

# **CANCER** AND THE **IMMUNE SYSTEM**

**THE VITAL CONNECTION**

A PUBLICATION FROM



**CANCER  
RESEARCH  
INSTITUTE**

The Leader in Immunotherapy



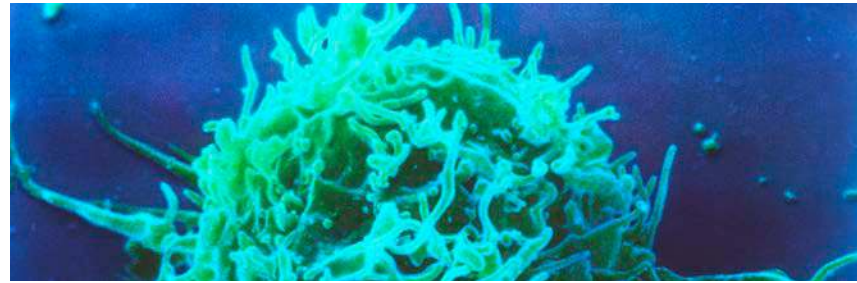
## **CANCER AND THE IMMUNE SYSTEM: THE VITAL CONNECTION**

Copyright © 1987 by Cancer Research Institute  
All rights reserved  
Revised 2003, 2016

Jill O'Donnell-Tormey, Ph.D.  
CEO and Director of Scientific Affairs  
Cancer Research Institute

Matthew Tontonoz, M.A.  
Science Writer  
Cancer Research Institute

# CONTENTS



<b>INTRODUCTION: REVISITING THE “C” WORD.....</b>	<b>3</b>
<b>PART 1: WHAT IS CANCER?.....</b>	<b>4</b>
1.1 Cell Division, Mutations, and Cancer.....	4
1.2 How Cancer Develops.....	6
1.3 Cancer Incidence and Mortality in the U.S.....	8
1.4 Cancer and the Immune System.....	9
<b>PART 2: THE HUMAN IMMUNE SYSTEM.....</b>	<b>12</b>
2.1 Innate Immunity: Our First Line of Defense.....	13
2.2 Adaptive Immunity: Learning the Enemy’s Tricks.....	15
2.3 Inflammation: Linking Innate and Adaptive Immunity.....	17
2.4 The Humoral Immune Response: Making Antibodies.....	20
2.5 The Cellular Immune Response: Making “Killer” T Cells.....	22
2.6 Tolerance and the Problem of Autoimmunity.....	24
<b>PART 3: CANCER IMMUNOTHERAPY.....</b>	<b>26</b>
3.1 Historical Origins.....	26
3.2 Non-Specific Immune Stimulants.....	26
3.3 Antibody Immunotherapies.....	28
3.4 Cancer Vaccines.....	32
3.5 Checkpoint Blockade.....	38
3.6 Adoptive Cell Therapy.....	42
3.7 Combinations: The Future of Cancer Immunotherapy.....	45
<b>GLOSSARY.....</b>	<b>46</b>
<b>REFERENCES.....</b>	<b>48</b>
<b>ACKNOWLEDGMENTS.....</b>	<b>50</b>

# Science

**Breakthrough of the Year**

## **Cancer Immunotherapy**

**T cells on the attack**



# INTRODUCTION

## REVISITING THE “C” WORD

Few words strike as much fear into the heart as “cancer.” As too many of us know, cancer is an often cruel disease that cuts lives short and causes significant suffering for both patients and families. Compounding this fear is the fact that cancer’s treatment is often considered worse than the disease itself.

But there is reason to believe that a new era in cancer treatment is upon us. Thanks to decades of research by countless scientists, we are now in a position to harness the power of our own immune system to fight cancer. This approach, called cancer immunotherapy, is proving to be a very effective way to combat this disease. By treating the patient, not the tumor, these therapies hold the potential for safer and more durable control of cancer. In fact, many believe that it is reasonable to start using that other “c” word—cure—to describe the long-lasting responses we are seeing.

As the one organization that has supported the field of cancer immunotherapy from the beginning, the Cancer Research Institute (CRI) is proud of the field’s recent accomplishments—including being named 2013 “Breakthrough of the Year” by *Science* magazine. From its inception in 1953, CRI has supported scientific research aimed at developing immune-based therapies for cancer.

Today, as FDA-approved immunotherapies become standard of care, we are more committed than ever to our long-term goal of fostering immunotherapy for all types of cancer.

CRI supports the development of cancer immunotherapy in several ways. Through research grants, we provide crucial funding to scientists conducting basic, translational, and clinical research into the immune system and cancer. Our Clinical Accelerator program allows us to work with industry partners to help speed the development of new immunotherapies, bringing lifesaving treatments to patients faster. Through our website, we educate patients and caregivers about the power of the immune system to fight cancer, and connect patients with clinical trials that may be their best source for treatment.

We recognize that patients and caregivers looking into cancer immunotherapy as a potential treatment option often face an uphill battle in understanding the technical language of immunology. To help explain the science behind immunotherapy, we have prepared this guide. It was written with the curious layperson in mind and answers a number of commonly asked questions about cancer, the immune system, and cancer immunotherapy.

“Many believe that it is reasonable to start using that other “c” word—cure—to describe the long-lasting responses we are seeing.



# PART 1

## WHAT IS CANCER?



The word “cancer” is an umbrella term used to describe diseases that result from abnormal **cell division**. Cell division is the process by which a cell duplicates its contents and then cleaves in two, creating two new daughter cells. Normal cells in the body know when to divide and when to stop dividing. Cancer cells do not. They divide without limit, resist death, and have the potential to invade other organs and tissues, with disastrous consequences.

Cancer is actually not one disease, but many. There are more than 200 different types of cancer, classified according to where they occur in the body, the specific cell type from which they arise, and, increasingly, the specific genetic mutations found within the cancer cells. Cancers that arise in the epithelium—the layer of cells covering the surface of the body and lining the internal organs and glands—are called **carcinomas**. Carcinomas can form in organs such as the lung, breast, colon, and stomach, or in glands such as the ovary and prostate. **Sarcomas** are cancers of the supporting tissues of the body such as bone, muscle, and blood vessels. Cancers of the white blood cells and the lymph glands are called **leukemias** and **lymphomas**, respectively. **Melanomas** arise from darkly pigmented cells, called melanocytes, located in the skin.

With so many possible ways to harm the body, it is not surprising that cancer takes a serious toll on public health. Apart from heart disease, more people in the U.S. die from cancer than from any other illness.

### 1.1 CELL DIVISION, MUTATIONS, AND CANCER

Though our bodies may seem like they are relatively static entities, they are actually in continual flux. The tissues and organs of our bodies are constantly changing as worn-out cells die and new ones are born to replace them.

Different parts of our body experience more cell division than others. Some cells—for example, those in the brain—divide rarely or not at all, while others—like the cells of the skin, gut, bone marrow, and reproductive tissues—divide frequently throughout life.

The number of cells in an average human being is approximately 30 trillion. On an average day, the human body produces and eliminates some 60 billion cells. This massive amount of cell division is the main reason why cancer is so common: each time a cell divides, there is a chance a mistake will occur.

For each cell division, the entire genetic material of the mother cell, in the form of DNA, must be faithfully copied and passed to the new daughter cells. DNA is made of subunits called nucleotides, which come in four different versions: adenine (A), guanine (G), cytosine (C), and thymine (T). One complete copy of DNA in a cell consists of a string of roughly 3 billion nucleotides—As, Gs, Cs, and Ts—arranged in a precise sequence that is unique to each individual. This vast DNA sequence, commonly thought of as the blueprint of life, must be accurately copied for the daughter cells to function properly.

If we compare DNA replication to the task of copying a 500-page book by hand, one can appreciate the number of errors that would begin to creep in each time the book is copied and then recopied. Fortunately, our cells are equipped with a quality control system that evolved to ensure proper replication: specialized molecules, called enzymes, not only copy but also proofread, edit, and correct errors in the newly manufactured DNA that is destined for the daughter cells. With this repair system, cells make fewer than one mistake in a billion nucleotides copied.

Nevertheless, mistakes do sometimes occur. When these mistakes change the DNA sequence—say an A becomes a G, or a C is dropped from the genetic text—we say that a **mutation** has occurred. If a mutation occurs within a **gene**, that gene may no longer be able to instruct the cell to produce the normal version of the protein it encodes. Cancer results when mutations occur in genes important for controlling cell division.

Mutations in two types of genes play important roles in cancer: **proto-oncogenes** and **tumor suppressor genes**. Proto-oncogenes are normal genes that provide cells with signals to

divide; they are the gas pedal of the cell division cycle. Tumor suppressor genes are normal genes that tell a cell to stop dividing; they are the brakes.

Mutations convert a proto-oncogene into an **oncogene**, which is like the gas pedal being permanently floored. Mutations in tumor suppressor genes are like cutting the brake cable. When both types of mutations occur in the same cell, the normal process of cell division is disrupted. The cell is now speeding out of control. Scientists have identified a number of different tumor suppressors and oncogenes in humans ([Table 1](#)).

TABLE 1. EXAMPLES OF TUMOR SUPPRESSORS AND ONCOGENES

TUMOR SUPPRESSORS	ASSOCIATED CANCER(S)
Rb	Retinoblastoma, sarcoma, bladder, breast, lung
p53	Brain, breast, colorectal, esophageal, liver, lung, sarcoma, leukemia, lymphoma
APC	Colorectal, lung
BRCA1, BRCA2	Breast, ovarian
INK4	Kidney
PTEN	Brain, melanoma, prostate, endometrial, kidney, lung
p16	Melanoma, pancreatic, brain, esophageal, non-small cell lung (NSCLC), sarcoma, bladder
ONCOGENES	ASSOCIATED CANCER(S)
HER2/neu	Breast, stomach, ovarian
BCR/ABL	Chronic myelogenous leukemia (CML)
EGFR	NSCLC, head and neck, colorectal, pancreatic
VEGF	Breast, colorectal, kidney, NSCLC, brain, cervical, ovarian
VEGFR	Kidney, stomach, NSCLC
BRAF	Melanoma, kidney
KRAS	Pancreatic
β-catenin	Colorectal
Bcl-2	Follicular cell lymphoma

What causes mutations? In addition to ordinary DNA replication mistakes, environmental insults such as certain chemicals, ultraviolet (UV) light, and radiation can damage DNA and cause it to mutate. For example, UV light causes DNA in our skin cells to break, which can lead to skin cancer. Cigarette smoke causes lung, bladder, and other cancers. Chemicals originating inside the body can also cause mutations, as is the case with DNA-damaging molecules called free radicals.

On some occasions, a normal cell becomes cancerous when a virus enters the cell and disrupts the genetic machinery. This is the case with cancers known to be caused by viruses—for example, cervical cancer (caused by the human papillomavirus, or HPV), liver cancer (caused by the hepatitis B or C virus), and Burkitt lymphoma and nasopharyngeal cancer (caused by the Epstein-Barr virus).

Mutations can also be inherited from one or both parents. A mutation that is present in a parent's sperm or egg cell will be passed to the newly fertilized egg during reproduction. Because every cell in an adult human is a descendant of that original fertilized egg, the mutation will be present in every cell of that person's body.

It is important to note a single mutation will not necessarily lead to cancer. Multiple mutations are usually required to transform a normal cell into a cancer cell, and this process takes time. This is why most environmentally caused cancers occur only after years of exposure to the cancer-causing **carcinogen**. Lung cancer caused by smoking, for example, usually occurs after several decades.

The situation is more complicated when inherited mutations are concerned. For someone who starts life with an inherited mutation, the chances of him or her developing cancer at a younger age are much greater, because they already have one hit in every cell of their body. If a second mutation occurs at some point—say as a result of cigarette smoking—then they will have two hits. This is why women with inherited mutations in the BRCA1 or BRCA2 gene are at much higher risk for developing breast and ovarian cancer than women without these mutations, and also tend to develop these cancers in their 30s and 40s rather than later in life.



SMOKING



VIRUSES



UV LIGHT

## 1.2 HOW CANCER DEVELOPS

Every cancer starts from a single cell that has been unleashed from the growth restraints placed on normal cells. Because the changes that took place within the cancer cell occurred as the result of mutations in DNA, they are passed on to each of the daughter cells arising from the original cancer cell. As these cells continue to divide and mutate, collections of abnormal cells



accumulate. The end result may be a mass of cancerous cells, called a **tumor** (Figure 1).

The cells of the tumor then push outward from their boundaries, infiltrating surrounding normal tissues. Small clumps of cells may dislodge from the tumor, enter blood or lymphatic vessels, and migrate to distant sites. Upon reaching a new destination, the cancer cells may move out of the circulation and invade the surrounding tissues, where they continue to multiply and form secondary tumors. This process of spreading to a distant site is called **metastasis**. Eventually, local invasion and metastasis disrupt the body's normal functions. By some estimates, metastasis accounts for 90 percent of cancer deaths from solid tumors.

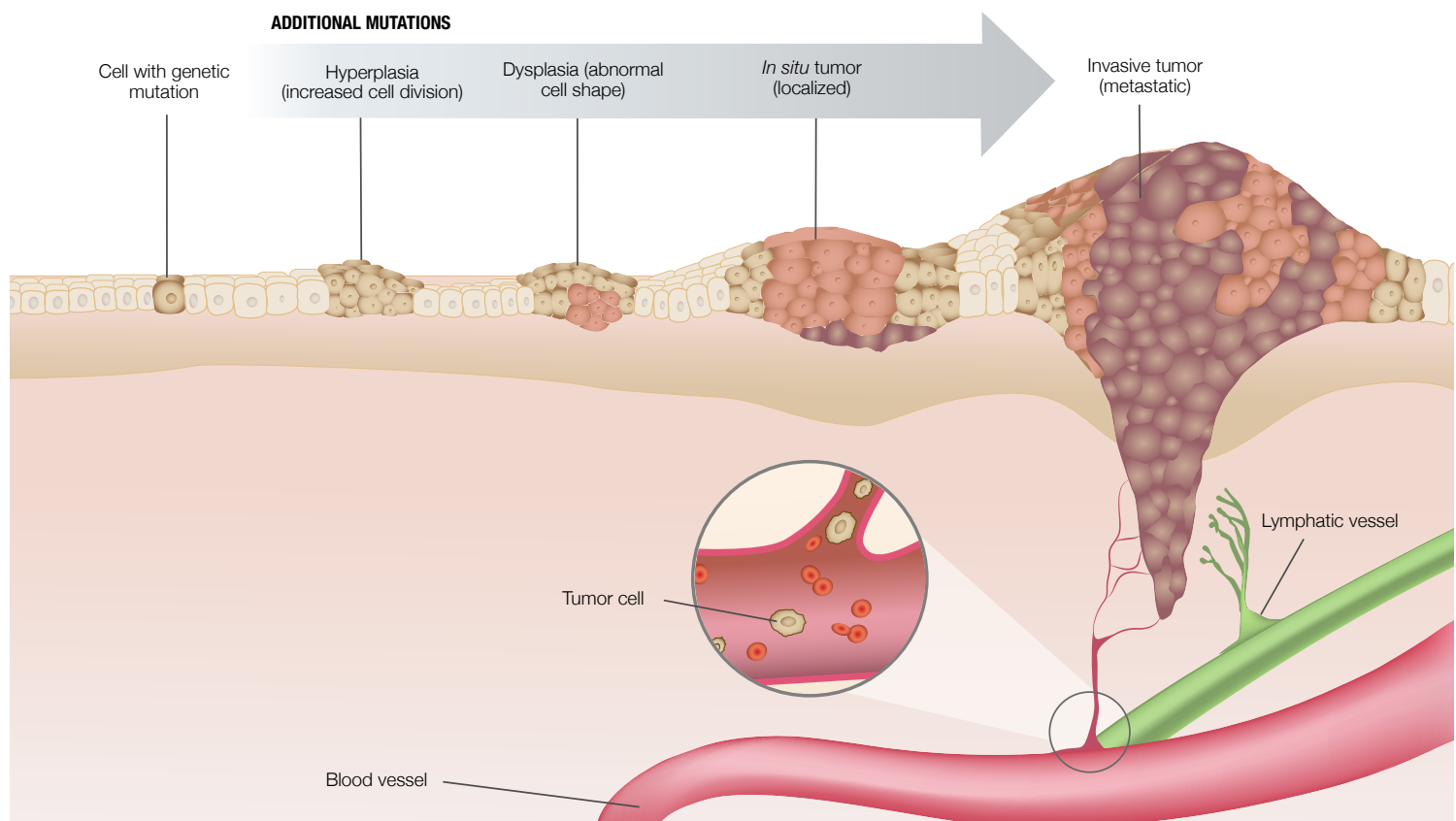
Tumors need nutrients in order to grow large. They actively promote the growth of new blood vessels and recruit these vessels to the tumor, a process called **angiogenesis**. (Certain drugs, called angiogenesis inhibitors, interrupt this process and are often used in cancer treatment.)

