies, however. Scientists usually picked out the cancer stem cells within a solid tumor by isolating cells with certain surface proteins, thought to be markers of stem cells. But it turned out that not all tumor-generating cells carried these markers, and other cells making up the bulk of the tumor sometimes did as well. Relying on markers "will fool you," says oncologist and cancer stem cell researcher William Matsui of Johns Hopkins University in Baltimore, Maryland.

Still, excitement about cancer stem cells inspired the U.S. National Cancer Institute and major companies starting in the late 2000s to launch small-scale safety trials of drugs aimed at signaling pathways active in stem cells. The pathways targeted were those controlled by the gene called Sonic

hedgehog and genes belonging to the Notch family, best known for shaping embryonic development. This first wave of trials, now winding up, has proved disappointing. Often the drugs caused serious side effects, most likely because they harmed normal stem cells, such as those needed to regenerate the gut lining, Matsui says. Even when these side effects could be managed, for most cancers the drugs showed no signs of efficacy in larger studies.

One hedgehog inhibitor did make it onto the market: vismodegib (Erivedge). But it is approved only for basal cell carcinoma, the most common skin cancer, in which the hedgehog signaling pathway goes awry in all of the malignant cells. As a result, it is unclear if the drug works by actually homing in on rare cancer stem cells.

Despite the early failures, a few compounds have shown enough promise in initial testing that their developers are moving them into larger efficacy trials. Wicha, an OncoMed co-founder and consultant to many companies targeting cancer stem cells, counts more than 60 ongoing trials at universities and companies, testing either antibodies or small molecules aimed at cancer stem cells. Most are designed primarily to gauge safety, while also looking for signs of efficacy. But a few are more advanced phase II trials in which patients are randomly assigned to receive either the experimental drug or a conventional drug or placebo—the classic way to determine whether a new drug is effective.

OncoMed's tarextumab, a monoclonal antibody targeted at proteins in the Notch pathway, is among those furthest in the pipeline. In a safety study that combined tarextumab with two conventional drugs for pancreatic cancer—a disease in which traditional chemotherapy rarely helps—83% of 29 patients' tumors were stable or shrank over periods of from 8 weeks to about a year, the company reported in 2014 at meetings. OncoMed last year began larger, phase II trials for tarextumab in pancreatic and lung cancer. The company says tarextumab and other drugs it is developing seem to work not by killing cancer stem cells, but by nudging them to differentiate into bulk tumor cells that get wiped out by the chemotherapy.

> ***"I think the onus is on all of us in the community that's developing cancer stem cell therapies to show beyond a doubt that these therapies really work."***
>
> **Max Wicha**, University of Michigan, Ann Arbor

Verastem's strategy is to screen approved drugs and other chemicals for their ability to block focal adhesion kinase (FAK), an enzyme that helps tumor cells stick to each other and also helps cancer stem cells survive. In the body, Weinberg believes, blocking FAK kills cancer stem cells directly and also makes it harder for these rare cells within a primary tumor to travel through the bloodstream and seed metastases.

The company's first candidate FAK inhibitor, a livestock antibiotic, did not pan out in the clinic. But according to before-and-after biopsies in 10 mesothelioma patients, who were given another FAK inhibitor called defactinib daily for nearly 2 weeks before having their tumor surgically removed, that drug seemed to knock down the portion of tumor cells carrying a specific stem cell marker. None of the patients' tumors grew in this safety study and in two cases they unexpectedly shrank, for reasons the company is still exploring, Verastem says. Defactinib is now being tested in phase II trials in people with lung cancer and in mesothelioma patients who responded to traditional chemo to prevent the cancer from recurring.

Reparixin, a drug initially developed by the Italian company Dompé to fight transplant rejection, seems to kill cancer stem cells by blocking a receptor that triggers their growth in response to inflammation, Wicha's team has reported. The receptor, which binds inflammatory molecules called cytokines, is found on the surfaces of the stem cells but not on other cells in the bulk tumor. After showing hints of efficacy against metastatic breast cancer in a safety trial, reparixin is now in a phase II trial in which it is given to women diagnosed with breast cancer for 3 weeks before surgery to see if it knocks down cancer stem cells in their tumors.

