BREAKTHROUGHS IN BIOSCIENCE / SPRING 2018

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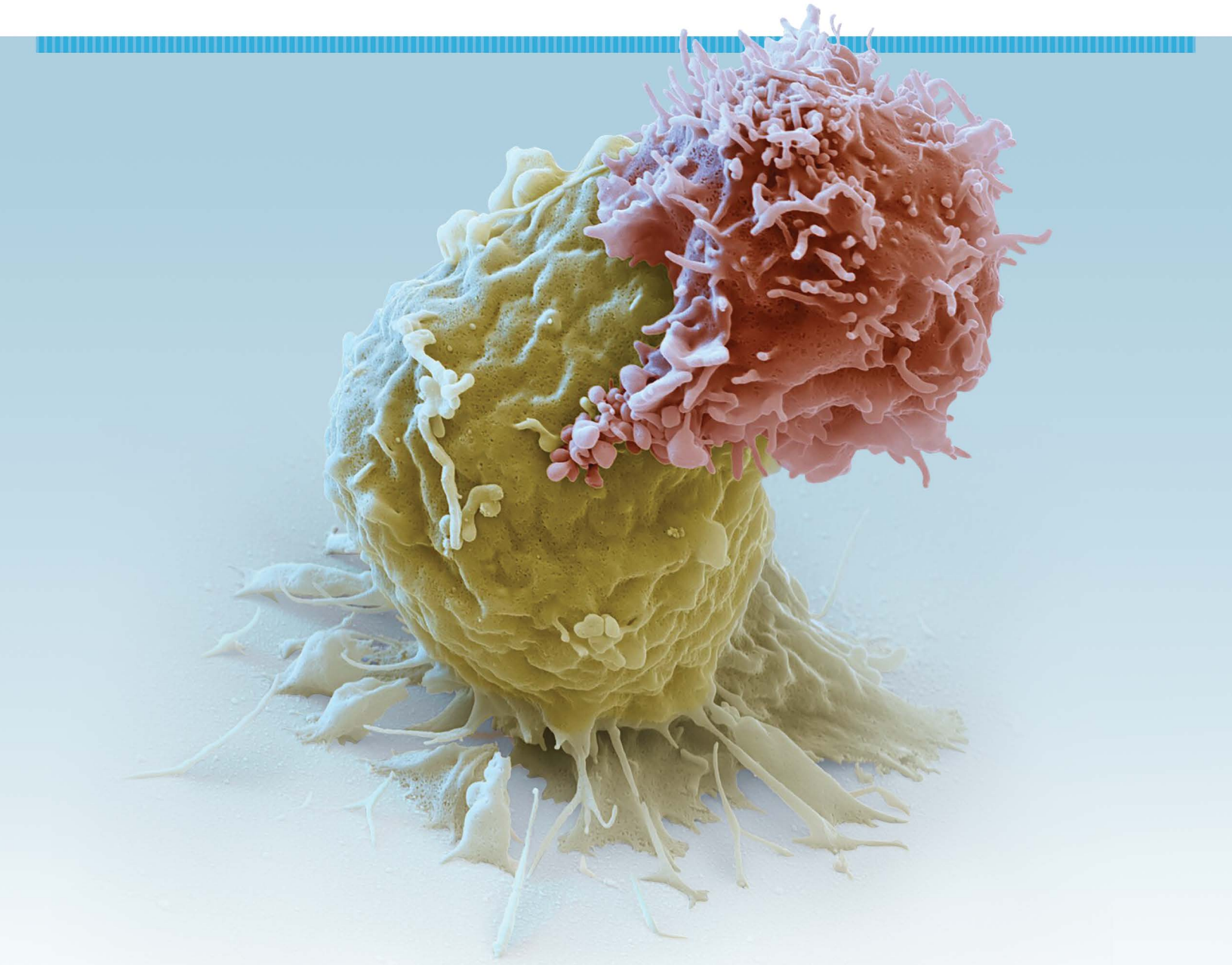

Cancer Immunotherapies

From Magic Bullets to Super T Cells

BREAKTHROUGHS IN BIOSCIENCE / CANCER IMMUNOTHERAPIES: FROM MAGIC BULLETS TO SUPER T CELLS

AUTHOR »

Margie Patlak writes about biomedical research and health from the Philadelphia region. She has written for *Discover, Glamour, Physician's Weekly, Consumer Reports on Health, The Washington Post, Los Angeles Times, Dallas Morning News*, and numerous other publications. She frequently writes for the National Institutes of Health and the National Academy of Sciences and currently works with a number of trade journals, such as *Endocrine News* and the *Journal of the National Cancer Institute*. This is her eleventh article in the *Breakthroughs in Bioscience* series.

SCIENTIFIC ADVISOR »

Jeffrey Sosman, MD, is an experienced clinical investigator whose career has been vested in the immune-based and targeted therapy of melanoma and renal cell carcinoma. He has played a significant role in the clinical development of the immune checkpoint inhibitors, both Ipilimumab (anti-CT-LA-4) and Nivolumab (anti-PD-1). In addition, Dr. Sosman has lead early trials demonstrating efficacy of BRAF inhibitors in patients with melanoma. He has been involved in efforts to identify mechanisms of resistance to checkpoint inhibitors and approaches to overcome de novo and acquired resistance. At the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, he is a Professor of Medicine and co-leads the Translational Research in Solid Tumors program in the Cancer Center. He is also the first Mary Hendrickson-Johnson Melanoma American Cancer Society Research Professor since 2008 for his long term achievements in melanoma clinical research.

SCIENTIFIC REVIEWER »

Tonya J. Webb, PhD
University of Maryland School of Medicine

ON THE COVER »

Color enhanced scanning electron microscope image of a CAR-T lymphocyte (pink) attacking a leukemia cell (yellow).
Image credit: Eye of Science/Science Source

This article was developed in collaboration with **The American Association of Immunologists**.