Not all cancers form solid tumors. Some, like those that arise in the blood or bone marrow, instead circulate throughout the body, crowding out normal blood cells. This is the case with the blood cancer leukemia.

The standard treatments for most cancers include surgery, chemotherapy, and radiation. Surgery—cutting out the disease—

### FIGURE 1. TUMORS DEVELOP IN STAGES

It takes more than one mutation to cause a tumor. Tumors develop over time as mutations accumulate in cells and as restraints on cell division are disrupted. Invasive tumors can metastasize, or spread, to other parts of the body.



may be effective when a tumor is small and localized and can be removed in full. **Chemotherapy** (“chemo”) refers to chemicals that kill cells by interrupting cell division. It is used when solid tumors have metastasized or when surgery is not an option. Chemo kills all actively dividing cells in the body. Because cancer cells divide frequently, they are likely to be killed by chemo. Many normal cells also divide frequently—such as those in the bone marrow and those lining the digestive tract—and are also killed by chemo, which accounts for the severe side effects of this treatment. **Radiation** is similarly non-specific, damaging the DNA of all cells in its path. Cells that acquire massive amounts of DNA damage are unable to function or reproduce, and consequently die.

Both chemotherapy and radiation can have powerful and immediate anti-cancer effects. Unfortunately, with the notable exceptions of some childhood cancers (such as acute lymphoblastic leukemia and Hodgkin lymphoma), thyroid cancer, and testicular cancer, chemotherapy and radiation are usually not curative when used to treat late-stage cancer; cancers have a tendency to become resistant to these treatments and come back.

Additionally, **targeted therapies** may be used alone or in combination with chemotherapy and radiation for certain types of cancers. Targeted therapies are more specific in their action than chemotherapy and radiation, often interfering with the action of a single molecule or cellular pathway. Therapies targeting oncogenes are becoming increasingly common. Some of these targeted therapies are immune molecules called antibodies (discussed in more detail later) (**Table 2**).

TABLE 2. SELECTED TARGETED THERAPIES

ONCOGENE	DRUG TRADE NAME (GENERIC)
HER2/neu	Herceptin (trastuzumab), Perjeta (pertuzumab)
EGFR	Tarceva (erlotinib), Erbitux (cetuximab), Vectibix (panitumumab)
VEGF	Avastin (bevacizumab)
VEGFR2	Cyramza (ramucirumab)
BCR/ABL	Gleevec (imatinib)
BRAF	Nexavar (sorafenib), Zelboraf (vemurafenib), Mekinist (trametinib)

### 1.3 CANCER INCIDENCE AND MORTALITY IN THE U.S.

According to the latest figures from the American Cancer Society (ACS), more than half a million people in the United States will die of cancer in 2016 alone, roughly 1,600 people per day. This makes cancer second only to heart disease as the leading cause of death in the U.S. The cost of cancer to the U.S. economy was estimated to be \$74.8 billion for 2013. According to the latest projections, one out of every four U.S. residents alive today will eventually die of cancer in the absence of major breakthroughs in prevention and treatment.

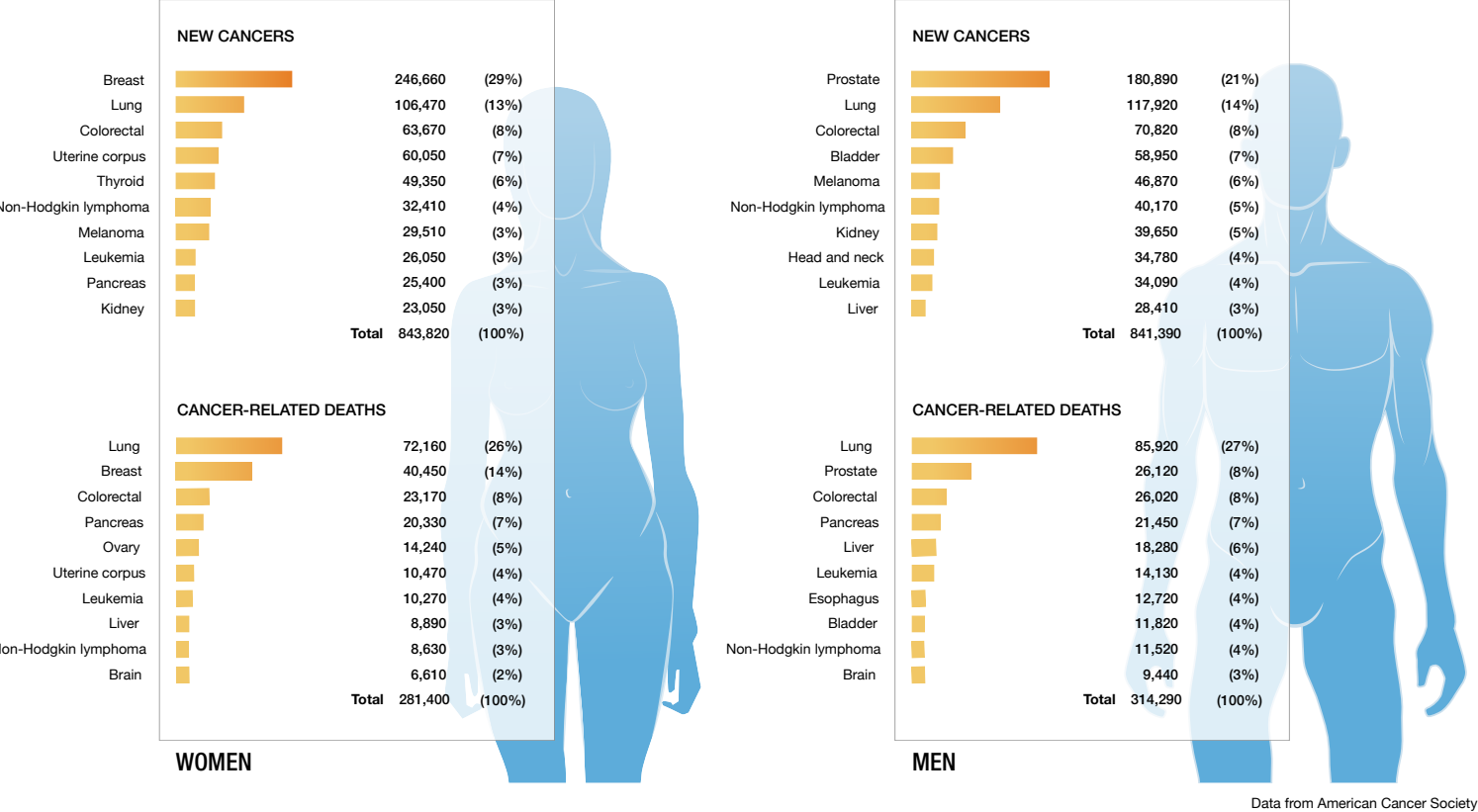
In terms of mortality rate, lung cancer is by far the biggest killer, followed by cancer of the breast, prostate, and colon and rectum. Even when men and women are considered separately, lung cancer is still the biggest killer (**Figure 2**).

There is mounting evidence that obesity increases the likelihood of developing several different types of cancer, including breast, colorectal, uterine, kidney, and others. With more than one-third (35 percent) of American adults classified as obese, this is troubling news.

The good news is that the rate of new cancer cases and deaths for all cancers combined, as well as for most of the top 10 cancers, has been declining. The ACS’s *Cancer Facts and Figures 2016* report shows that the incidence rate—the number of new cancer cases per 100,000 persons per year—for all cancers combined declined by an average of 1.8 percent per year between 2008 and 2012 in men, while staying roughly the same in women. Rates of cancer deaths have been declining continuously for two decades, dropping about 23 percent since 1991. This translates to about 1.7 million fewer cancer deaths over that time period. The reduction in deaths from cancer has been attributed to better screening and advances in treatment, as well as to the decline in the prevalence of smoking. These positive findings reflect the progress that has been made against cancer.

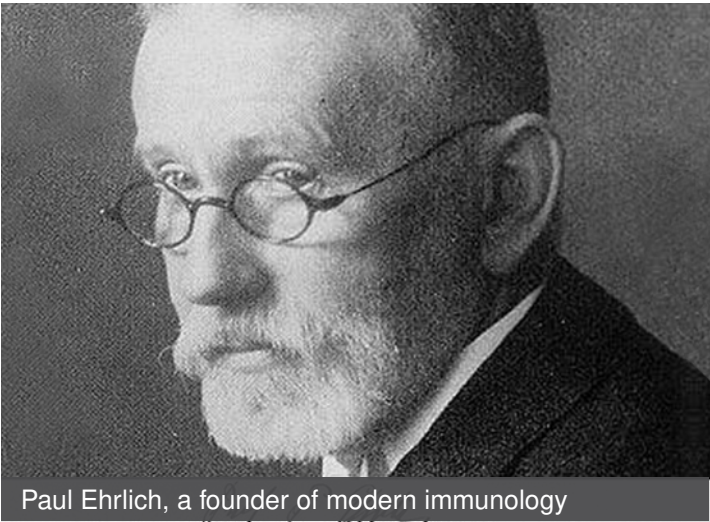
Nevertheless, cancer remains a devastating illness for far too many individuals and the need for better treatments is urgent.

FIGURE 2. 2016 U.S. CANCER INCIDENCE AND MORTALITY



# 1.4 CANCER AND THE IMMUNE SYSTEM

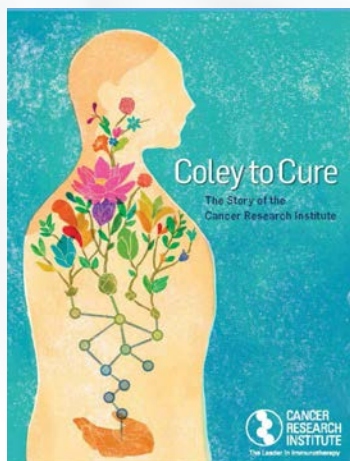
The idea that the immune system can aid in the fight against cancer has deep roots. In 1909, the German scientist Paul Ehrlich proposed that the incidence of cancer would be much higher were it not for the action of our immune system in recognizing and eliminating tumor cells. Half a century later, two scientists, Lewis Thomas and Frank Macfarlane Burnet, took Paul Ehrlich's original idea a step further and proposed the model of "immunosurveillance," where cells of the immune system actively patrol the body looking for cancerous cells and eliminate them as they arise. This idea became a grounding principle of the new field of **cancer immunology** that took shape beginning in the 1950s.



It would take several more decades before the concept of immunosurveillance was given firm scientific support. An important breakthrough came in 2001, when CRI Scientific Advisory Council associate director Robert D. Schreiber, Ph.D., founding medical director Lloyd J. Old, M.D., and member Mark J. Smyth, Ph.D., showed that mice genetically engineered to lack important immune system components had higher rates of cancer. This work provided some of the first, clear evidence in support of the immunosurveillance hypothesis. Other support came from observations made in humans. For example, patients with HIV/AIDS, whose immune systems are severely compromised, were noted to have higher rates of a rare cancer called Kaposi sarcoma. And patients receiving organ transplants whose immune systems were medically suppressed to prevent organ rejection also had higher rates of several types of cancer. These data clearly showed that the immune system helps to protect us from cancer.

Since the validation of the immunosurveillance hypothesis, researchers have learned a great deal more about how the immune system works, including why it sometimes fails to protect us from cancer. They have also learned a variety of ways to manipulate the immune system to make it a more effective cancer fighter.

In the next part, we introduce the structure and function of the immune system, focusing on how its different organs and cells cooperate to protect us from disease. Following that, we explore in detail the modern forms of cancer immunotherapy that researchers have developed based on a scientific understanding of how the immune system functions.



For more on the historical development of cancer immunology as a field, see [Coley to Cure: The Story of the Cancer Research Institute](#).







# PART 2

## THE HUMAN IMMUNE SYSTEM

The environment in which we live contains a wide range of **pathogens**—microbes such as bacteria, viruses, and fungi that can cause disease. It is the primary job of our immune system to defend the body against these pathogens, preventing infections. As we will see, the **immune system** also plays an important role in protecting us from cancer (Figure 3).

In broad terms, the immune system is divided into two arms: the innate and adaptive immune systems. The **innate immune system**

is evolutionarily ancient, shared across animals as diverse as sea sponges, fruit flies, and humans. It consists of defenses that are inborn and always active. The **adaptive immune system** is a much more recent evolutionary development, having evolved only 500 million years ago. It consists of defenses that must be primed over time to become fully active. The two systems cooperate to protect us from harm (Table 3).

### FIGURE 3. ANATOMY OF THE IMMUNE SYSTEM

The immune system is an integrated series of organs, tissues, cells, and molecules that cooperate to protect us from pathogens and cancer.

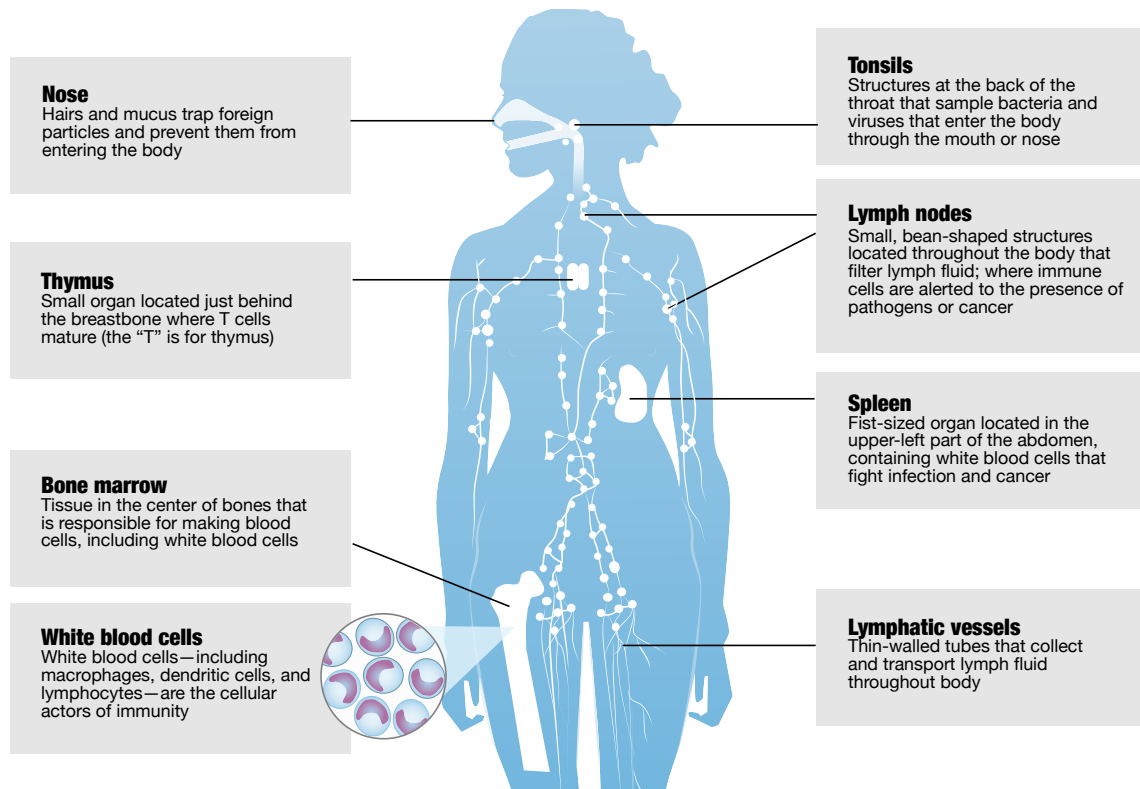


TABLE 3. TWO ARMS OF THE IMMUNE SYSTEM

INNATE IMMUNE SYSTEM		ADAPTIVE IMMUNE SYSTEM
EXTERNAL DEFENSES	INTERNAL DEFENSES	<ul style="list-style-type: none"><li>• Humoral (antibody-mediated) immune response</li></ul>
<ul style="list-style-type: none"><li>• Skin, hair, cilia</li></ul>	<ul style="list-style-type: none"><li>• Inflammatory response</li></ul>	<ul style="list-style-type: none"><li>• Cell-mediated immune response</li></ul>
<ul style="list-style-type: none"><li>• Mucus and chemical secretions (e.g., lysozyme in tears)</li></ul>	<ul style="list-style-type: none"><li>• Complement proteins</li></ul>	<ul style="list-style-type: none"><li>• Memory cells</li></ul>
<ul style="list-style-type: none"><li>• Digestive enzymes in mouth, stomach acid</li></ul>	<ul style="list-style-type: none"><li>• Phagocytic cells (macrophages, neutrophils, natural killer cells)</li></ul>	

## 2.1 INNATE IMMUNITY: OUR FIRST LINE OF DEFENSE

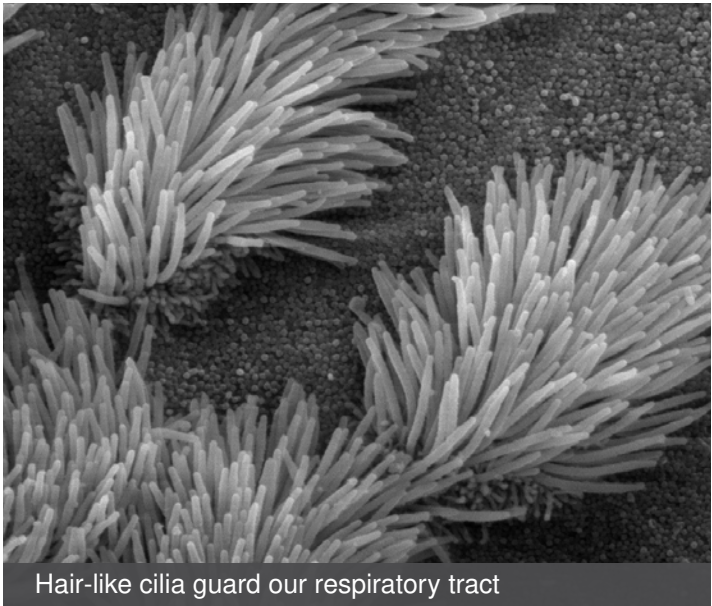
The innate immune system consists of mechanical, chemical, and cellular defenses that work to keep pathogens from gaining a foothold in the body. The skin, for example, acts as a kind of coat of armor, preventing pathogens and dangerous substances from entering the body. In addition, the skin produces acidic substances that make it difficult for many kinds of bacteria to grow on it. Certain harmless bacteria and fungi thrive on our skin; these also constitute a defense mechanism, as they tend to compete with and crowd out harmful microbes.