SOME ARE NOT OPTIMISTIC about the new drugs. Harvard University cancer biologist William Kaelin, a prominent skeptic of the cancer stem cell hypothesis, says that even if these cells exist as a small, distinct population in solid tumors—he's not convinced—tumors can resist chemotherapy in many ways. It's misleading to suggest that "if you kill the cancer stem cells, your work is done," he cautions.

Even if the trial results are encouraging, it won't be easy to tease out the effects of the experimental drug. For one thing, many trials combine a drug targeting can-

A cancer hypothesis on trial

Some efficacy trials of drugs aimed at cancer stem cells, often combined with conventional tumor treatments.

COMPANY	DRUG	TARGET	CANCER	STAGE	COMBINATION
OncoMed	Tarextumab	Notch 2,3 receptors	Pancreatic, lung	Phase II	Yes
	Demcizumab	DLL4 (Notch ligand)	Ovarian	Phase II, mid-2015	Yes
Verastem	VS-6063	Focal adhesion kinase	Mesothelioma, lung	Phase II	No
Boston Biomedical (Sumitomo Dainippon)	BBI608	STAT3, ß-catenin, Nanog	Colon	Phase III halted*	No
			Gastric, esophageal	Phase III	Yes
			Colon, other cancers	Phase II	Yes
	BBI503	Multiple kinases	Solid tumors	Phase II	No
Stemline Therapeutics	SL-401	Interleukin-3 receptor	Leukemia	Phase I/II	No
Dompé	Reparixin	Chemokine receptors 1 and 2	Breast	Phase II	No

*Failed to meet efficacy endpoint

cer stem cells with more conventional cancer therapy. The true test would be to give only a drug that kills the stem cells, says stem cell biologist Irving Weissman of Stanford University in Palo Alto, California. If cancer stem cells exist as proponents envision, then eliminating them should eventually wipe out a tumor because the bulk tumor cells cannot divide indefinitely and will eventually die, he explains.

But giving people only a cancer stem cell drug is not practical because it would be so slow to take effect—perhaps taking many weeks or months to wipe out a human tumor, says developmental biologist Michael Lewis of Baylor College of Medicine in Houston, Texas, who suggests this is why some early trials found no efficacy. What's more, ordinary cancer cells may sometimes "revert" to cancer stem cells, work in Weinberg's and other labs suggests. If so, conventional cancer drugs may also help kill cancer stem cells—and disentangling the effects of stem cell versus conventional treatments could be even more difficult.

A sign that the new drugs are working as advertised would be a drop in the fraction of stem cells within a person's tumor. But monitoring tumors' stem cell content isn't easy. Serial biopsies of the tumor are needed to track any change, which requires cooperating oncologists, consent from the patient to an invasive procedure, and a means of storing and shipping the tumor samples for analysis.

How to quantify the cancer stem cells in a biopsy is another puzzle. Just counting the number of cells with a particular stem cell marker, such as a surface protein, isn't enough for many in the field. The gold standard for proving that putative cancer stem cells are actually that is to inject them at various doses into immune-deficient mice and see if they form tumors. But these "limited dilution" tests require four or five cell doses, several groups of mice, and at least a half-dozen mice per group. "It's time-consuming and expensive," notes stem cell researcher Mick Bhatia of McMaster University in Hamilton, Canada.

Joanna Horobin, chief medical officer for Verastem, says that for "pragmatic" reasons her company isn't trying to measure cancer stem cells with a battery of tests in its trials. Instead, its approach is to answer "different questions in different studies, then put the whole thing together." For example, in a study of mesothelioma, the company showed that chemotherapy alone correlated with an increase in stem cell markers in patients' remaining tumor tissue, as would be expected if those cells resist treatment. Together with the drop in stem cells seen in mesothelioma patients who received a Verastem drug shortly before surgery, "it starts to create the case," Horobin says.