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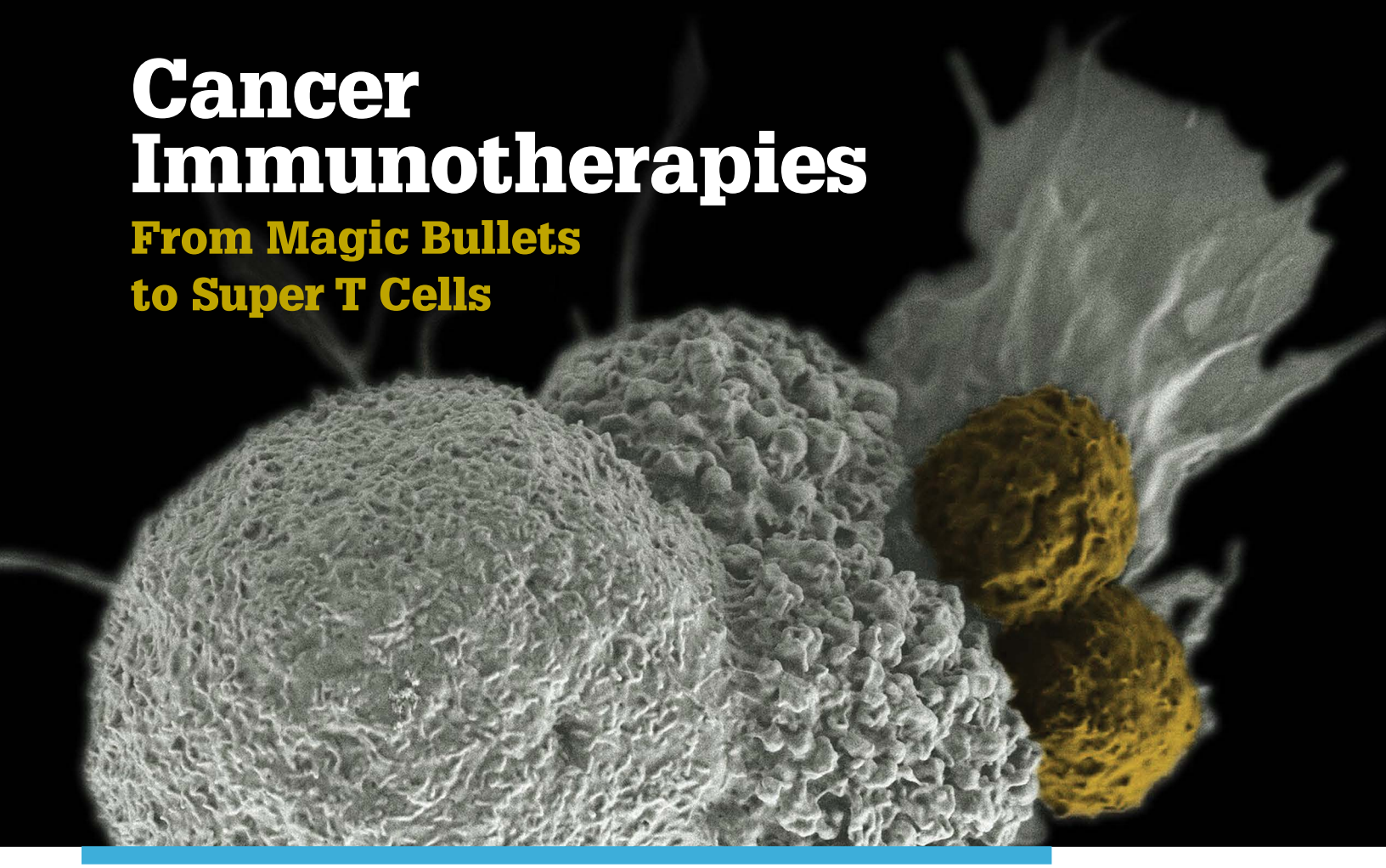

A cancer cell (gray) being attacked by two cytotoxic T cells (gold). *Image credit: National Cancer Institute*

SANDRA WAS ONLY 22 YEARS OLD, **but she was already running out of time. Shortly after Sandra got engaged, doctors discovered the nagging cough she had been experiencing was due to metastatic melanoma. A deadly cancer had grown from a malignant mole she hadn't noticed, spreading to her lungs and brain. Chemotherapy and radiation treatments slowed, but could not stop its relentless reach. Fortunately, Sandra's doctor was able to offer her a new experimental treatment that was designed to unleash the power of her immune system so it could more effectively fight off her tumors. Within three months of Sandra's treatment with this innovative treatment, called immunotherapy, her doctors were surprised to see that every trace of melanoma had disappeared on her CT scan. This marked the beginning of a remission that has lasted more than ten years, and has let Sandra enjoy a high quality of life. Since her recovery, she has even had two children.**

Sandra can count herself as one of the tens of thousands of cancer patients who have benefited from cutting-edge immunotherapies that have provided new therapeutic options. These therapies have proven effective in treating advanced skin, kidney, lung, bladder, head and neck cancers, and certain types of colorectal cancers—with response and survival rates that far outperform traditional treatments. Immunotherapy is different from conventional cancer treatment strategies, such as chemotherapy and radiation, which directly battle tumor cells. Instead, immunotherapies work by strengthening patients' own natural arsenals for fighting cancer.

GLOSSARY

ADOPTIVE CELL THERAPY (ACT): an immunotherapy in which T cells are removed from a patient, grown, and sometimes modified in the laboratory, and then re-injected into the patient

ANTIBODY: a protein produced by B cells that latches onto an antigen as part of an immune response to the antigen

ANTIGEN: a foreign or abnormal substance in the body that provokes an immune response

B CELL: a white blood cell that matures in the bone marrow and produces antibodies

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Immunotherapy encompasses multiple approaches, including harnessing or enhancing immune cells, hormones, and other weapons of the immune system to destroy tumor cells, as well as releasing the brakes on the immune response that tumors trigger. These innovative therapies are ushering in a promising new era in cancer treatment that would not have been possible without the strong foundation of basic research in immunology and tumor cell biology built by scientists for more than a century. These researchers pursued answers to basic questions such as, "how does a virus cause leukemia in mice," "why are tumors able to evade an immune response despite many immune cells surrounding them," and "what causes white blood cells to multiply?" Without answers to these key questions, Sandra would not be raising a family or be counted among the many patients who have benefited from cancer immunotherapies.

1890s | COLEY PHENOMENON

One of the earliest hints that the body's immune response could combat cancer came from the work of the New York City surgeon Dr. William Coley in the 1890s (Figure 1). A pioneer in immunotherapy at a time when very little was known about the immune system, Coley was intrigued by reports that a few patients with incurable advanced cancers experienced remissions, if not cures, after battling deadly bacterial infections. Frustrated by the futility of surgery for treating his patients with advanced malignancies, Coley injected them with heat-killed bacteria, later referred to as "Coley's toxins," hoping to induce a similar response that would rid their bodies of cancer.

Coley claimed that in nearly half of the 93 cancer patients that he treated with his "toxins," the tumors shrank or disappeared altogether. When attending medical conferences, Coley would sometimes bring along one of his former patients. This young man had once been bedridden by a large, inoperable abdominal tumor. After Coley injected the patient with his "toxins," the man developed a raging fever. But within a short period of time after the fever subsided, the cancer mass disappeared. The patient went on to live another 26 years until he died of a heart attack.

Unfortunately, other investigators were not able to duplicate Coley's findings, perhaps because their toxins did not match the immune-stimulating capacity of those used by Coley. Or perhaps they discontinued the toxin injections before the patient had developed a fever, which Coley believed was needed for an anti-tumor response. At the time, doctors were starting to use radiation therapy to treat cancer with more consistently effective results. This caused many of them to disregard or be skeptical of Coley's findings, especially since he couldn't fully explain them. As noted by immunologist Dr. Lloyd Old at a research symposium, "Science had to catch up with the Coley phenomenon, and the cellular and molecular language of inflammation and immunity had to be understood before the forces that Coley unleashed could be predictably translated into tumor cell destruction."