Our nose provides a first line of defense against foreign airborne particles entering our bodies. Nose hairs block large particles from entering the respiratory tract. Further down the respiratory tract, a slippery substance called mucus traps dust, pathogens, and other foreign debris. Hair-like projections called cilia then sweep these particles away from the respiratory tract and toward the esophagus, where they can be coughed up or swallowed. Pathogens entering our bodies orally, principally in food, are destroyed by gastric juice—a potent mixture of acid and enzymes that chew up the invading pathogens into harmless molecular bits.

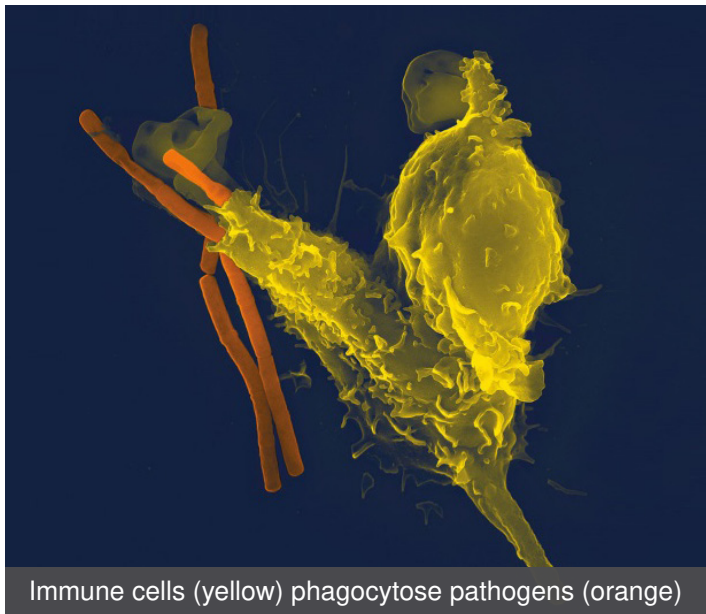
Other parts of the body that are exposed to the environment have additional defenses. Our tears and saliva contain an enzyme called lysozyme that cuts and destroys the cell wall surrounding some bacteria. And urine helps flush bacteria from the urethra.

These physical and chemical defenses do a very good job of keeping pathogens out of the body. But they are not 100 percent effective. Breaches in these barriers do sometimes occur and pathogens succeed in lodging themselves within the body. What happens then?

The next line of defense consists of innate immune cells charged with the task of fending off invaders. Innate immune cells include **macrophages** (“big eaters”), **neutrophils**, **dendritic cells**, and



Hair-like cilia guard our respiratory tract



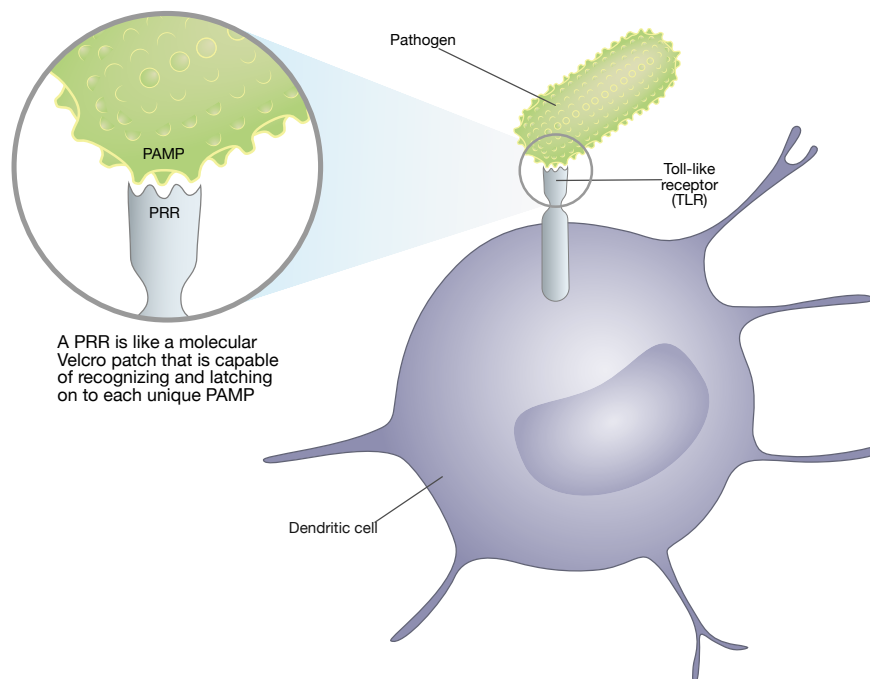
**natural killer cells.** These cells perform numerous important jobs, including gobbling up dangerous pathogens and cellular debris through a process called **phagocytosis** (“cell eating process”). They also release inflammatory molecules that alert the body to the presence of danger.

How do cells of the innate immune system recognize dangerous invaders? These cells have evolved the ability to recognize distinct molecular targets on commonly encountered pathogens. These targets are known as **pathogen-associated molecular patterns (PAMPs)**, and they are found on all microorganisms. PAMPs are recognized by distinct **pattern recognition receptors (PRRs)** located on cells of the innate immune system. A PRR is like a molecular Velcro patch that is capable of recognizing and latching on to each unique PAMP (**Figure 4**).

Examples of PAMPs include lipopolysaccharide (also known as endotoxin) and peptidoglycan found in bacterial cell walls; double-

#### FIGURE 4. INNATE IMMUNE CELLS RECOGNIZE MOLECULAR PATTERNS ON PATHOGENS

Pathogen-associated molecular patterns (PAMPs) are found on all microorganisms and are recognized by distinct pattern recognition receptors (PRRs) found on cells of the innate immune system.



stranded DNA found in viruses; and a molecule called flagellin found in bacterial flagella. It is estimated that several hundred PRRs exist in the vertebrate innate immune system and that they are so vital to life that they are encoded in our genes. An important class of PRRs is Toll-like receptors (TLRs). As we will see later, molecules that bind to these receptors can be potent components of cancer immunotherapies.

## 2.2 ADAPTIVE IMMUNITY: LEARNING THE ENEMY'S TRICKS

Unlike the innate immune system, which is present from birth and is constantly active, the adaptive immune system takes time to develop. It is essentially non-existent at birth, but as we grow and are exposed to new germs, it learns to recognize them.

The adaptive immune system is characterized by three unique features: specificity, diversity, and memory.

We say the adaptive immune system is *specific* because it recognizes and defends us against particular threats—a specific virus, for example. It is very important that the immune system be able to discriminate among targets so that it does not attack components of our own body.

The adaptive immune system is described as *diverse* because it has the remarkable ability to detect essentially any foreign molecule it encounters—billions of different targets. The upside of this immense capacity for recognition is that we can acquire immunity to just about every pathogen we may encounter in our lifetime. The downside is that it takes time—on the order of 4-7 days—to mount an immune response against a new invader. By the time the adaptive immune system is capable of responding, we may already be quite sick.

Fortunately, the adaptive immune system has *memory* and will remember past invaders. When the adaptive immune system encounters a pathogen it has seen before, the immune response is much quicker—on the order of hours. This immunological memory provides us with protection throughout our life. We call this protection **immunity**. Unfortunately, as we enter advanced age, our adaptive immune system begins to falter—a fact with important consequences for cancer development.

The adaptive immune system is made up of specialized white blood cells called **lymphocytes**, which possess an exquisite capacity to recognize pathogens and other harmful invaders. Unlike the cells of the innate immune system, which come equipped with only a limited set of pathogen-identifying receptors that are encoded in the genes, lymphocytes can generate a nearly infinite variety of new receptors. Through a process of genetic re-shuffling and re-stitching of DNA, developing lymphocytes can assemble new receptors that are fine-tuned for recognizing distinct enemies.

There are two main types of lymphocyte: **B cells** and **T cells**. Like all blood cells, lymphocytes are born in the bone marrow. As they mature, they exit the **bone marrow** and circulate in the blood. T cells develop to maturity in the **thymus gland** (the “T” stands for thymus). Mature B and T cells are deployed to **lymph nodes** and other lymphoid organs where they lie in wait for pathogens.

In addition to the receptors through which they recognize invaders, lymphocytes also produce and release chemicals that enable them to communicate with each other—for example, to alert other immune cells that there is an infection in the body. The **cytokines** are an important class of such chemicals.

Long before scientists knew about the cells and molecules making up the adaptive immune system, physicians made use of its powers to improve health. The adaptive immune system forms the basis of **vaccination**, which humans have practiced for centuries. The earliest known form of vaccination, called variolation, was first practiced in China in the 10th century as a way to prevent the disease smallpox. It involved exposing healthy people to the infectious material found in a smallpox lesion. The person would get sick, though often not as bad as when contracting the disease naturally; if they didn't die, they would be protected against a future exposure.

Variolation was introduced into Europe by the wife of the British ambassador to Turkey, Lady Montagu, who observed the practice being performed in Istanbul in 1718. In 1796, an English physician named Edward Jenner hit upon an improvement over variolation. Jenner observed that milkmaids who contracted cowpox, a relatively mild disease caught from the cows they milked, seemed to be protected from the related but more deadly smallpox. He hypothesized that the cowpox had somehow

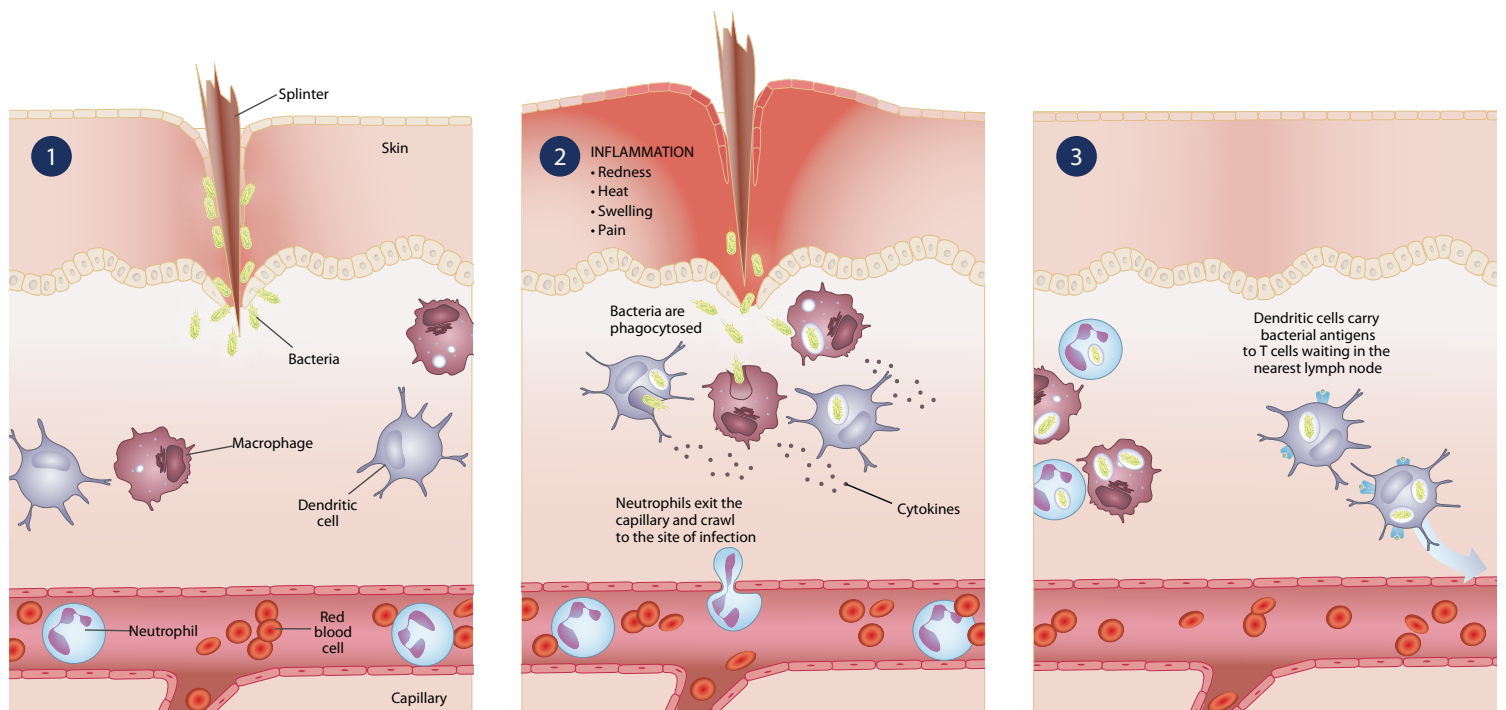
conferred protection against smallpox to the milkmaids. To test this hypothesis, he injected the material obtained from a cowpox pustule from the hand of an infected milkmaid into the arm of an 8-year-old boy. Six weeks later, after the boy had recovered from the cowpox infection, Jenner inoculated him with smallpox (an experiment that would be considered highly unethical today!). As Jenner had hoped, the boy never developed the disease. Jenner's technique became known as "vaccination," derived from the Latin word "vacca," meaning cow. Even without a scientific understanding of how his method worked, Jenner had discovered a safe and effective way to prevent people from developing a serious illness.

It was not until the late 19th century, when the germ theory of disease was developed, that scientists understood what was in Jenner's vaccine that made it effective, and even longer until scientists understood the nature of the immune response that it triggered. Today we know why Jenner's vaccine was effective: it primed the boy's immune system to make memory lymphocytes

that recognized the smallpox virus. This pioneering immunological work eventually gave rise to vaccines against many other diseases, including rabies, diphtheria, yellow fever, polio, mumps, hepatitis B, measles, rubella, influenza, whooping cough, and tetanus.