Timothy Hoey, senior vice president for cancer biology at OncoMed in Redwood City, California, notes that the company does have some limiting dilution data on patient samples suggesting that its drugs are hitting cancer stem cells. But the assay OncoMed relies on most is a gene expression signature that it believes indicates how much of a tumor sample is made up of stem cells. Looking at changes in this pattern in a tumor before and after a person receives treatment "connects the preclinical studies to the clinical studies," Hoey says. He notes that these studies have been presented at meetings but the results, including the so-far undisclosed gene signature, still need to be published in a research journal. "I think it's important for the field," he says.

Still in development are tests that will examine the cells released into the bloodstream by solid tumors. Wicha says his team is working closely with engineers and genomics experts on the University of Michigan campus to develop assays that can reliably identify circulating cells with stem cell–like properties.

For now, cancer patients, researchers and physicians, and investors in companies such as Verastem will anxiously wait for data to roll in from the clinical trials. For those with a stake in treatments, the results could bring hope. For researchers debating the reality of cancer stem cells, though, they may not bring resolution. Says Jeremy Rich of the Cleveland Clinic in Ohio, who is studying stem cells in brain cancer, "Even if we're wildly successful, which I don't think we will be, I don't think there will be a black-and-white answer." ■

Taking aim at the seeds of cancer

According to the cancer stem cell model, chemotherapy kills bulk tumor cells but leaves rare, stem cell–like tumor cells untouched (left). These cells then seed the tumor's regrowth. Killing the stem cells may lead to tumor regression over time (center), but combining a cancer stem cell drug with chemotherapy could be faster (right).

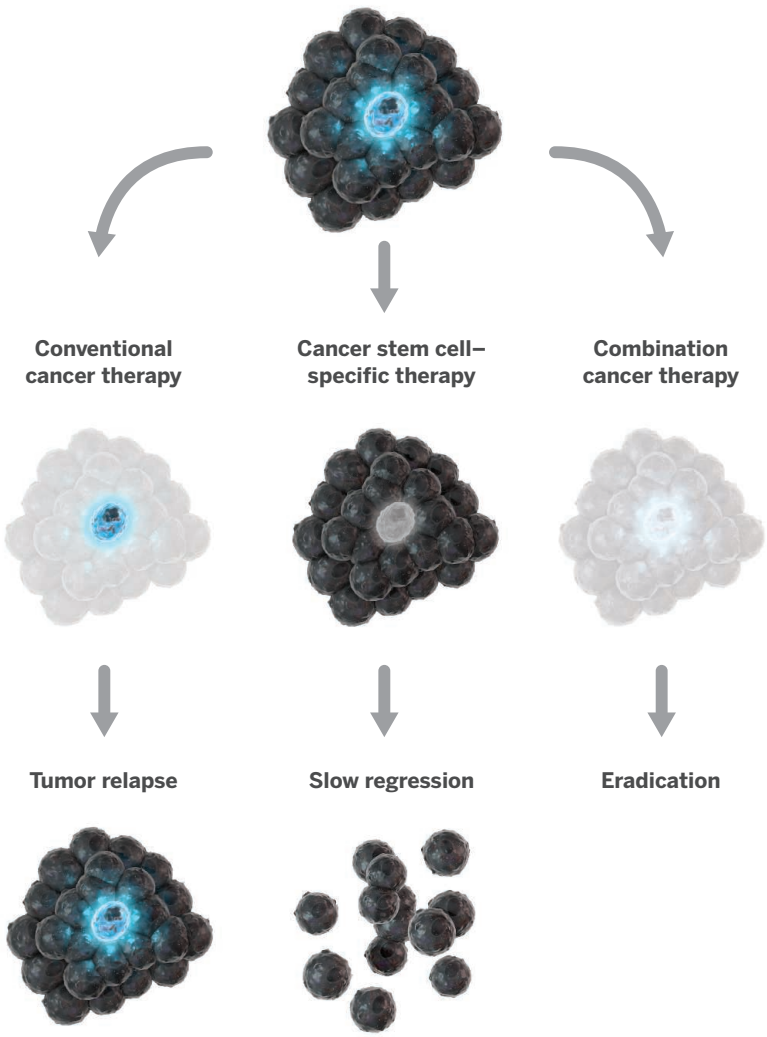


ILLUSTRATION: C. BICKEL/*SCIENCE*