FIGURE 1 / DR. WILLIAM COLEY » Dr. William Coley (center) and colleagues at the Hospital for the Ruptured and Crippled. *Image credit: National Library of Medicine.*

1900s | MAGIC BULLETS

Fortunately, scientific explanations for Coley's findings began to emerge over the next century, thanks to the contributions of numerous researchers in basic biological science. In 1890, the German scientist Emil Behring discovered that if he injected one guinea pig with the serum of a second guinea pig that had recently recovered from diphtheria, the first animal would be protected from deadly diphtheria toxins. This led his colleague, chemist Paul Ehrlich, to suggest in 1900 that there must be "magic bullets" in the blood that seek out and destroy specific toxins. Behring termed these agents **antibodies** (Figure 2). Ehrlich imagined

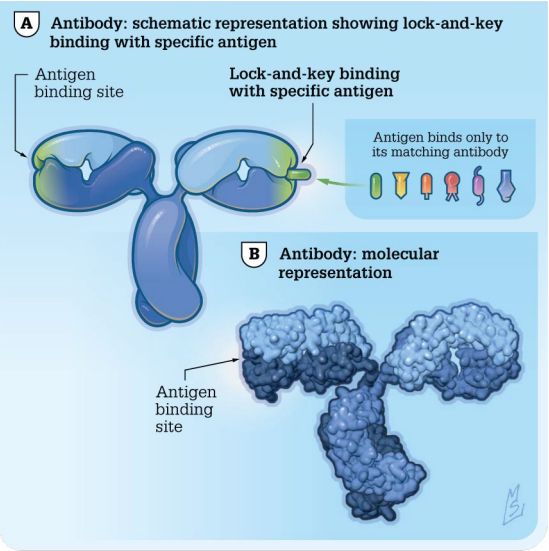

FIGURE 2 / ANTIBODIES AND EMIL BEHRING » Description of antibody and antigens (top); Emil Behring injecting a guinea pig held by an assistant (bottom). *Image credits: (top) © Michael Linkinhoker, Link Studio, LLC; (bottom) Stock Montage/Contributor/Getty Image.*

that antibodies latched onto specific toxins and other foreign substances (**antigens**) in the bloodstream with the specificity of a lock to a key, unleashing an immune attack on anything else in the body that also bore those antigens. (See the *Breakthroughs in Bioscience* article, "Magic Bullets and Monoclonals: An Antibody Tale.")

Recognizing that tumors might also have abnormal antigens that could stimulate an immune attack, Ehrlich proposed in 1909 that the immune system could counter or eliminate cancers in the same way it does for infections. This concept gave birth to the hope that understanding the interface between a tumor and the immune system could provide effective cancer treatments. But it wasn't until the 1960s that researchers made further progress in cancer immunotherapy thanks to a serendipitous discovery in mice.

1960s-1970s | BASIC LEUKEMIA RESEARCH LEADS THE WAY

By 1948, it was known that antibodies were produced by **B cells** that circulate in the blood stream as a component of white blood cells (Figure 3). But up until 1961, little was known about the cells that *trigger* antibody production by B cells, other than that they were also a type of white blood cell. The cells in this portion of the blood did not garner much interest in the scientific community because they were thought to be short-lived. The fact that they could not be maintained in cell culture impeded researchers' ability to study them in the laboratory.

But scientists began paying more attention to white blood cells in the 1960s thanks to experiments performed by Dr. Jacques Miller, a medical researcher at the National Institutes of Health (NIH). With government support, he was trying to understand how a virus caused leukemia in mice. It was thought that the virus infected the thymus, a small organ whose function was unknown at the time. Miller removed the thymus from mice expecting it would make them immune to the virus and prevent leukemia. Instead, the missing thymus made the animals more susceptible to all types of infectious diseases. Miller went on to determine that the thymus is an organ where a key component of the immune system, **T cells**, matures and develops. Without functional T cells, the mice's immune systems did not function properly. Miller not only divided up white blood cells into two distinct populations—T cells and B cells—he also showed that without mature "helper" T cells, antibody-producing B cells were unable to make antibodies. These discoveries filled in an important piece of the puzzle concerning what is needed for an effective immune response.

Then in 1976, Dr. Doris Morgan, working with Dr. Robert Gallo at the National Cancer Institute (NCI), tried to use a nutrient broth that had previously grown T cells to promote the expansion of patients' leukemia cells for her cancer investigations. Previous investigators had

GLOSSARY

CYTOKINE: a protein secreted by immune cells that regulates an immune response

CHIMERIC ANTIGEN RECEPTOR: a synthetic receptor that has features of both antibodies and T cell receptors

CO-STIMULATOR: a molecule produced by certain cells of the immune system that triggers an immune response when antigens are present

CYTOTOXIC T-LYMPHOCYTE ASSOCIATED PROTEIN 4 (CTLA-4): a molecule produced by certain cells of the immune system and by tumor cells that suppresses an immune response

CYTOTOXIC T CELLS: a subset of T cells that kill infected or cancerous cells as part of an immune response

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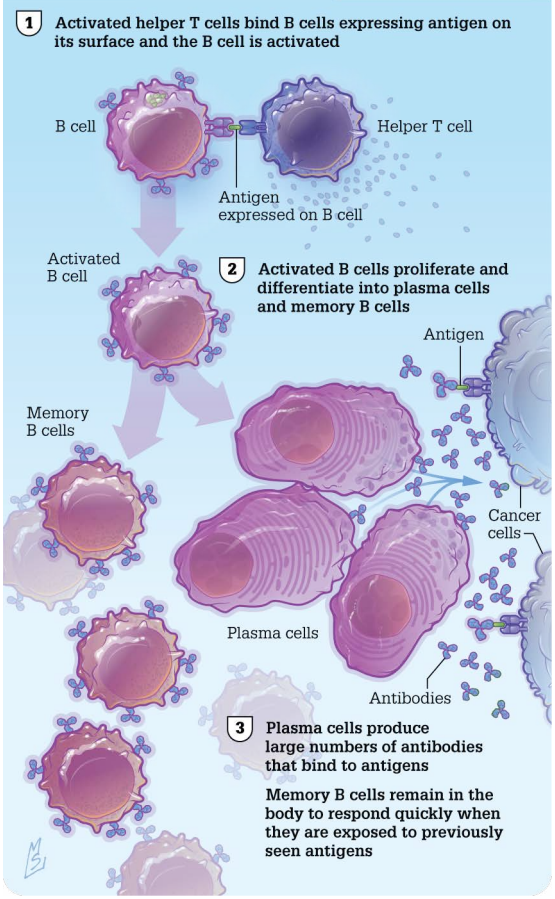

FIGURE 3 / B CELLS » In the 1960s, Jacques Miller discovered the importance of a second type of white blood cell – the T cell. Helper T cells orchestrate an immune response by activating B cells. Activated B cells develop into memory B cells, sentries that stick around after an initial response to prevent reinfection, or plasma cells, which produce large numbers of antibodies. *Illustration: © Michael Linkinhoker, Link Studio, LLC.*

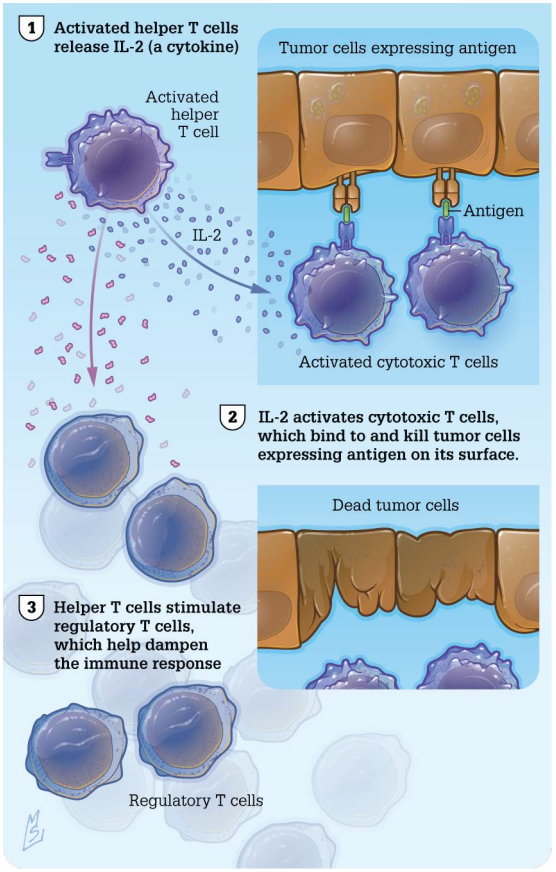

FIGURE 4 / T CELLS » T cells have receptors on their cell surface. When these receptors latch onto antigen, they release interleukin-2 and several other cell hormones to help orchestrate a complex immune response. T helper cells activate cytotoxic T cells, which bind to and kill cells expressing the same antigen on its surface. Stimulation of regulatory T cells can dampen the immune response. *Illustration: © Michael Linkinhoker, Link Studio, LLC.*

reported that this culture medium fostered the growth of immature white blood cells, which were akin to leukemia cells. At first, Morgan was frustrated to discover that, rather than stimulating the growth of the cancer cells as she expected, this culture medium caused T cells to proliferate. But then she and her colleagues were intrigued by what was causing the T cells to multiply.