**FIGURE 5. INFLAMMATION: HOW IMMUNE RESPONSES GET STARTED**





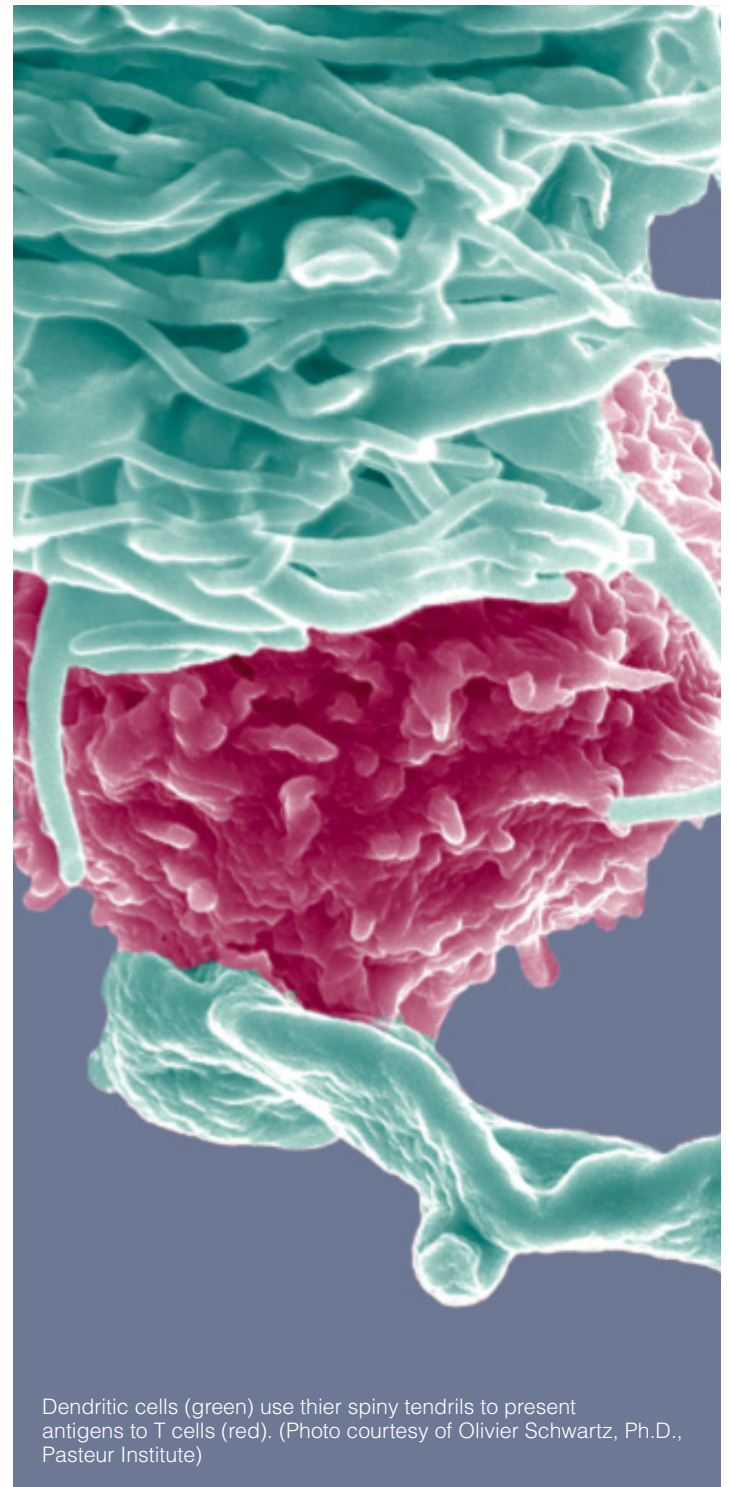
## 2.3 INFLAMMATION: LINKING INNATE AND ADAPTIVE IMMUNITY

The innate and adaptive arms of the immune system cooperate to defend the body against infections and cancer. Consider what happens when a person gets a splinter in her finger. The splinter breaches the physical barrier of the skin, part of the innate immune system. The splinter will likely transfer several hundred bacterial cells into the wound.

A few minutes after sustaining the splinter wound, the injured site will become red, warm, swollen, and painful: this is **inflammation**. Inflammation is a direct effect of the accumulation of immune cells and fluid at the site of injury. It is a normal process that contributes to both immune defense and wound healing (**Figure 5**).

The earliest immune responders to the site of injury will be cells of the innate immune system, including macrophages and neutrophils. Macrophages reside in most tissues and are usually the first immune cells to encounter pathogens. Macrophages are able to recognize the pathogens as foreign and harmful because they come bristling with PAMPs on their surface. At the site of infection, macrophages “eat up”—phagocytose—the invading pathogens. Macrophages also release cytokines that recruit other innate immune cells, principally neutrophils, to the site. Neutrophils normally circulate in the blood, but when they receive a cue from macrophages, they can leave the blood vessel and enter the infected tissue where they help to destroy the invaders. Once the dangerous pathogens have been eliminated and the wound has healed, the redness, warmth, swelling, and pain will abate.

Inflammation plays an important role in cancer, as well. Like wounds, developing tumors cause tissue damage that triggers an inflammatory response. This inflammatory response can help nip developing tumors in the bud by destroying the cancerous cells. But, unfortunately, inflammation can also play a negative role in cancer. Inflammation that goes on for too long—chronic inflammation—can actually promote the development of some cancers by creating an environment that damages DNA and encourages metastasis. This relationship between chronic inflammation and tumor development has led scientists to liken tumors to “wounds that do not heal.”



Dendritic cells (green) use their spiny tendrils to present antigens to T cells (red). (Photo courtesy of Olivier Schwartz, Ph.D., Pasteur Institute)

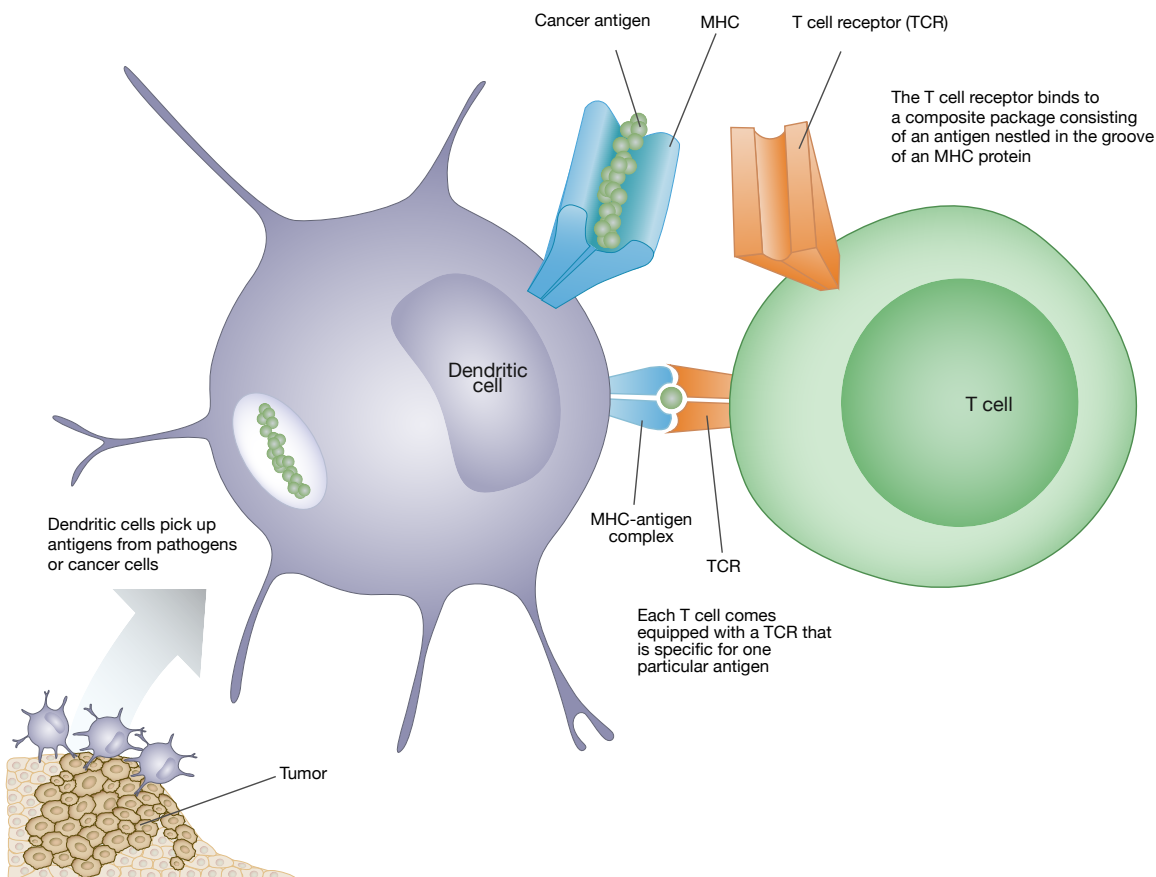
In addition to destroying pathogens and cancer directly, another role for cells of the innate immune system is to “present” targets to cells of the adaptive immune system—a process called **antigen presentation**. They do this by engulfing pathogens or cancer cells and then posting bits of these antigens into the groove of a protein called the **major histocompatibility complex (MHC)**, which protrudes from the cell surface. You can think of MHC as the bun of a hotdog; the antigen to be displayed sits in MHC much like a hotdog sits in a bun. The MHC-antigen complex is then recognized by a corresponding **T cell receptor (TCR)** on a T cell; this binding of the TCR to the MHC-antigen complex is how T cells “see” their targets (**Figure 6**).

The best **antigen-presenting cells (APC)** in the body are dendritic cells, but macrophages can also do this important work. By serving as the initial trigger of an adaptive immune response, these APCs are the link between the two arms of the immune system, innate and adaptive.

There are two types of MHC proteins, class I and class II. Macrophages and dendritic cells use MHC class I and II molecules to display antigens. Other cells in the body use just MHC class I molecules. In an analogous manner, different subsets of T cells use slightly different machinery to bind the MHC-antigen. While all T cells have a TCR that detects antigen, different subsets of T cells

### FIGURE 6. HOW T CELLS RECOGNIZE INFECTED AND CANCEROUS CELLS

T cells are alerted to the presence of pathogens or cancer in the body by professional antigen-presenting cells. T cells “see” antigens with the help of their T cell receptor.



have different “co-receptors” that assist in MHC binding. “Helper” T cells use a molecule called CD4 as a co-receptor, while “killer” T cells use CD8.

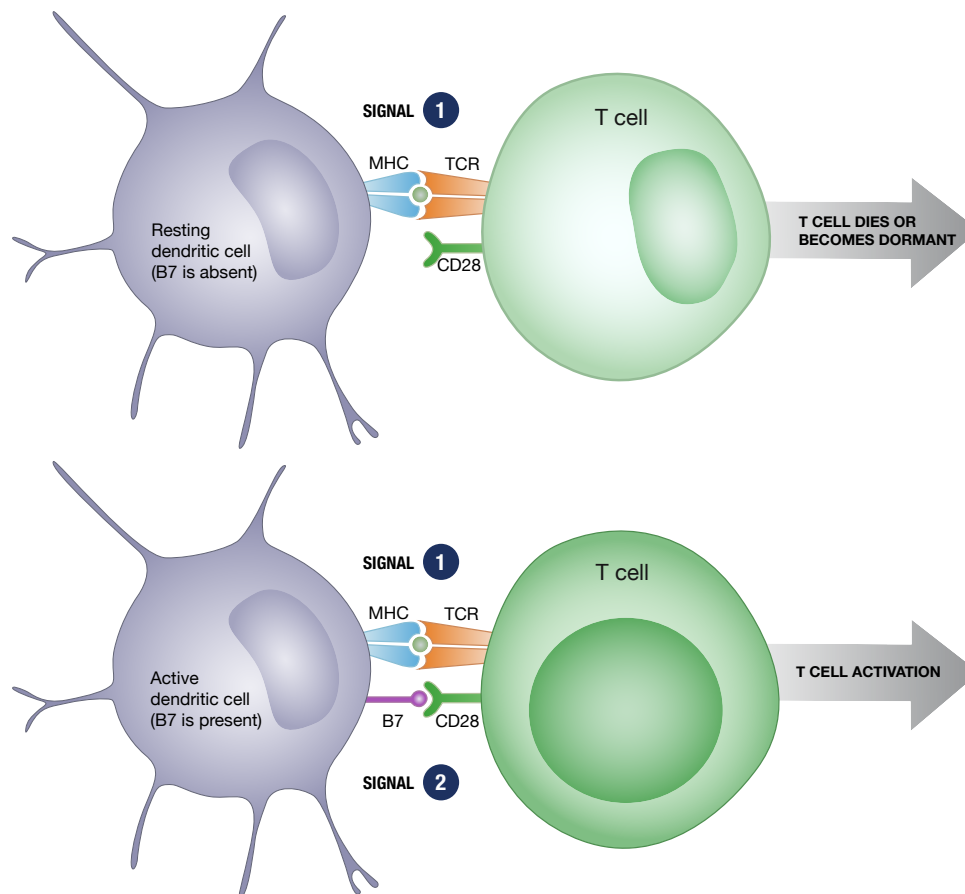
The antigen recognition step discussed above is a crucial one for the immune response, but it's not the only one. In fact, two separate signals are required to activate a T cell and turn it into an immune fighter. Binding of the TCR to the MHC-antigen complex can be considered “signal 1.” This is the antigen recognition step. The second signal is provided by the binding of a molecule called B7 on the APC to a molecule called CD28 on the T cell (“signal 2”).

This second signal is referred to as **co-stimulation**. Both signals must occur to activate a T cell; if signal 2 is missing, the T cell will become dormant or die.

The presence or absence of signal 2 is an important way that the immune system learns to recognize “self” from “non-self.” B7 is present on APCs that have encountered “danger signals” such as pathogens or inflammatory cytokines. B7 is absent on resting APCs. Therefore, T cells are only stimulated to attack an antigen when it is presented in the context of a danger signal; otherwise they learn to stand down (**Figure 7**).

### FIGURE 7. CO-STIMULATION: TWO SIGNALS ARE REQUIRED TO ACTIVATE A T CELL

In addition to the signal provided by MHC-antigen (“signal 1”), another signal is required to activate a T cell and turn it into an immune fighter. This second signal (“signal 2”) is provided by co-stimulatory molecules such as B7.



## 2.4 THE HUMORAL IMMUNE RESPONSE: MAKING ANTIBODIES

Coursing through our blood at any given moment are many different types of **antibodies**. Antibodies are Y-shaped molecules that bind to precise molecular targets, called **antigens**, on pathogens and cancer. By binding to a pathogen or cancer cell, antibodies mark their target for destruction by the immune system.

This destruction can occur in two different ways. First, binding of antibodies to antigens makes it easier for macrophages and other innate immune cells to engulf the invading bacteria or cancer cell. Second, it activates **complement**—a group of proteins that literally punch holes in the target cells causing them to lyse, or burst (**Figure 8**).

Antibodies play a crucial role in what's called the **humoral immune response**—the cascade of events that leads to the destruction of invading bacteria and other pathogens found circulating in the blood. (“Humoral” means “of the humors,” an older way of referring to blood and bodily fluids.)

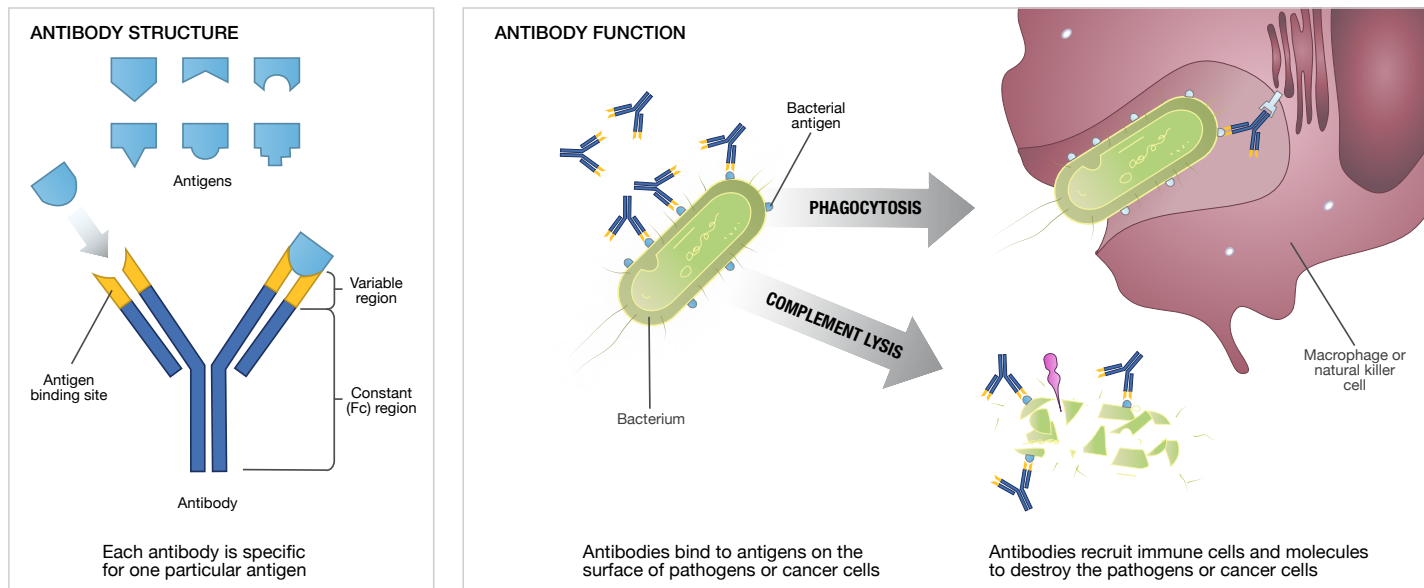
The humoral immune response begins when invading bacteria are eaten by an antigen-presenting cell (APC) such as a dendritic cell or macrophage. The bacterium is digested and its antigens are processed and presented in combination with MHC (class II) on the surface of the APC. The MHC-antigen complex is then recognized by a type of immune cell called a **helper T cell**. Additional co-stimulatory signals lead to activation of the helper T cell.

Once activated, the helper T cell releases cytokines including interleukin-2 (IL-2), which causes helper T cells to multiply. The proliferating helper T cells release other cytokines that signal a B cell (that also specifically recognizes the antigen) to begin multiplying and differentiating into **plasma cells**.