Other researchers in Gallo's lab eventually discovered it was **interleukin-2 (IL-2)** that T cells had secreted into the culture medium that was triggering their multiplication. This was the first of many proteins, called cytokines, made by cells that researchers would discover fired up an immune response. Over the next 10 years, further investigations revealed that T cells have receptors on their cell surface. When these receptors latch onto antigen, they release IL-2 and several other cytokines to help orchestrate a complex immune response. This response includes not just the generation of antibodies by B cells, but also the production of **killer or "cytotoxic" T cells**, which directly detect and kill any cells bearing the same antigen triggers on their cell surface. In parallel, another part of this response is the generation of **regulatory T cells** that dampen the immune response to keep it in check (Figure 4).

T cells, which had long stood in the shadow of B cells that produce antibodies, were now pushed to the forefront, not just in immunology, but in cancer research as well. Other studies continued to reveal the vital role

T cells and the cell hormones they release play in detecting and destroying tumors.

1980s-1990s | CELLULAR WARRIORS AND WEAPONS

In the 1980s, investigators showed that high doses of IL-2 caused major shrinkage in the tumors in five to 10 percent of patients with advanced kidney cancer and melanoma. Those patients who responded to the treatment remained in remission for many years. These results reinforced the notion that one could harness an individual's own immune system to rid the body of cancer. The Food and Drug Administration (FDA) approved IL-2 treatment for kidney cancer in 1992 and for advanced melanoma in 1998.

Encouraged but not satisfied with the IL-2 results, Dr. Steven Rosenberg at NCI used the agency's government support to search intensely for those immune cells that specifically seek out and destroy tumor cells (Figure 5). He removed tumors from experimental animals and cultured them with IL-2 for several weeks to activate the T cells within the tumors and expand their numbers. While observing these cultures under the microscope, he found that the tumor cells were besieged by what he called "**tumor-infiltrating lymphocytes**" **(TILs)**. Analysis of the TILs revealed that they were composed of killer and helper T cells. These T cells were highly specific for the tumor cells and rarely attacked normal cells. "They were the best available evidence that at least some humans with cancer do indeed mount a specific immunologic reaction against their tumors," Rosenberg said in a 1990 *Scientific American* article. He went on to show that the TILs could cause tumor regression in mice, even in those that had widespread cancer.

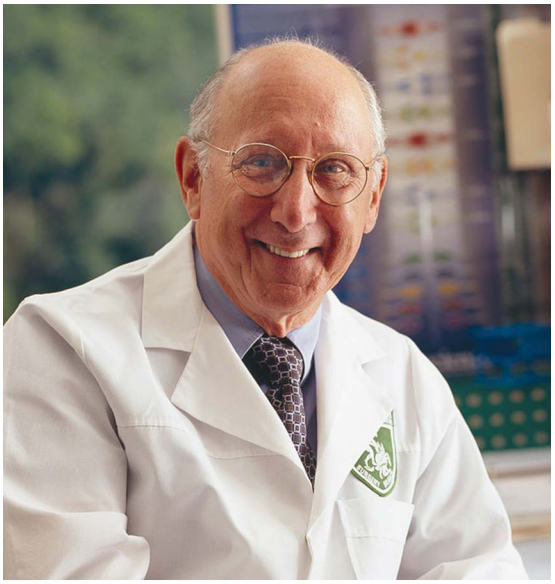

FIGURE 5 / DR. STEVEN ROSENBERG
Image credit: National Institutes of Health.

These exciting findings prompted Rosenberg to treat patients with advanced cancers with his experimental **adoptive cell therapy (ACT)**. After surgically removing a portion of their tumor, he cultured these tumor cells with IL-2 and then re-injected the patients with the TILs stimulated by the cytokine. Noticing that some tumor fragments elicited TILs that recognized and attacked tumor antigens more effectively than others, Rosenberg started selecting subsets of TILs with greater anti-tumor action to expand in culture and then re-infuse in patients (Figure 6). About half of the nearly two hundred patients with metastatic melanoma treated with this

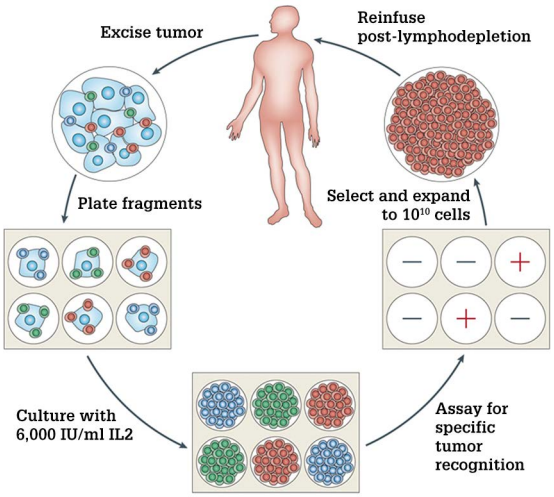

FIGURE 6 / ADOPTIVE CELL THERAPY (ACT) » ACT works by removing a portion of a patient's tumor, placing small pieces of the tumor in individual growing compartments, and promoting the proliferation of the T cells contained in the tumor piece by administering interleukin 2 (IL-2). Each individual T cell population is then tested to see which one reacts against the tumor tissue. Those that positively react are expanded and infused back into the patient after they have undergone chemotherapy to rid the body of its naturally occurring white blood cells (lymphodepletion). The hope is that the infused T cells attack the patient's tumor. *Image credit: Reprinted by permission from Springer Nature, Nature Reviews Cancer, Adoptive cell transfer: a clinical path to effective cancer immunotherapy, SA Rosenberg, NP Restifo, JC Yang, RA Morgan, and ME Dudley, 2008.*

GLOSSARY

HELPER T CELLS: a subset of T cells that help prompt B cells to produce antibodies

IMMUNE CHECKPOINT: a molecule produced by immune cells that suppresses an immune response

INTERLEUKIN-2 (IL-2): a cytokine that activates T cells

NEOANTIGEN: a new antigen produced by a tumor cell that is not produced by normal cells

PROGRAMMED DEATH PROTEIN 1 (PD-1): a molecule produced by immune and tumor cells that suppresses an immune response

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procedure responded. Of these, one-quarter of patients experienced durable and marked clinical improvements, including complete regression of their tumors lasting more than five years. Essentially, these patients seemed cancer-free. Rosenberg's lab has also recently had some success with ACT by selecting TILs that target antigens derived from some of the many genetic mutations commonly found in cancer cells. These antigens have been named "**neoantigens**" since they are unique to cancer cells and not found in normal cells.

However, Rosenberg's exciting results with his ACT required that researchers administer chemotherapy to patients as a means of temporarily ridding the body of most of its immune cells to create space for the infused immune cells. Dr. Nicholas Restifo's team at NCI discovered that pretreatment with chemotherapy enables the transferred T cells to predominate in an immune response to the tumor. It triggers the release of a number of cytokines that activate these tumor-killing cells to expand in number in the cancer patient. However, there may be another important reason why ACT was only successful when patients were pretreated with chemotherapy. Some researchers suspected that the chemotherapy could eliminate regulatory T cells, which put the brakes on an effective immune response against the tumor. Understanding those brakes through basic research was the next important advance.

1970s-1990s | BRAKES ON AN IMMUNE RESPONSE

Several researchers observed that T cells often swarmed the margins of tumor samples obtained from patients, and they were puzzled as to why those white blood cells were not able to enter and destroy the tumors. Studies showed that these T cells were exhausted and had lost their potent anti-tumor activity. Presumably this lethargy explained why they were less effective at destroying tumors. But what was the cause? Like everything else in immunology, the answer to this question proved to be complex and required years of research on several different fronts.

Starting back in the 1960s and 1970s, immunologists observed a curious phenomenon—more than just antigens are required for a full-blown immune response. Paradoxically, if pure antigens are injected alone into mice, it dampens the production of corresponding antibodies. On the other hand, studies also showed that using the culture medium of white blood cells previously exposed to antigens prompted a more robust

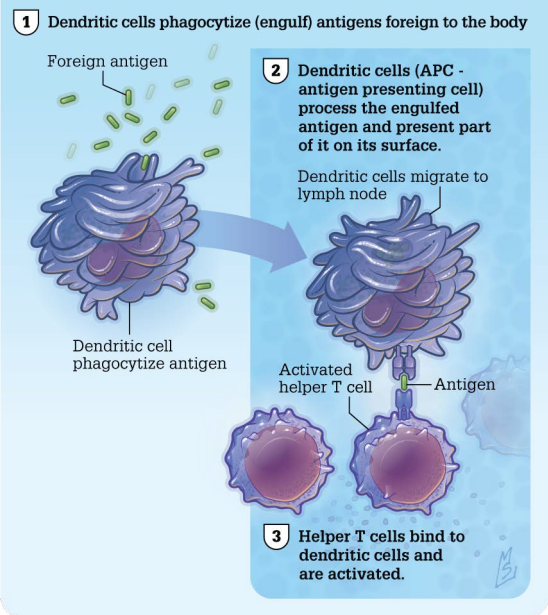


FIGURE 7 / DENDRITIC CELLS » Ralph Steinman at Rockefeller University studied dendritic cells, which looked branch-like under the microscope, and discovered that these branches snag foreign antigens, engulf them, and present them to T cells. T cells bind the presented antigen and other co-stimulatory receptors, are activated, and are able to mount an immune response. *Illustration: © Michael Linkinhoker, Link Studio, LLC.*

immune response than just the antigens alone. In 1974, Australian researchers Drs. Kevin Lafferty and Alistair Cunningham put these findings together and proposed that to stimulate an effective immune response two signals are needed—antigens and another "**co-stimulator**" provided by certain white blood cells. Exposure to antigens in the absence of the co-stimulator results in a suppressed immune response, which explained earlier findings. But the identity of the co-stimulator was not revealed until many years later.

Meanwhile in the early 1970s, immunologist Dr. Ralph Steinman at Rockefeller University was peering through a microscope at a slide of white blood cells and saw strange branched cells unlike any other blood cell he had noticed before. Curious, he used funding from the NIH to develop techniques to enrich these cells in his cultures, and then conducted a number of experiments to see what role the tree-shaped cells he called dendritic cells may play in immune responses (Figure 7). This research revealed that the numerous branches on the cells snag antigens, process them, and then present the antigens along with co-stimulatory signals to

T cells. Without dendritic cells to mediate this interaction, T cells would ignore the antigens and not trigger an immune response.

For this groundbreaking research, Steinman received the 2007 Albert Lasker Award for Basic Medical Research, shortly after he was diagnosed with pancreatic cancer. At the time, he stated in an interview "I think dendritic cells provide the potential for a whole new type of therapy in cancer, but we need research and patience to discover the rules, to discover the principles." Unfortunately, there was not enough time for Steinman to benefit personally from his discoveries. Just as the 2011 Nobel Prize in Physiology or Medicine was announced for Steinman, he succumbed to metastatic pancreatic cancer.

Steinman's legacy made immunologists and cancer researchers realize it is not just antigens and T cells that are key to triggering an immune response, but the context in which those antigens are presented to the T cells is also critically important. Dendritic cells help provide the needed context with all the required signals. Such a realization spurred multiple investigations aimed at unraveling what exactly those signals were.

In the 1980s, Drs. Ronald Schwartz and Marc Jenkins at the National Institute of Allergy and Infectious Diseases reported that dendritic cells supplied the key second signal (co-stimulation) that T cells needed to become active. If T cells encounter antigens without this signal, they shut themselves down instead of launching an attack. The molecular nature of this signal became apparent shortly after, when other laboratories identified a specific protein that appears on the surface of dendritic cells. When this protein latches onto a specific receptor on T cells, it triggers these cells to make IL-2 and other cytokines needed for their multiplication expansion and activation.

Then in 1991, Dr. Peter Linsley and his colleagues at Oncogen and the University of Washington reported they had found that same molecular signal dendritic cells provide also latches onto a different receptor on T cells called **cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)**. The researchers initially assumed CTLA-4 was another T cell receptor involved in co-stimulation of T cell activity. But then studies in mice and in cultures of T cells by Dr. Jeffrey Bluestone, while at the University of Chicago, and Dr. James Allison, then at the University of California-Berkeley, suggested something different (Figure 8). These studies, which were supported by grants from NIH, indicated that CTLA-4 surfaces on T cells only two to three days *after* they are activated, and then suppresses their expansion and activity. In other words, CTLA-4 turned off an immune response rather than stimulated one.

FIGURE 8 / DR. JEFFREY BLUESTONE AND DR. JAMES ALLISON. *Image credits: (left) Steve Babuljak; (right) The University of Texas MD Anderson Cancer Center.*

Putting all the pieces of evidence together, Allison suggested that antigens binding to the T cell receptor work similarly to a key turning the ignition of a car. The antigen binding turns on the immune response, but only when the T cell interacts with the co-stimulator, provided by dendritic and other immune cells. This binding activates T cells, causing them to expand and attack cells that have the antigen. On the other hand, CTLA-4, a molecule produced by some immune cells and tumor cells, operates like the car's brakes, stopping an immune reaction shortly after production of IL-2. In this way, CTLA-4 checks an immune reaction, and consequently was called an **immune checkpoint**. This discovery led Bluestone to develop a drug that mimics CTLA-4 for patients with the disorder rheumatoid arthritis, which is due to an over-active immune system that attacks normal tissues. But CTLA-4 could also help explain the inability of tumor antigens to activate T cells and led Allison to pursue a different idea. Allison hypothesized that if he could make a drug that blocked CTLA-4, he could release the brakes on the immune system, allowing it to mount a more robust attack on tumor cells.