Plasma cells are the cells that actually produce and secrete antibodies. The antibodies released by the plasma cells bind specifically and tightly to antigens on the surface of invaders that survived the initial attack by macrophages. Each plasma cell is programmed to make one specific antibody, which can bind to one specific antigen (**Figure 9**).

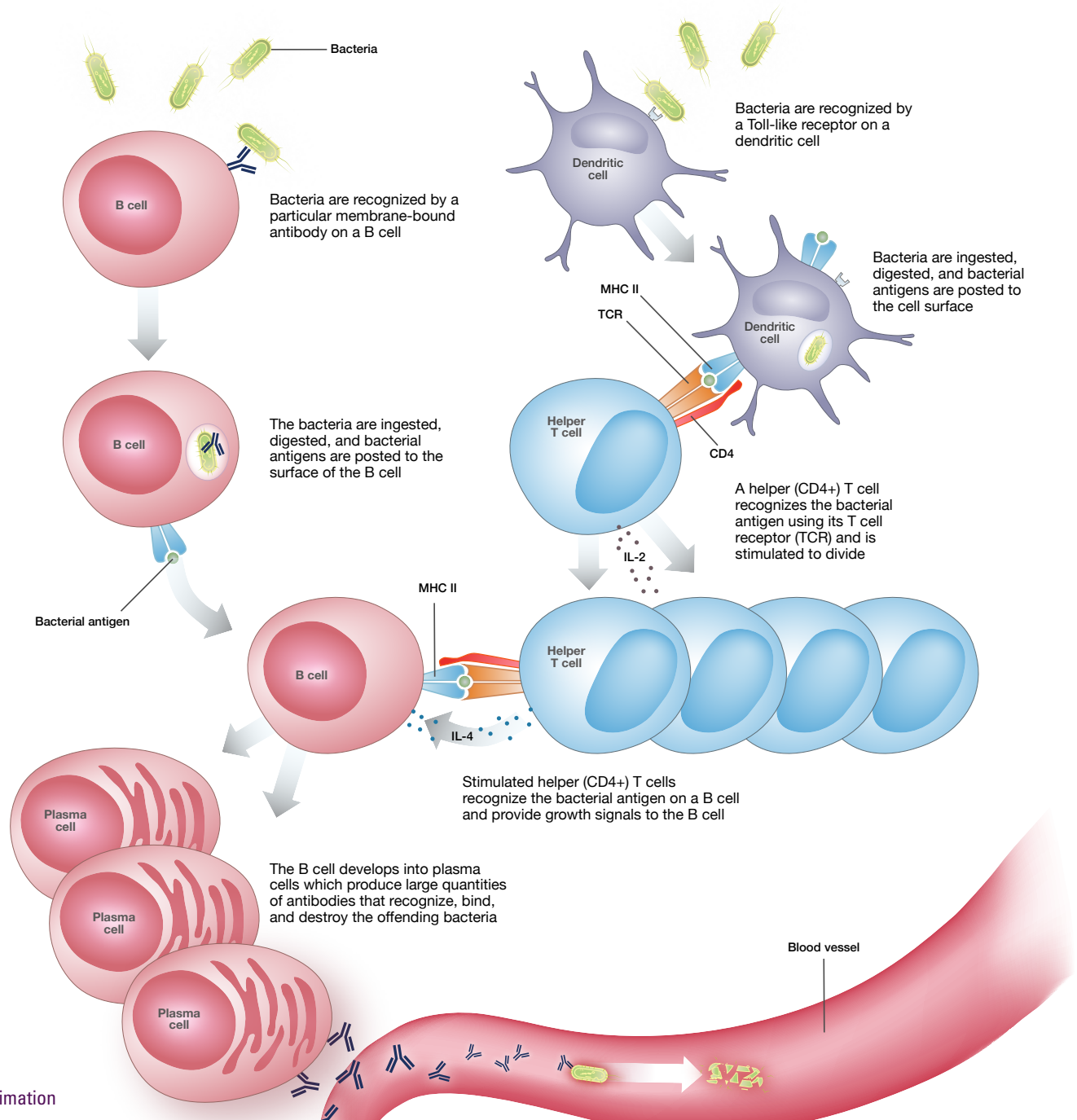
### FIGURE 8. ANTIBODIES BIND TO ANTIGENS ON PATHOGENS AND CANCER

Antibodies are proteins that protect the body from free-floating pathogens in the blood or lymph fluid. Antibodies bind to antigens on pathogens, which marks them for destruction by other cells and molecules of the immune system. They can also bind to antigens on cancer cells.



## FIGURE 9. HUMORAL (ANTIBODY-MEDIATED) IMMUNE RESPONSE

The humoral immune response produces antibodies, which protect the body from free-floating pathogens and toxins circulating in the blood and lymph fluid.





As we will see, antibodies can be modified in the laboratory for use in cancer immunotherapy, where their great specificity for targets is a very useful feature.

## 2.5 THE CELLULAR IMMUNE RESPONSE: MAKING “KILLER” T CELLS

In addition to defending the body against free-floating pathogens swimming in the blood, the immune system must also defend the body against intracellular pathogens—viruses mainly—which reproduce inside cells. Killing intracellular pathogens is the job of the **cellular immune response**, which makes “killer” T cells to attack infected cells.

The cellular immune response begins when an antigen-presenting cell, such as a dendritic cell, encounters its target—a flu virus, for example. The dendritic cell devours the virus and then presents the digested viral antigens on its surface in conjunction with MHC (class II) proteins. A helper T cell recognizes the displayed antigen on the dendritic cell and binds to the MHC-antigen complex. Through the action of additional co-stimulatory signals, the T cell becomes activated.

The activated helper T cell releases chemical messengers such as the cytokines IL-2 and interferon gamma (IFN- $\gamma$ ), which stimulate killer T cells to take action against cells that have been infected

with the same virus. The flu virus, for instance, is likely to infect epithelial cells lining the respiratory tract. Inside epithelial cells, viral proteins are digested, and subsequently posted on the cell surface in conjunction with MHC (class I) proteins.

The activated killer T cells are now ready to encounter epithelial cells infected with the flu virus. The activated killer T cells bind to the MHC-antigen complex on the surface of the infected epithelial cells. This binding causes the killer T cell to release a potent chemical called perforin, which perforates the cell membrane of the infected cells, causing the cells to burst. As the viral infection is brought under control, the killer T cells are turned off. Memory T cells remain behind to respond quickly if the same virus attacks again (**Figure 10**).

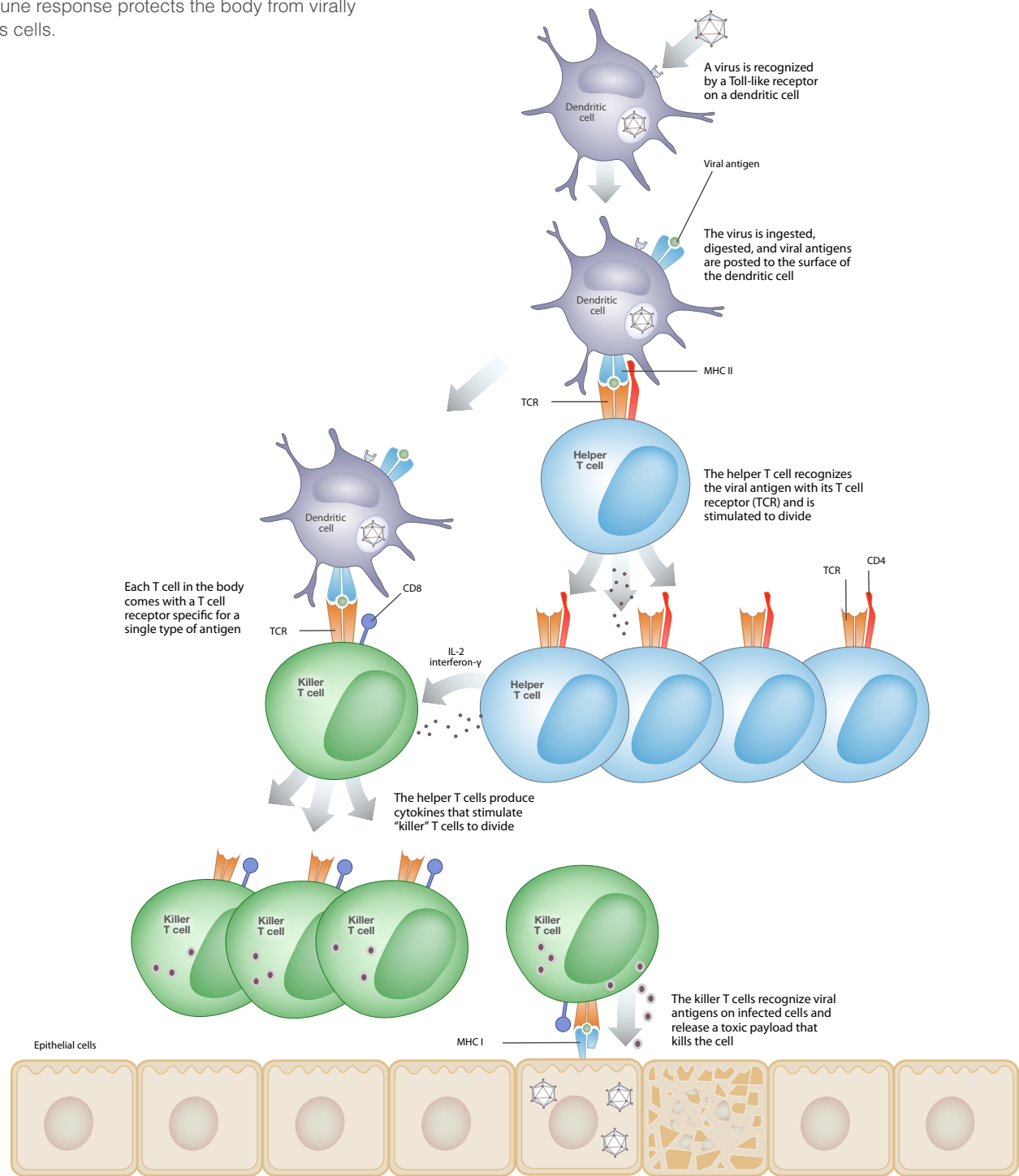
Although we have explained the humoral and cell-mediated immune response primarily in the context of pathogens like bacteria and viruses, it is important to note that the immune system reacts in a similar manner when it encounters cancer cells, which it may also recognize as foreign or “non-self.” Scientists have discovered that the immune system is capable of destroying tumor cells, and that the body defends itself against cancer in much the same way that it defends itself against infection. Obviously, the system is far from perfect, since cancer will affect one in three people in their lifetime. But with deeper understanding of the immune system, the hope is that our anti-cancer defenses can be strengthened (**Table 4**).

TABLE 4. SUMMARY OF HUMORAL AND CELL-MEDIATED IMMUNITY

	HUMORAL	CELL-MEDIATED
Cell type	B cells	T cells
Mechanism of killing	Circulating antibodies	Direct cell-to-cell contact
Purpose	Primary defense against extracellular pathogens, e.g., certain bacteria and free-floating viruses	Primary defense against intracellular pathogens—e.g., viruses, fungi—and cancer
Type of cancer antigens recognized	Extracellular cancer antigens, like HER2 and other overexpressed cell-surface proteins	Intracellular cancer antigens, like NY-ESO-1 and mutated proteins

**FIGURE 10. CELL-MEDIATED IMMUNE RESPONSE**

The cell-mediated immune response protects the body from virally infected and cancerous cells.



## 2.6 TOLERANCE AND THE PROBLEM OF AUTOIMMUNITY

T cells play a crucial role in both humoral and cell-mediated immune responses, and therefore in protecting us from all kinds of infections, as well as cancer. To be effective, T cells must be able to distinguish between foreign antigens and self-antigens. If a T cell were to stimulate an immune response against a normal protein found in the body, for instance, the consequences would be disastrous—akin to the body waging war against itself. In fact, this does happen occasionally, and the result is an **autoimmune disease**—such as rheumatoid arthritis, multiple sclerosis, or lupus—in which the immune system attacks parts of the body.

To avoid this kind of collateral damage, young T cells are subjected to a strict training program in the **thymus**, an organ located in the chest. As part of their training, the developing T cells are exposed to as many self-antigens as possible and any T cell that reacts against these self-antigens is eliminated. This rigorous training regime ensures that the remaining T cells are “tolerant” of self-antigens and will attack only non-self-antigens. As we have seen, “co-stimulatory” signals (e.g., from B7 on an antigen-presenting cell to CD28 on a T cell) play an important role in determining **tolerance**. T cells become tolerant to self-antigens that are presented to a T cell in the absence of a co-stimulatory signal.

Another important way that the body guards against autoimmunity is through the action of **regulatory T cells (Tregs)**, which are T cells that rein in the actions of activated killer T cells. Tregs are an important area of research in cancer immunology because it appears that in some cases anti-cancer immune responses are curtailed prematurely by the action of these guardian cells. Scientists are interested in selectively counteracting these cells to unleash more powerful immune responses against cancer.





Rheumatoid arthritis, an autoimmune disease, is caused by chronic inflammation of the joints in the hands and feet.

# PART 3

## CANCER IMMUNOTHERAPY

The aim of cancer immunotherapy is to harness, strengthen, and sustain the power of the immune system to fight cancer. Though not as well-known as chemotherapy and radiation, immunotherapy actually predates both of these cancer treatments. It was, in fact, the first non-surgical treatment of cancer.

### 3.1 HISTORICAL ORIGINS

The connection between cancer and the immune system was first uncovered more than 100 years ago, long before an in-depth knowledge of the immune system existed. In the early 1890s, William B. Coley, M.D., a New York-based surgeon, stumbled upon a surprising finding in case files at the hospital where he worked: a patient's cancer had regressed after he came down with an acute bacterial infection. Suspecting that the bacterial infection was in some way responsible for the regression of the tumor, Coley decided to try an experiment in which he deliberately injected live bacteria into a patient with inoperable cancer to see whether the patient's tumor would regress. To his astonishment, the experiment worked, and the patient lived for another 26 years until a heart attack took his life.

Coley continued to pursue his approach and ultimately developed a mixture of killed bacteria that became known as Coley's mixed bacterial toxins, or simply "Coley's toxins." He and other physicians treated over 1,000 cancer patients with these toxins, with varied success. Though the treatment clearly worked in some cases, the results were unpredictable, and neither Coley nor the medical community at large could explain precisely why his mixture worked when it did. This was due to the fact that the science of immunology was still rudimentary at the time. As other cancer treatments—first radiation, then chemotherapy—became popular, Coley's method faded from view and was virtually forgotten for years.



William B. Coley

Renewed interest in Coley's work was sparked by his daughter, Helen Coley Nauts, who, in the 1940s, began compiling and disseminating information on patients treated with the toxins. Nauts's work showed clearly that many patients had indeed benefited—sometimes achieving complete remissions—from Coley's toxins. Gradually, as scientists learned more about the immune system, they began to understand how Coley's preparation worked: the bacterial products of which it was composed had acted as immune stimulants, goading the immune system into killing the cancer cells along with the bacteria.



Helen Coley Nauts

To support research into this area, Nauts founded the Cancer Research Institute in 1953, which ever since has funded the work of scientists studying the link between cancer and the immune system. Today, cancer immunology is a thriving field and Coley has come to be regarded as the "Father of Cancer Immunotherapy."

### 3.2 NON-SPECIFIC IMMUNE STIMULANTS

The first modern immunotherapies took their cue directly from Coley's toxins and were essentially extensions of his work. They were attempts at using bacterial products to generate an intensified immune response against cancer. Because these treatments rev up the immune system against all types of pathogens and cancers, they are sometimes referred to as "non-specific immunotherapy" (as opposed to a "specific immunotherapy," which generates an immune response against specific cancer targets). Although first introduced decades ago, these treatments can still be helpful in some instances and may be the best treatment for certain cancers.

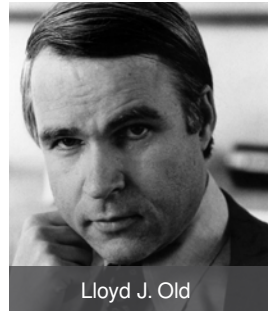


## BACILLUS CALMETTE-GUÉRIN (BCG)

The first modern non-specific cancer immunotherapy was Bacillus Calmette-Guérin (BCG), a weakened form of the bacterium that causes tuberculosis. In the early 20th century, BCG was used as a vaccine against tuberculosis. In the late 1950s, researchers began experimenting with BCG in cancer. A now-classic paper published in 1959 by former Cancer Research Institute medical director Lloyd J. Old, M.D., and colleagues described the effect of BCG on tumor growth in mice. Old injected BCG into mice and then transplanted tumors into them. Remarkably, he found that mice injected with BCG had increased resistance to tumor growth compared to mice that had not received BCG. The BCG-treated mice also lived longer. In the early 1970s, other scientists funded by the Cancer Research Institute found that BCG could be used successfully to treat cancer in humans.