GLOSSARY

REGULATORY T CELLS: a subset of T cells which suppress an immune response

T CELL: a white blood cell that matures in the thymus and orchestrates an immune response

TUMOR-INFILTRATING LYMPHOCYTES (TILS): a subset of T cells that attack tumor cells

TUMOR MICROENVIRONMENT: the environment surrounding a tumor, including blood vessels, immune cells, supportive cells and tissues, cell hormones, and other signaling molecules

1990s | CHECKPOINT BLOCKERS

In 1995, Allison produced antibodies that blocked CTLA-4, and tested them in mice with tumors. He was shocked by his results. "I was expecting anti CTLA-4 to slow tumors a little bit, but the tumors completely melted," said Allison in an interview with the *Journal of Clinical Investigation*. Allison's research group then tested the effectiveness of the CTLA-4 blocker on a variety of cancers in animal models and found that it nearly always eliminated the tumors (Figure 9). Their research indicated that by treating the immune system, rather than the tumor, researchers could rid the body of cancer—at least in mice.

Subsequent clinical tests of a humanized version of the checkpoint blocker showed that around 20 percent of 5,000 metastatic melanoma patients receiving this drug, called ipilimumab, survived for at least 5 years. These results were remarkable considering that with standard treatments, 90 percent of these patients would have died within a few years. In 2011, the FDA approved the drug for metastatic melanoma.

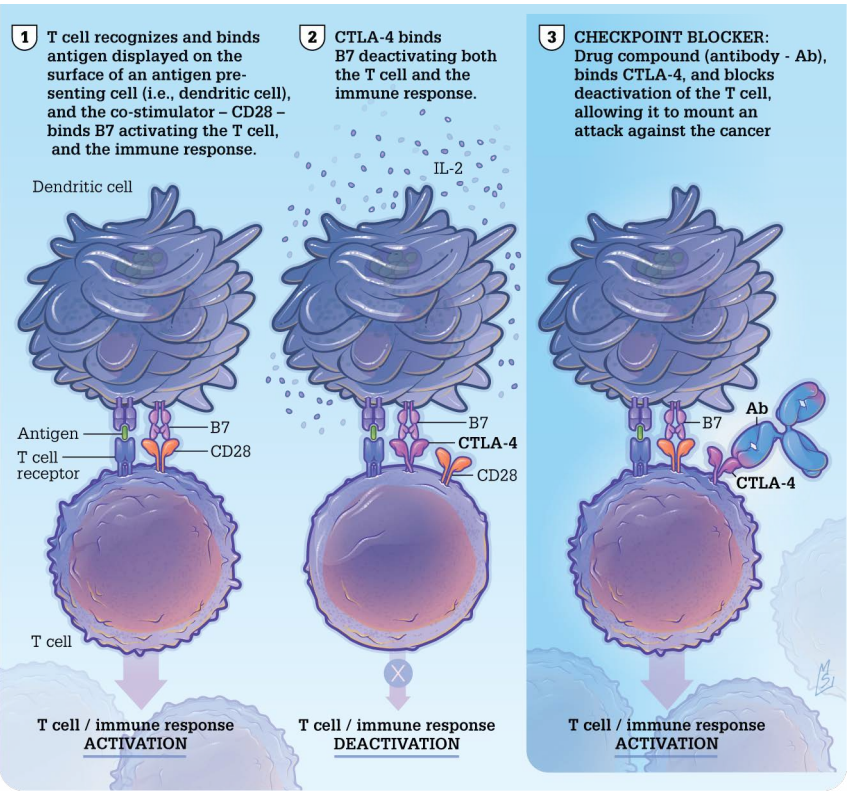


FIGURE 9 / CHECKPOINT BLOCKER, CTLA-4 » Schematic showing how CTLA-4 antibodies block deactivation of an immune response. *Illustration: © Michael Linkinhoker, Link Studio*

In an article in the *Journal of Clinical Investigation*, Dr. Jillian Hurst noted that Allison's basic research resulted in a highly successful cancer therapy and "serves as the ultimate example of how a basic science finding can change the practice of medicine."

Basic research also led to the discovery of another type of immune checkpoint that tumor cells use to evade an immune response. Around the same time that Allison was performing his research on CTLA-4, a cell biologist in Japan was trying to understand the signals that lead cells to destroy themselves. Formally known as programmed cell death, this process is observed in almost all bodily tissues, and differs from cell death resulting from injury or infection. Curious about what prompted programmed cell death, Dr. Tasuku Honjo of Kyoto University looked for genes activated when programmed cell death occurred in cultures of cancerous T cells. He then determined the proteins those genes encoded. In 1992, Hongo reported that he had found the molecular "grim reaper" for cells, a protein he called programmed cell death (**PD-1**). To get a better sense of PD-1's function, he collaborated with Dr. Gordon Freeman and Dr. Arlene Sharpe of Harvard University to genetically engineer mice so they couldn't produce this protein (Figure 10). They were surprised to discover that the immune systems in these mice went haywire, attacking various organs. At that point, it became clear that the researchers had uncovered that PD-1 is another checkpoint, or brake, on the immune system (Figure 11). Without PD-1 checking immune responses, the immune system attacks normal tissues, resulting in autoimmune disorders.

Further research uncovered that PD-1 is a cell receptor, and by 2002 it was clear that many cultured tumor cells and dendritic cells bear the ligand (protein that binds to a receptor) on their surface. Oncologist Dr. Drew Pardoll at Johns Hopkins then proposed, and later studies confirmed, that tumor cells were co-opting the PD-1 brake on the immune system to prevent an immune attack on themselves. These cancer cells produced the PD-1 ligand (PD-L1) on their surface, which then snagged the PD-1 protein on T cells. This interaction triggered these T cells to self-destruct or be ineffective. This could partly explain why earlier investigators saw tumors that had survived being surrounded by T cells that had homed in on their antigens. Other studies in humans showed that high levels of the PD-L1 in tumors corresponded with worse disease prognosis in cancer patients.

FIGURE 10 / DR. ARLENE SHARPE
Image courtesy of The American Association of Immunologists

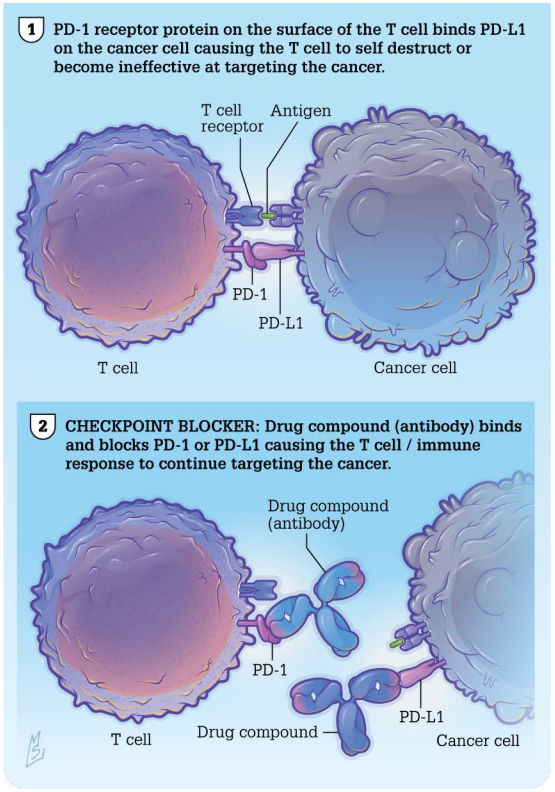


FIGURE 11 / CHECKPOINT BLOCKERS, PD-1 AND PD-L1 »
Schematic showing how PD-1 and PD-L1 antibodies allow for the immune system to continue targeting the cancer. *Illustration © Michael Linkinhoker, Link Studio, LLC.*

These basic research findings led several companies to develop compounds that block PD-1 or PD-L1 as potential cancer treatments. Clinical tests on some of these compounds have found them to be effective in patients with several different types of cancers, including metastatic melanoma, lung, bladder, kidney, head and neck cancer, and treatment-resistant Hodgkin's lymphoma. For many patients, these drugs have stemmed the growth of their tumors for long periods of time. By 2016, the FDA approved a small number of drugs that target PD-1 (pembrolizumab and nivolumab) or PD-L1 (atezolizumab, avelumab, and durvalumab) as immunotherapy treatments for certain cancers.