Despite extensive clinical testing showing that BCG was effective, the approach remained controversial. It was not until 1990 that BCG was approved by the FDA as first-line treatment for early forms of bladder cancer, for which it is still used as a mainstay of therapy.

BCG is now known to be a Toll-like receptor (TLR) agonist and a potent activator of the immune response.



Lloyd J. Old

## CYTOKINES

Cytokines are molecules that immune cells use to communicate with each other. They are often used as a form of non-specific immunotherapy.

Cytokines promote tumor immunity in several ways. Some cytokines, such as tumor necrosis factor alpha (TNF $\alpha$ ) and interferon alpha (IFN $\alpha$ ), interact directly with tumor cells, inducing them to either commit suicide or stop growing. Other cytokines, such as IL-2 and GM-CSF, activate important immune cells such as natural killer (NK) cells, T cells, and dendritic cells.

Cytokine therapy is approved for clinical use in several cancers. For example, IL-2 is FDA approved for the treatment of melanoma and kidney cancer. IFN $\alpha$  is FDA approved for the treatment of melanoma, chronic myelogenous leukemia, hairy cell leukemia, follicular non-Hodgkin lymphoma, and Kaposi sarcoma.

Because they stimulate the immune system in a general way, cytokines are often combined with other immunotherapies.

Increasingly, cytokines are also being studied for their role in contributing to cancer development. One way they might do this is by promoting chronic inflammation, which has been linked to the development of several types of cancer, including colorectal, bladder, and pancreatic cancer. Therapies designed to reduce the levels of inflammatory cytokines in and around tumors are currently



being tested as a way to improve responses to immunotherapies (Table 5).

ADJUVANTS

Adjuvants are non-specific immune stimulants, often given along with vaccines to generate a stronger immune response. Adjuvants are believed to improve vaccines by creating an optimal environment for the presentation of antigens to cells of the adaptive immune system. Several different adjuvant molecules are being studied in clinical trials.

Commonly used adjuvants in experimental vaccine trials include bacterial products and TLR agonists. Recall that TLRs are the “taste bud” molecules found on dendritic cells and macrophages that detect bacteria and other infectious pathogens. TLR agonists, which bind to TLRs, make good adjuvants because they spur antigen-presenting cells into a frenzy of antigen presentation, including the antigens derived from the vaccine.

GM-CSF, which stimulates dendritic cells to develop, is also often used as an adjuvant with cancer vaccines.

3.3 ANTIBODY IMMUNOTHERAPIES

All the immunotherapies discussed in the previous section have in common the fact that they are not directed at any particular antigen in particular; they stimulate immune cells in a general way. By contrast, the immunotherapies discussed in this section are specific immunotherapies—ones that target particular antigens.

MONOCLONAL ANTIBODIES

As we have seen, antibodies are proteins made by immune cells that circulate in the blood and protect us from free-floating pathogens and cancer cells. Since the mid-1970s, it has been possible to generate abundant quantities of specific antibodies in the laboratory for use as medicines. These purified molecules, called **monoclonal antibodies**, can be likened to heat-seeking missiles, selectively targeting one specific antigen.

Monoclonal antibodies can provide therapeutic benefits in several different ways. They can physically interfere with important signaling molecules on cancer cells and halt their growth; they can promote destruction by macrophages or NK cells; and they

TABLE 5. SELECT CYTOKINES IN IMMUNOTHERAPY

NAME	FUNCTION	TESTING STATUS
Interleukin (IL)-2	Enhances cytotoxic (“killer”) T cell and NK cell function	FDA approved for melanoma and renal cell carcinoma
IL-7	Enhances T cell function	Phase II testing
IL-10	Inhibits tumor antigen presentation	Phase I testing
IL-12	Enhances cell-mediated immune response; inhibits angiogenesis	Phase II testing
IL-15	Enhances cytotoxicity	Phase II testing
IL-18	Enhance tumor antigen presentation and cytotoxicity	Phase I testing
IL-21	Enhances T cell and NK cell function	Phase I/II testing
GM-CSF	Enhances tumor antigen presentation	FDA approved for use with bone marrow transplants; phase III testing with cancer vaccines
Interferon (IFN)-alpha	Enhances tumor antigen presentation and cytotoxicity	FDA approved for melanoma, chronic myelogenous leukemia, hairy cell leukemia, follicular non-Hodgkin lymphoma, and Kaposi’s sarcoma
IFN-gamma	Enhances tumor antigen presentation and cytotoxicity	Phase I testing
Tumor necrosis factor-alpha	Induces tumor-cell death	Phase III testing

can activate complement that causes tumor cells to burst (**Figure 11**).

Monoclonal antibodies can also be fitted with poisons or radioactive isotopes that deliver a deadly payload to cancer cells.

To date, nearly 20 monoclonal antibodies have been approved by the FDA for use in cancer treatment (**Table 6**). Three of the most common are Rituxan®, Herceptin®, and Avastin®.

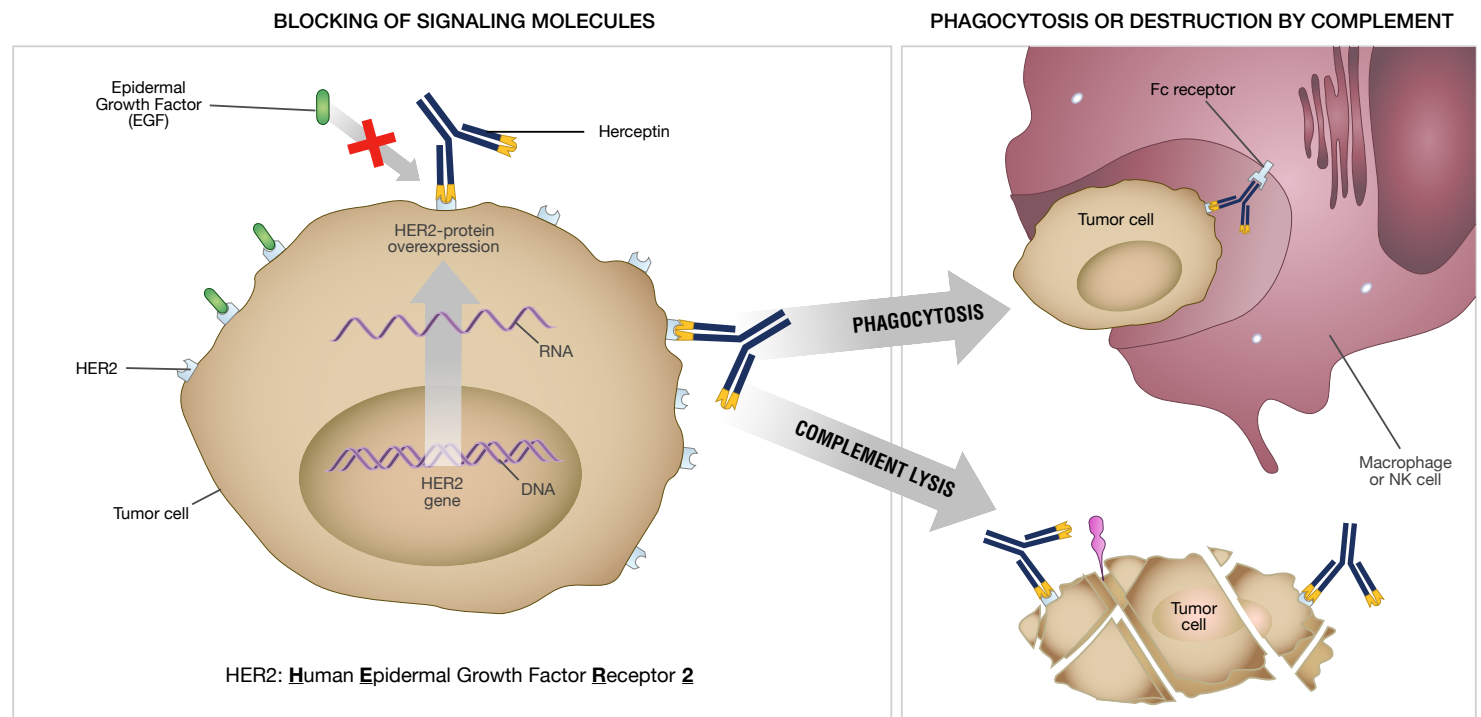
Rituxan (rituximab) is a monoclonal antibody specific for an antigen called CD20, found on the surface of both normal and cancerous B cells. Rituxan destroys cells displaying the CD20 marker by promoting phagocytosis and complement lysis. Rituxan was

approved by the FDA in 1997 for the treatment of relapsed or refractory B cell non-Hodgkin lymphoma. In February 2006, Rituxan received FDA approval as first-line treatment of diffuse large B cell lymphoma (DLBCL) in combination with chemotherapy (R-CHOP). It is also approved for the treatment of CD20-positive chronic lymphocytic leukemia (CLL).

While the CD20 marker is found on both normal B cells and cancerous B cells, it is absent from the plasma cells that make antibodies, so humoral immunity is not completely interrupted by this treatment. And because new B cells are continually born, blood levels of B cells will likely return to normal following treatment with Rituxan.

### FIGURE 11. MONOCLONAL ANTIBODIES BIND TO SPECIFIC ANTIGENS ON CANCER CELLS

Monoclonal antibodies can inhibit cancer in several ways, including by blocking important signaling molecules on cancer cells and by recruiting immune cells and molecules to the tumor site.



Herceptin (trastuzumab) is another FDA-approved antibody, specific for the human epidermal growth factor receptor 2 (HER2). HER2 is overexpressed in 25-30 percent of primary breast cancers. Herceptin blocks the activity of this important growth receptor, and leads to cancer cell death. Herceptin was approved by the FDA in 1998 for the treatment of HER2-overexpressing breast cancer. Herceptin is commonly used in combination with standard chemotherapy, but may also be given as a single agent in women who have previously been treated with chemotherapy. Herceptin is also now approved for patients with HER2-overexpressing metastatic gastric cancer.

Avastin (bevacizumab) is a monoclonal antibody that binds to human vascular endothelial growth factor (VEGF), preventing the interaction of VEGF with its receptor on the surface of endothelial cells. VEGF is a molecule that some cancers use to recruit new blood vessels into a tumor. Avastin interrupts this process and

therefore starves a tumor of nutrients. Avastin is FDA approved for the treatment of colorectal, lung, brain, kidney, cervical, and ovarian cancers.

The plus side of monoclonal antibodies is that they are usually well-tolerated, with minimal side effects, and often lead to significant cancer reductions. (The side effects that do result can be attributed to the fact that the antigens targeted by the monoclonal antibodies are not truly cancer-specific, so some normal cells are also killed—although fewer than in chemotherapy.) The down side is that they are rarely curative; even when remissions are achieved, many cancers will eventually develop resistance to the drug and come back. Another limitation is that they do not work well on bulky tumors, which tend to be inaccessible to antibodies.

In addition to these FDA-approved antibodies, a number of new antibodies for cancer are in clinical development.

**TABLE 6. SELECTED FDA-APPROVED MONOCLONAL ANTIBODIES FOR CANCER**

TRADE NAME (GENERIC NAME)	TARGETS	INDICATIONS	FDA APPROVED
Rituxan (rituximab)	CD20	B cell non-Hodgkin lymphoma, chronic lymphocytic leukemia	1997
Herceptin (trastuzumab)	HER2	HER2+ breast cancer, HER2+ gastric cancer	1998
Erbix (cetuximab)	EGFR	Colorectal cancer, head and neck cancer	2004
Avastin (bevacizumab)	VEGF	Colorectal, lung, brain, kidney, cervical, and ovarian cancers	2004
Vectibix (panitumumab)	EGFR	Colorectal cancer	2006
Arzerra (ofatumumab)	CD20	Chronic lymphocytic leukemia	2009
Adcetris (brentuximab vedotin)	CD30	Hodgkin lymphoma	2011
Perjeta (pertuzumab)	HER2	HER2+ breast cancer	2012
Kadcyla (ado-trastuzumab emtansine)	HER2	HER2+ breast cancer	2013
Gazyva (obinutuzumab)	CD20	Chronic lymphocytic leukemia	2013
Cyramza (ramucirumab)	VEGF2	Gastric, lung, and colorectal cancers	2014
Unituxin (dinutuximab)	GD2	Neuroblastoma	2015
Darzalex (daratumumab)	CD38	Multiple myeloma	2015
Empliciti (elotuzumab)	SLAMF7	Multiple myeloma	2015
Portrazza (necitumumab)	EGFR	Lung cancer	2015

CD, cluster of differentiation; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor

## BISPECIFIC ANTIBODIES

A newer form of antibody therapy involves **bispecific antibodies**—antibodies genetically engineered to recognize two different targets. These two-pronged proteins are formed by physically joining two different monoclonal antibodies together.

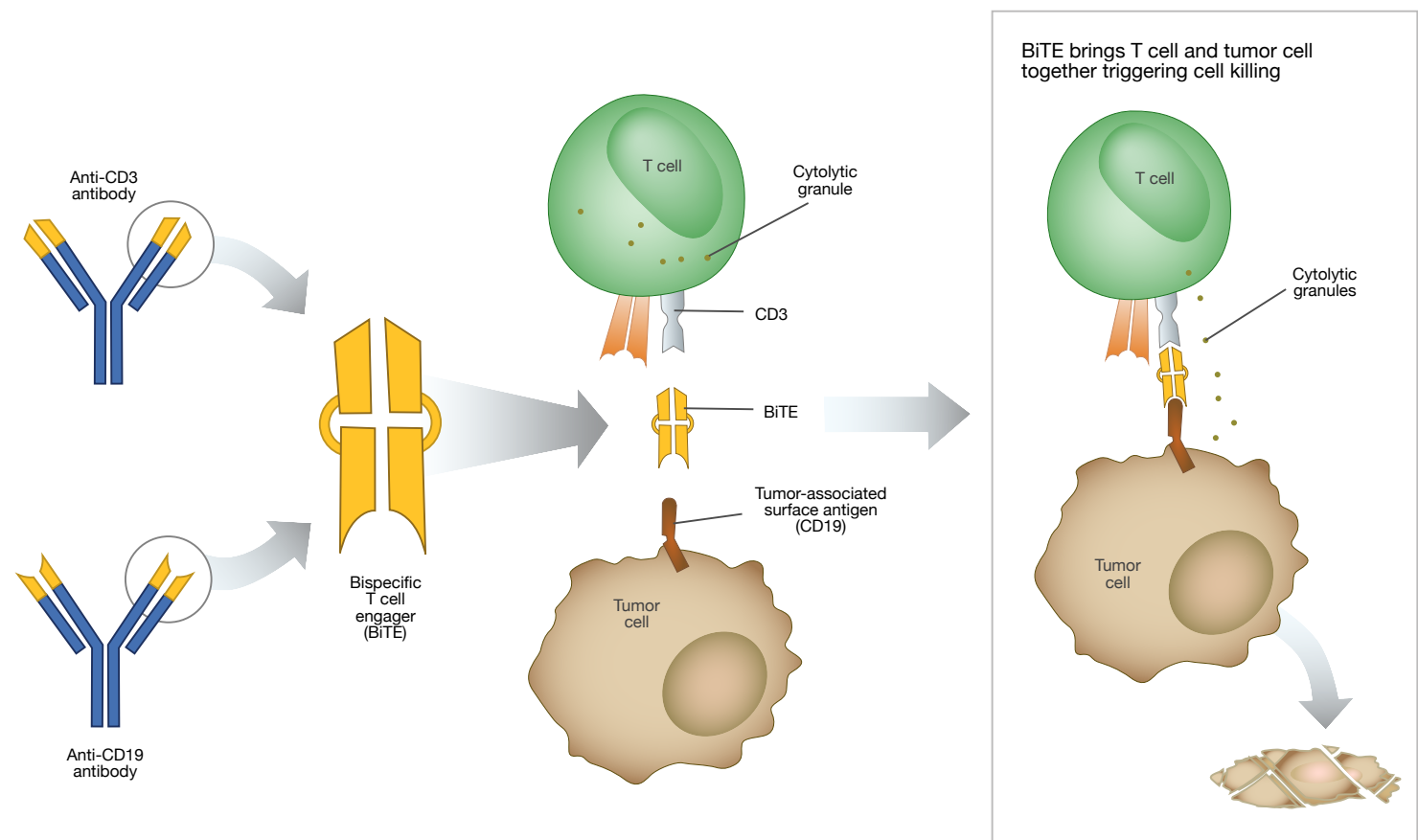
The most common form of bispecific antibodies are bispecific T cell engagers (BiTEs), which bind to a tumor antigen on one end and a “killer” T cell on the other. Through this dual action, the BiTE brings the T cell in close contact with the tumor cell, allowing the T cell to kill the tumor cell.

One BiTE is currently FDA approved for the treatment of B cell leukemia. Called Blincyto® (blinatumomab), this drug recognizes CD3 on T cells and CD19 on B cells. The FDA approval of this drug was based on a phase II clinical trial showing that, of the 185 patients treated, 41.6 percent achieved complete remission with Blincyto (**Figure 12**).