While many patients undergoing cancer immunotherapy treatment enjoy an improved quality of life, these new checkpoint-blocking drugs are not free of potentially serious side effects. In some cases, the drugs unleash an immune response against healthy tissues. Such autoimmune reactions include colitis, hepatitis, rashes, diabetes, and other conditions that result in hormonal deficiencies. These reactions have severe consequences in a small fraction of patients. Physicians can usually mitigate the autoimmune responses by treating patients early with immune suppressants, which do not appear to hamper the anti-tumor effects of the checkpoint blockers.

TODAY AND BEYOND | THE PERFECT THERAPEUTIC STORM

Because they target different phases of a T cell immune response, various combinations of a CTLA-4 blocker and drugs targeting PD-1 or PD-L1 are showing impressive results for certain types of cancers. For example, an unprecedented 58 percent of metastatic melanoma patients given one FDA-approved combination therapy experienced shrinkage of their tumors, including more than 15 percent of patients in whom the combination therapy caused tumors to completely vanish on scans. Initial findings suggest that most responses are durable, with some lasting more than three years.

Scientists suspect that a multi-pronged approach using radiation therapy, chemotherapy, or targeted therapies added to checkpoint blockers will be even more effective. When standard cancer treatments kill tumor cells, the disintegration of the tumor cells releases more tumor antigens, making these tumor antigens more likely to encounter immune cells fired up by the checkpoint blockers. "The result could be a perfect therapeutic 'storm' of killing tumor cells and allowing their debris to be recognized more avidly by the immune system…It is finally time to start thinking realistically about long-term remissions, even cures, because we can now combine standard therapies that target the tumor with immunotherapies that boost a patient's

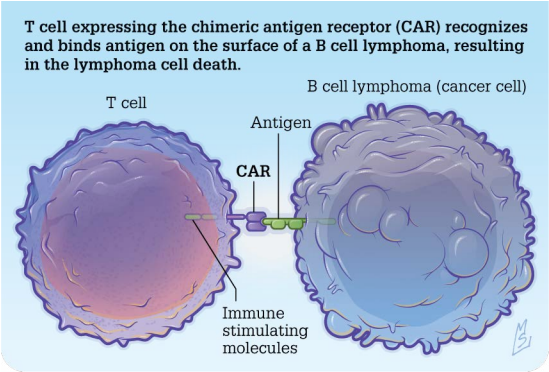


FIGURE 12 / CHIMERIC ANTIGEN RECEPTOR » The external portion of a chimeric antigen receptor (CAR) has the specificity of an antibody, which can recognize cancer antigens. The inside portion of the receptor has signaling capabilities that activate the T cell once it finds a tumor cell. Activated T cells can then kill the cancer cells. *Illustration: © Michael Linkinhoker, Link Studio, LLC.*

own defenses," wrote Dr. Jedd Wolchok in a 2014 issue of *Scientific American*.

Checkpoint blockers also open up the possibility of making tumor vaccines more effective. Researchers since Coley's time have tried to develop various kinds of anti-cancer vaccines. However, these vaccines often failed clinical testing because they were not able to overcome the immune suppression induced by the tumor. With checkpoint blockers now doing this job, there is more hope that using tumor vaccines in combination with checkpoint blockers will be an effective dual approach to treating cancers.

The number of clinical studies testing immunotherapies, either singly or in combination, for numerous types of cancers has exploded exponentially of late. There are now more than 250 clinical studies underway that test the combination of a checkpoint blocker with standard or experimental cancer therapies. Some of these studies test checkpoint blockers in patients with early-stage cancer, in whom the treatments are expected to be even more potent than what has been seen in patients with late-stage metastatic cancer.

Clinicians are also testing other checkpoint blockers, since CTLA-4 and PD-1 turned out to be just the tip of the iceberg for immune checkpoints. The more that investigators in basic research looked for immune suppressors, the more they found immune cells or tumor cells that produced them. Such basic science research has also uncovered new molecules akin to the protein produced by dendritic cells that co-stimulate an immune response, as well as new T cell receptors for these stimulator proteins. Investigators have now discovered more than a half-dozen cell surface receptors that act as stimulators or suppressors of an immune response to tumors. Experimental drugs targeting these receptors are already undergoing testing in cancer patients. Basic immunology research has also uncovered many of the molecular signals that these immune regulators trigger. Such signals include growth factors, enzymes, and cell hormones. The complexity is astounding; investigators have identified more than 20 different subtypes of T cells, and close to 40 different types of interleukins, some that stimulate and some that suppress the immune system.

In the past decade or so, basic science investigations have also tremendously improved understanding of the crosstalk between tumor cells and their neighbors. In addition to mature white blood cells, these neighbors include immature blood cells and connective tissue cells that tumors recruit as part of an inflammatory and wound repair response. This response is often ineffective and instead feeds tumors with their growth-stimulating compounds. The wound response also fosters the development of dendritic cells suited for supporting tissue remodeling but not the antigen presentation needed for an anti-tumor response. Researchers have discovered that cells within close proximity of the tumor (**tumor microenvironment**) activate specific molecular signals to suppress an immune response to cancer. This suggests that there are additional targets for cancer immunotherapies, and investigators are just beginning to explore this possibility.

"We are starting to appreciate that tumors can hijack a number of potent regulators of the immune system in order to survive," stressed cancer researcher Dr. David Munn of the Medical College of Georgia in a 2016 issue of *Current Opinion in Immunology*.

The explosion of knowledge on how to elicit an effective immune response to cancer, and on how tumors disrupt the immune response, is increasing the number of potential weapons physicians can use in the war against cancer. It has also enabled researchers to genetically engineer super T cells that have shown remarkable effectiveness in treating certain types of cancers in the clinic.

1990s TO TODAY AND BEYOND | SUPER T CELLS TO THE RESCUE

A groundbreaking treatment in cancer, the production of super T cells, is based on decades-old basic

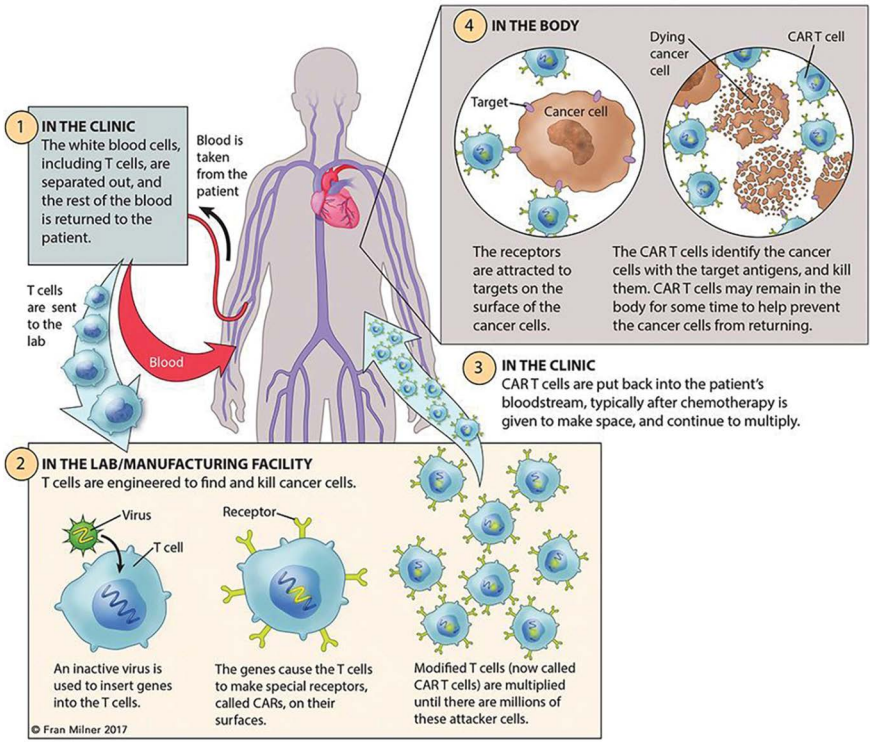
biological research showing that T cell receptors can only latch onto tumor antigens presented by dendritic cells when the antigens are inserted into clefts on the cells' surface. These clefts are comprised of a collection of proteins, called the major histocompatibility complex (MHC), that serve as molecular fingerprints for subgroups of individuals. But many tumor cells suppress both the production of the MHC cleft and the multiplication of dendritic cells that can present tumor antigens, contributing to the failure of T cells to destroy tumors in some patients receiving ACT.

Seeking to resolve this problem, in 1989 Israeli immunologists Drs. Gideon Gross, Tova Waks, and Zelig Eshhar of the Weizmann Institute of Science swapped the antigen-recognizing portion of T cell receptors with the antigen-recognizing portion of antibodies, which can latch onto antigens without the antigens being inserted into MHC clefts. That way, the "**chimeric antigen receptor**," (CAR) would have the specificity of antibodies as well as the cell-killing capabilities of T cells (Figure 12). These scientists initially did not achieve success in killing tumor cells with the artificial immune cells they produced. That success didn't happen until other researchers modified the CARs so that they included immune-stimulating signals normally generated by interactions with dendritic and other immune cells. This improved the potency and duration of the T cell response.

In 2010, Rosenberg started using this new immunotherapy for cancer, called CAR T-cell therapy, to experimentally treat patients with cancers that were derived from B cells, such as B-cell leukemia and B-cell lymphoma. The treatment involves harvesting a cancer patient's T cells from their blood, genetically engineering the T cells to have CARs targeting an antigen commonly found on B cells, and re-infusing these super T cells back into patients after their immune systems have been temporarily depleted by chemotherapy (Figure 13). CAR T-cell therapy is not as time-consuming as Rosenberg's original ACT, yet has generated stunningly high success rates in certain blood cancers derived from B cell lymphocytes, and is showing early promise in the treatment of solid tumors. Six out of the first eight patients Rosenberg treated with CAR T-cells went into remission.

In 2011, Drs. Carl June, David Porter, and their colleagues at the University of Pennsylvania tested CAR T-cell therapy in children and adults with treatment-resistant leukemia, including those that did not respond

FIGURE 13 / CAR T-CELL THERAPY » Schematic of CAR T-cell Therapy. *Image used with permission of The Leukemia & Lymphoma Society (Chimeric Antigen Receptor (CAR) T-Cell Therapy Facts, 2017).*



to bone marrow transplants. These patients were not expected to live through the end of that year. Of the 30 patients treated, 27 went into complete remissions that lasted for years. "The outcomes were really quite astounding," Porter reported at a National Academy of Sciences workshop in 2016 in Washington, DC. Similar response rates were reported by Dr. Michel Sadelain and his colleagues at Memorial Sloan Kettering Cancer Center. Complete and long-lasting remissions for other blood cancers initially tested on small numbers of patients have ranged between 26 and 75 percent.

Researchers continue to test CAR therapies in larger numbers of patients, and it is too early yet to say whether the treatment can essentially cure patients of their cancer. But many of the first patients treated continue to do well, including the first child June's group treated with super T cells. Emily Whitehead was six-years old at the time, had failed prior cancer treatments, and wasn't expected to reach second grade. She's now 12 years old, has no signs of her cancer, and leads the normal life of a middle-schooler. However, as many as a quarter of those leukemia patients who initially go into

remission after receiving this CAR therapy relapse six months later because their tumors stop producing the type of antigen targeted by the genetically engineered T cells. But a large portion—perhaps as many as three-quarters--of these patients may be successfully treated with a currently experimental CAR therapy that targets a different antigen, one study suggests. Ultimately, combination therapy with both types of CAR therapies might be the best option for patients with leukemia that doesn't respond to standard treatments, Dr. June noted in a *New York Times* article, stating that such combination therapy "should make it 'game over' for leukemia."

Similar to other immunotherapies, CARs targeting B cells can cause serious reactions to the potent cytokines the treatment unleashes. These reactions include life-threatening drops in blood pressure, fluid retention, and fever—all of which can often be alleviated with immune suppressants. The treatment also depletes antibody-generating B cells that are needed to fight infections, but that depletion can be countered with antibody infusions. Various drug companies are working to bring CAR therapy into the general market. In August of 2017, the FDA approved a CAR T-cell therapy developed by Novartis for the treatment of certain leukemias. The treatment is the same one Emily Whitehead received. In October of 2017, FDA approved a similar treatment that was initiated by Dr. Rosenberg at NCI and further developed and commercialized by Kite Pharma for some types of lymphomas.

When compared to cancers comprised of circulating tumor cells in the bloodstream, solid cancerous masses tend to be more challenging to treat with CAR therapies. One problem is that the antigens on the cells in solid tumors are also frequently found on the surface of cells in healthy organs. Since CAR T-cells target specific antigens regardless of whether they appear on tumor cells or healthy cells, treating solid tumors with this therapy is more likely to cause severe adverse reactions. Investigators are currently testing CAR therapies in nearly 40 clinical trials on patients with cancers involving solid tumors, such as breast, prostate, and lung cancer. In these cases, they are using CAR T-cells that target antigens which are overproduced by the tumors, in the hopes of avoiding damage to healthy tissue. These treatments require careful administration to reach the sweet spot in which mostly tumor cells are killed and not normal tissues that also have the antigens.

"While no one can predict exactly where the research will lead, one thing is certain, the future of cancer immunotherapy is bright indeed," noted June and his colleague Dr. Laura Johnson in a recent issue of *Cell Research*. Rosenberg added in a *Scientific American* article "What was once an intuition is now becoming a reality. Immunotherapy for cancer can be effective."

We've come a long way since Coley first tinkered with cancer immunotherapy more than a century ago. Now we have more reliable and effective treatments based on science rather than intuition. The productive interplay between basic immunology and tumor biology research has led to more than a half-dozen new FDA-approved cancer treatments in just the last 5 years. These innovative treatments, which release the brakes on an immune response to cancer, are expected to be followed by more effective combination therapies in the next five years, as well as treatments that use super T cells to combat tumors. This explosion of new drugs for cancer stem from the findings of numerous curious basic research scientists that rigorously explored the black box of what constitutes an effective immune response to tumors. Such basic research provided the clues for the development of unexpected new therapies – today's magic bullets in the treatment of malignancies.

ADDITIONAL SUGGESTED READING

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