Another BiTE, called Removab® (catumaxomab), was approved in the European Union in April 2009. Removab binds to CD3 on T cells and EpCAM on cancer cells. It is used to treat malignant ascites, an accumulation of cancer cells and fluid in the abdominal cavity.

### FIGURE 12. BISPECIFIC ANTIBODIES BIND TO TWO DIFFERENT TARGETS

Bispecific antibodies are genetically engineered proteins that consist of two different antibodies fused together. They can be used to bring T cells and cancer together to trigger killing.





## 3.4 CANCER VACCINES

We are all familiar with vaccines that protect us from infectious diseases like hepatitis, measles, mumps, and flu. These vaccines work by presenting our immune system with a weakened or killed version of a virus or bacterium (or fragment thereof) so that an adaptive immune response can be generated against the offending pathogen before we ever come down with the disease. Cancer vaccines are similar, in that the goal is to present cancer antigens to the immune system in order to trigger an immune response against cancer.

There are two broad classes of cancer vaccines: **preventive vaccines** and **therapeutic vaccines**. Preventive cancer vaccines are most like typical vaccines against infectious diseases in that the aim is to prevent cancer from forming in the first place. Therapeutic cancer vaccines, on the other hand, are designed to stimulate an immune response against an existing cancer, in the hope of destroying it.

### PREVENTIVE VACCINES

Several cancers are known to be caused by viral infections. In these cases, it is possible to prevent the cancer by vaccinating against the virus.

Three preventive cancer vaccines are currently FDA approved, one for liver cancer and two for cervical cancer. Liver cancer is caused by infection with the hepatitis B and C viruses. Vaccinating against the hepatitis B virus with a vaccine is an effective way to prevent liver cancer. (A hepatitis C vaccine does not yet exist.)

Cervical cancer is caused by infection with human papillomavirus (HPV). The cervical cancer vaccine, called Gardasil®, was developed by CRI-funded scientist Ian Frazer, M.D., FRCPA, and approved by the FDA in 2006. Gardasil now protects against nine types of HPV that cause approximately 90 percent of all cases of cervical cancer worldwide.

Another preventive vaccine in development is one built around a non-viral antigen called MUC1. MUC1 is a protein found on epithelial cells, such as those that line the intestine. It is overexpressed in many cancers, including colorectal and breast cancers. In mouse models of colorectal cancer, vaccination against MUC1 is effective at preventing cancer. A pilot study of the vaccine in humans is enrolling. This study will be the first immunoprevention trial based on a tumor antigen (as opposed to a viral antigen).

### WHOLE TUMOR CELL VACCINES

An early form of cancer vaccination involved removing tumor cells from a patient, mashing them up, and injecting the crude



Professor Ian Frazer AC, who developed the cervical cancer vaccine Gardasil

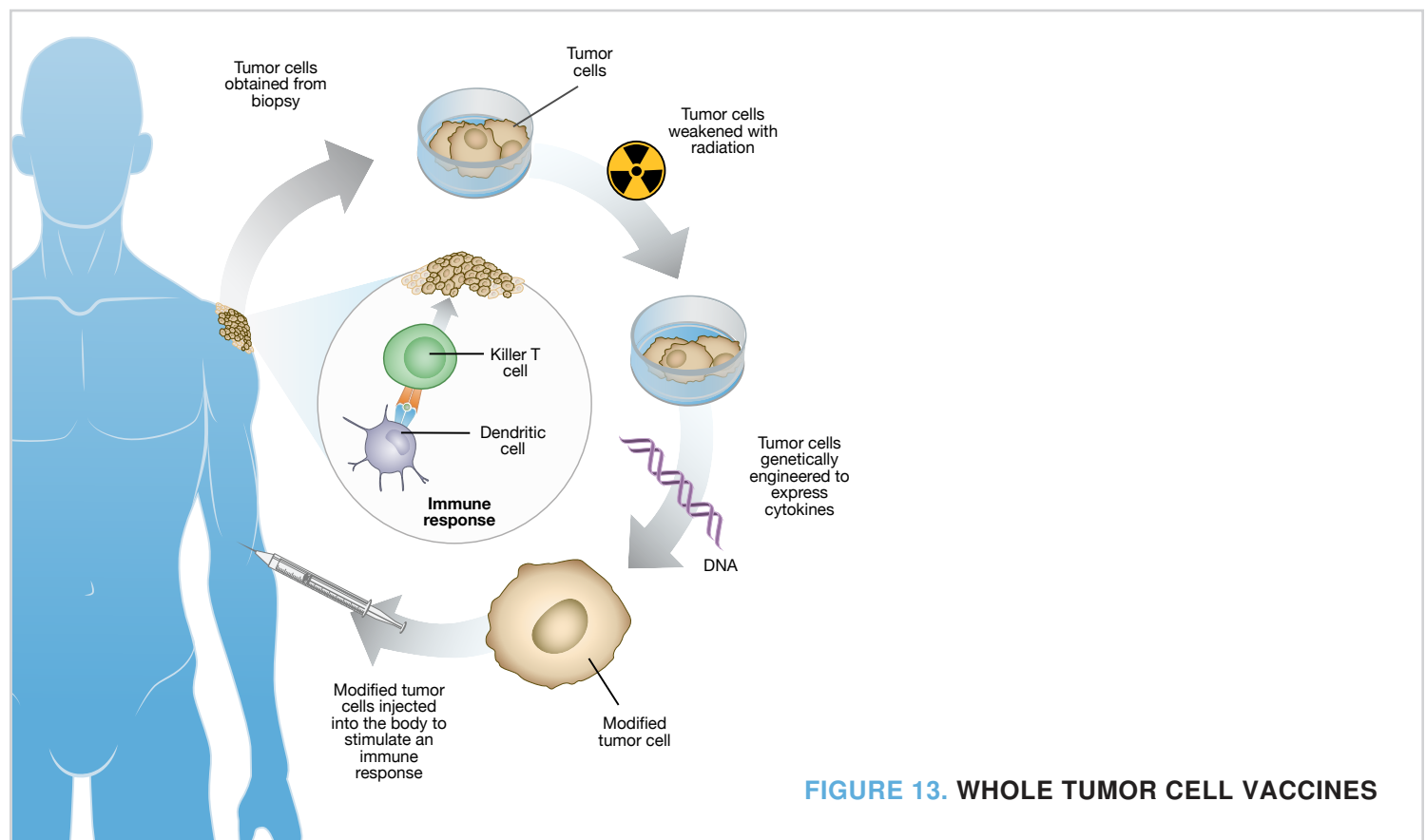
mixture back into the patient. Since then, cancer vaccines have come a long way, advancing along with our understanding of cancer antigens and the importance of adjuvants.

A modern approach to whole tumor cell vaccines involves extracting whole tumor cells from the patient, genetically modifying them with important cytokines, blasting the cells with radiation to weaken them, and then transferring them back into the patient in the presence of an adjuvant (Figure 13).

Scientists have shown that the use of cytokines, such as GM-CSF, can dramatically improve the efficacy of tumor-based vaccinations. These experiments, conducted by CRI-funded scientists Glenn Dranoff, M.D., and Drew M. Pardoll, M.D., Ph.D., led to the development of the vaccine GVAX, which consists of irradiated tumor cells that have been genetically engineered to

express GM-CSF, the most potent recruiter of antigen-presenting cells (e.g., dendritic cells) to tumor sites. GVAX is injected intradermally (under the skin). The idea behind GVAX is that the irradiated tumor cells will die in the body, releasing tumor antigens to dendritic cells. The dendritic cells will then be stimulated by the GM-CSF to take up the antigens and present them to T cells, which will then go on the attack. GVAX is currently being tested in multiple clinical trials for multiple types of cancer, including pancreatic, prostate, and breast cancer.

The main advantage of whole tumor cell vaccines is that scientists do not have to isolate a specific antigen from tumor cells. This also means that many tumor antigens are presented to the immune system at one time, generating a broader response. A disadvantage is that it is difficult to measure specific immune responses without knowing which specific antigens are present in the vaccine.



**FIGURE 13. WHOLE TUMOR CELL VACCINES**

## ANTIGEN-SPECIFIC VACCINES

All vaccines involve the same basic principle: present the immune system with antigens it will recognize as foreign or dangerous. In cancer vaccines, the antigens are derived from a cancer cell. A cancer antigen is one that is uniquely or preferentially found on or in cancer cells. Scientists have discovered numerous cancer antigens, and many of these antigens are being used in vaccines that are currently being tested in clinical trials.

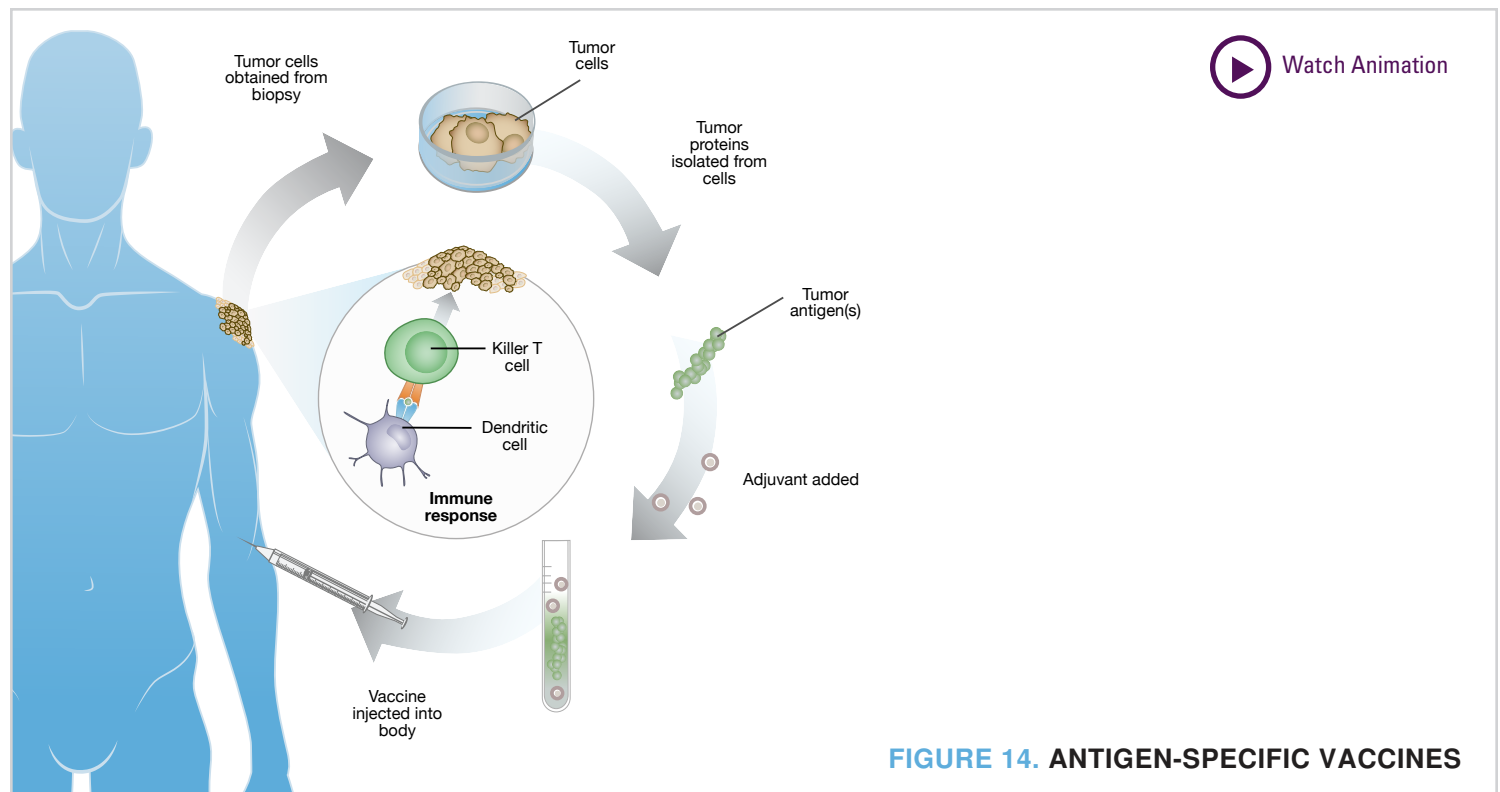
For simplicity, cancer antigens can be grouped into two different categories: shared and unique. Shared cancer antigens are ones that tumor cells from multiple patients have in common. Examples of this type of antigen are MAGE and NY-ESO-1, two proteins that are normally found in a developing embryo but not in adult tissues (with the exception of the testis). Cancers often reactivate these proteins, which may play a role in cancer's ability to divide uncontrollably. NY-ESO-1 is found in about 20-40 percent of breast, prostate, ovarian, lung, bladder, and head and neck cancers, as well as in melanoma.

Unique antigens are ones that are restricted to a particular patient's tumor. These are often mutated proteins which differ from normal proteins by having slight changes in DNA sequence. These antigens are sometimes called **neoantigens**, because they arise anew in a particular tumor.

With either type of cancer antigen, the vaccine approach is generally the same: deliver the antigen (or antigens) to the immune system, along with an adjuvant, in the hope of triggering an immune response against cancer cells bearing these antigens (**Figure 14**).

Two large phase III trials testing a cancer vaccine consisting of the shared cancer antigen MAGE in melanoma and non-small cell lung cancer were completed in 2014. Unfortunately, these trials did not show improved survival for patients receiving the vaccine.

Another strategy involves using shared antigens in a slightly different way—to prevent recurrence after conventional therapy. NeuVax™ (nelipepimut-S) is a vaccine made with a portion of the



**FIGURE 14. ANTIGEN-SPECIFIC VACCINES**

HER2/neu protein that is commonly overexpressed in breast cancer. It also contains an adjuvant molecule, GM-CSF. The vaccine is being tested in a phase III trial (referred to as “the PRESENT trial”) as a means to prevent recurrence of breast cancer after standard treatment with surgery, chemotherapy, and/or radiation.

Instead of vaccinating against one specific cancer antigen, another strategy is to use a medley of them. This, in essence, is the approach of the vaccine called Prophage, developed by former CRI postdoctoral fellow and current Scientific Advisory Council member Pramod K. Srivastava, M.D., Ph.D. Prophage uses what are called heat-shock proteins (HSPs) to deliver cancer antigens to the immune system. HSPs are found in every cell of the body, where they serve to protect cells against stress and also assist in protein folding. HSPs are good candidates for cancer-antigen delivery because they come equipped with a full sampling of antigens from the entire suite of proteins in a tumor cell. Furthermore, the immune system is primed to respond to the presence of HSPs in such a way to facilitate antigen presentation: macrophages detect the HSPs at the site of injection and swallow them up, which sets the antigen-presentation process rolling. The macrophages will then present the cancer antigens to T cells, which can then go and attack the cancer. Prophage, which was approved for use in Russia in 2008, has been used to treat melanoma and kidney cancer, and is now being tested in clinical trials for the treatment of glioblastoma, a deadly form of brain cancer.

The newest form of antigen-specific vaccination involves neoantigens. Using advanced genomic methods, scientists can

identify these specific antigens, which distinguish cancer cells from a patient's normal cells, and then create a vaccine. Studies in mice have shown this to be a very promising approach, which is beginning to be tested in patients in clinical trials.

## VIRUS-BASED VACCINES

Throughout history, doctors have periodically noticed a connection between viral infections and cancer remissions. In 1910, an Italian physician observed that a woman suffering from cancer of the cervix went into remission while she was receiving a rabies vaccine. In the 1950s, American doctors noticed that a young boy with leukemia experienced a remission of his cancer after coming down with chickenpox. These serendipitous discoveries led doctors to attempt to treat cancer by deliberately infecting patients with viruses. While this approach was sometimes effective at reducing the extent of a patient's cancer, it was also often dangerous, as many patients came down with serious infections.

Modern virus-based vaccines are much improved and come in essentially two forms. In the first, viruses are used as vectors, or delivery vehicles, to transport tumor-associated antigens to the immune system. The two most common of such viruses are the vaccinia virus (used originally to vaccinate against smallpox) and the fowlpox virus (a type of bird virus that is not harmful to humans). These viruses are genetically engineered to contain the gene for a tumor-associated antigen, such as NY-ESO-1. When injected into the body, the proteins making up the virus are recognized as foreign by the immune system. The viral proteins are taken up by dendritic cells, which present both these viral antigens and the



cancer antigen to T cells, triggering an adaptive immune response against both the virus and the cancer.

This is similar to the approach used in a vaccine called PROSTVAC for the treatment of prostate cancer. This vaccine uses fowlpox and vaccinia viruses that have been genetically engineered to contain prostate-specific antigen (PSA), a protein found on prostate cells that is often elevated in cancer, as well as multiple co-stimulatory molecules. The virus is digested by antigen-presenting cells (APCs), which present antigens from the virus, including PSA, to T cells. The interaction of these APCs with T cells initiates a targeted immune response and T cell-mediated tumor cell destruction.

The second major virus-based vaccine approach uses **oncolytic viruses**, which preferentially reproduce inside tumor cells and kill them by causing them to burst. These viral vaccines have both a direct and an indirect effect on cancer. By infecting tumor cells and causing them to burst, the oncolytic virus can kill cancer cells directly at local tumor sites. But these viruses can also indirectly kill cancer by triggering an immune response.

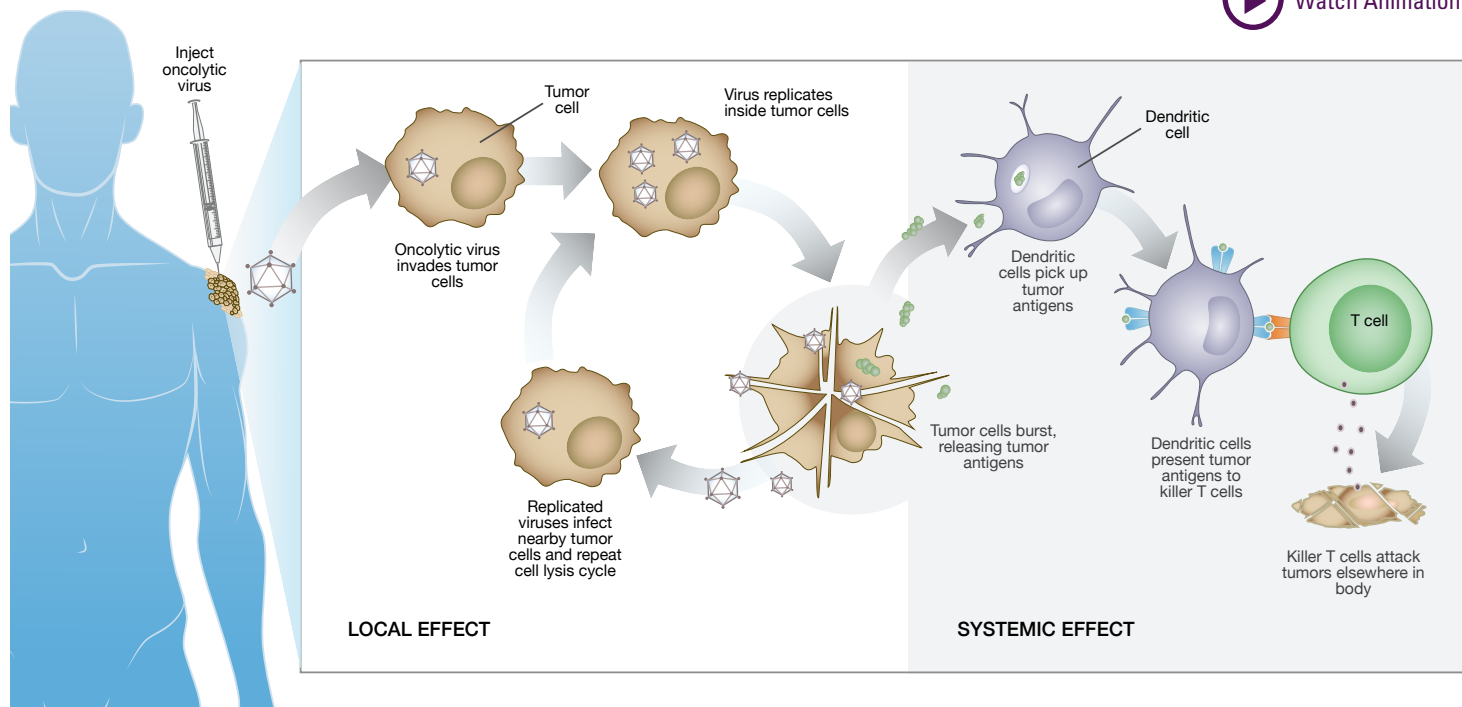
When the virally infected cancer cells burst, tumor antigens are released from these cells. The tumor antigens are then presented by APCs to T cells, which can then kill cancer cells elsewhere in the body bearing the same antigens.

This is how the vaccine Imlygic™ (talimogene laherparepvec) works. Imlygic consists of a modified herpes virus that has been genetically engineered to replicate in cancer cells and produce GM-CSF. Imlygic is injected into accessible tumor sites, where the virus kills cancer cells and releases tumor antigens that can stimulate an immune response. Imlygic was approved by the FDA in 2015 for the treatment of advanced melanoma that has recurred following surgery and is non-resectable. It is also being tested with the checkpoint inhibitors Yervoy® (ipilimumab) and Keytruda® pembrolizumab, to see whether the two might work better than one (more on these later) (Figure 15).

## DENDRITIC CELL-BASED VACCINES

Recall that T cells only recognize antigens that have been properly processed and presented on the surface of an antigen-presenting

**FIGURE 15. ONCOLYTIC VIRUS-BASED VACCINES**



 Watch Animation



cell bound to a protein called MHC. The best antigen-presenting cells in the body are dendritic cells. A promising vaccine strategy is to use a patient's own dendritic cells as the starting material for the vaccine. Commonly, the dendritic cells are obtained from the patient's blood, and then treated in the lab with cancer antigens and cytokines. The cells are then returned to the patient where they stimulate T cells to mount an immune response (Figure 16).

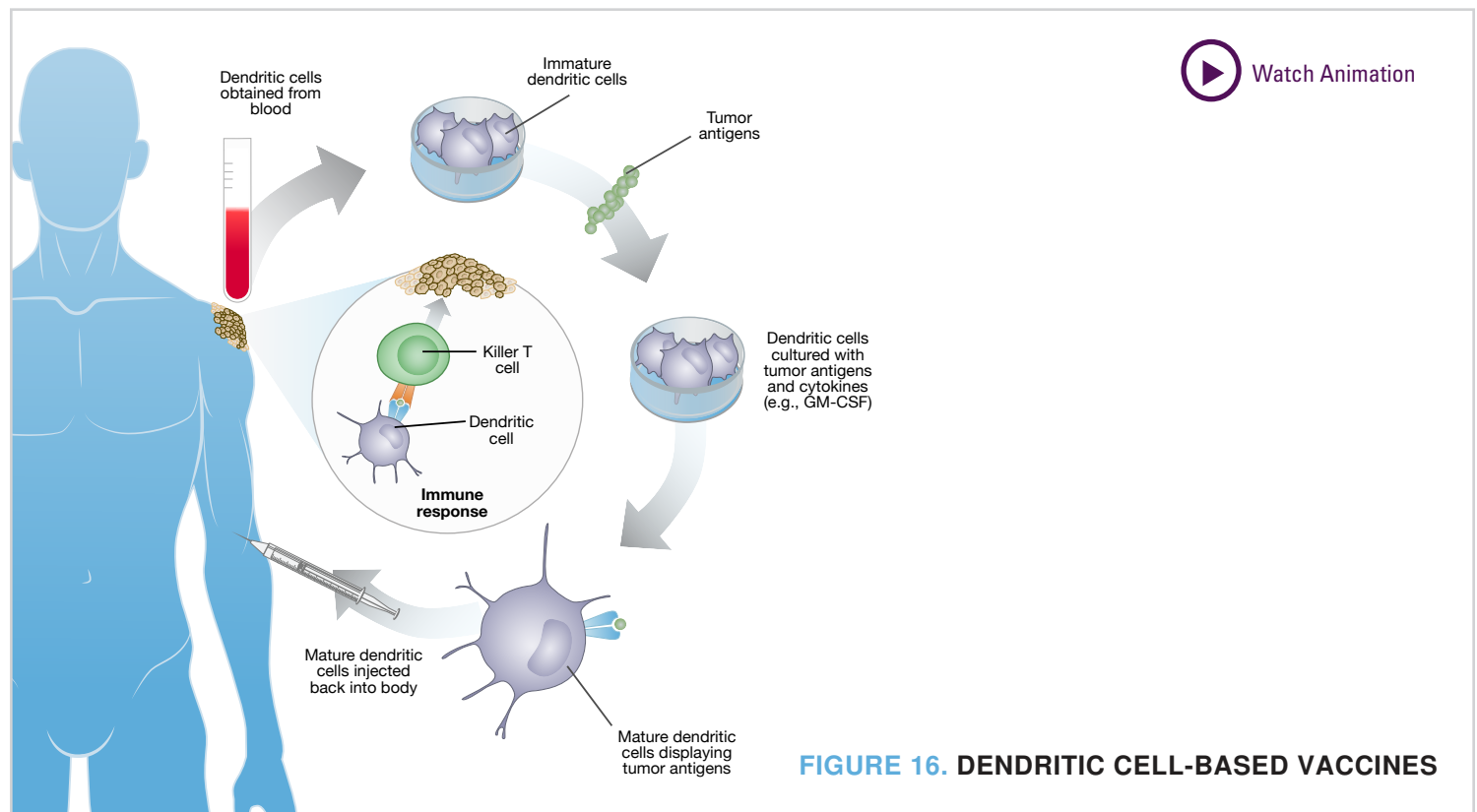
The main advantage of dendritic cell-based vaccination is that dendritic cells produce all the molecules required for eliciting an immune response, unlike other forms of cancer immunotherapy where adjuvants and co-stimulatory molecules are required to boost the ensuing immune response.

A therapeutic cancer vaccine called Provenge® (sipuleucel-T) was approved by the FDA in 2010 for the treatment of prostate cancer. Provenge is made by removing immature white blood cells, including dendritic cells, from a patient's blood, culturing them

in the lab with the cancer-associated antigen prostatic acid phosphatase (PAP), and then returning them to the patient.

Provenge has been shown to extend, by an average of 4 months, the lives of patients with metastatic prostate cancer. Ongoing research is devoted to improving the effectiveness of this and other dendritic cell-based vaccines.

Several additional dendritic cell-based vaccines are being tested in large phase III trials. One, called AGS-003, is designed to treat metastatic kidney cancer. To create AGS-003, doctors remove a small sample of tumor during surgery. From this tumor tissue, genetic material encoding the tumor antigens is obtained. Next, a blood sample is taken from the patient to obtain immature dendritic cells. These dendritic cells are combined with the tumor antigens and cytokines and allowed to mature. They are then given back to the patient, where they stimulate T cells to mount an immune attack against cancer cells bearing these antigens.



A list of cancer vaccines currently being tested in late-stage clinical trials is included in [Table 7](#).

### 3.5 CHECKPOINT BLOCKADE

One of the most promising forms of contemporary immunotherapy is known as **checkpoint blockade**. In this approach, antibodies are used to “release the brakes” on immune cells, revving them up. The approach takes advantage of the fact that the immune system already knows how to fight cancer—it just needs a little help.

Checkpoint blockade therapy works on immune cells called T cells, one of the two main classes of lymphocytes that carry out adaptive immunity. The antibodies that bind to the braking molecules are called **checkpoint inhibitors**.

To understand how checkpoint blockade therapy works, it's necessary to know a little bit more about the different molecules

found on T cells. As we have seen, T cells come equipped with a T cell receptor (TCR) that they use to recognize specific antigens on cancer cells. In addition, several distinct “co-stimulatory” receptors are present. Some of these co-stimulatory receptors, such as CD28, provide signals for the T cell to attack; other receptors, such as CTLA-4, provide signals to stand down.

Scientists often use the analogy of a car. If the T cell receptor is the ignition switch, then the CD28 molecule is the gas pedal, telling the T cell to go. The CTLA-4 molecule, on the other hand, is the brake, helping to keep the immune system in check so it doesn't speed out of control. Checkpoint inhibitors are designed to temporarily let up on the brakes so that the immune response can mount a stronger attack against cancer ([Figure 17](#)).

James P. Allison, Ph.D., who currently serves as director of CRI's Scientific Advisory Council, was the first to realize the potential of checkpoint blockade as a way to treat cancer. In the mid-

TABLE 7. CANCER VACCINES IN PHASE III CLINICAL TRIALS AS OF 2015

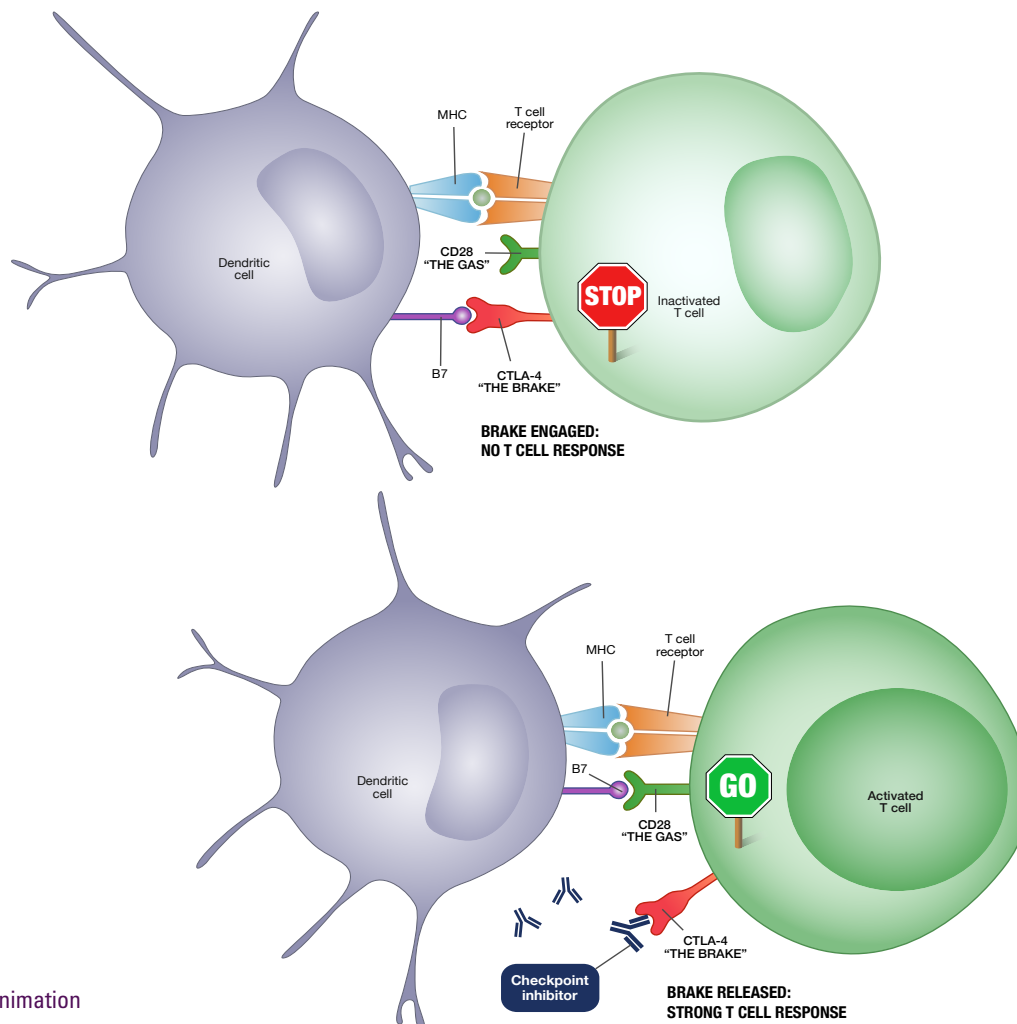
TRADE NAME (GENERIC)	COMPANY	ANTIGEN	CANCER	TYPE OF VACCINE
HyperAcute-Pancreas (algenpantucel-L; PILLAR)	NewLink Genetics	multiple	pancreatic	modified whole tumor cell
Vaxira (racotumomab)	Recombio SL, CIMAB	anti-HEN-glycolil (NGc) GM3 (NGcGM3)R2	lung	antibody
NeuVax (nelipepimut-S; E75)	Galena Biopharma, Inc.	HER2/neu	breast	peptide
DCVax-L	Northwest Biotherapeutics	multiple	brain	dendritic cell
ProstAtak (AdV-tk)	Advantagene, Inc.	multiple	prostate	modified virus
DCVAC	Sotio	multiple	prostate	dendritic cell
GV1001	Kael-GemVax Co., Ltd.	telomerase	lung	peptide
TG4010	Trangene	MUC1	lung	modified virus
PROSTVAC	Bavarian Nordic, Inc.	PSA	prostate	modified virus
DC-TC	NeoStem, Inc.	multiple	melanoma	dendritic cell
AGS-003	Argos Therapeutics	multiple	kidney	dendritic cell
Imprime PGG	Biothera	multiple	colorectal	antibody
Rintega (rindopepimut; CDX-110)	Celldex Therapeutics	EGFRvIII	brain	peptide

1990s, Allison showed that an antibody directed against CTLA-4 in mice could quickly and permanently cure them of their tumors. This dramatic laboratory finding led to the antibody drug, called ipilimumab, which was then tested in clinical trials for the treatment of melanoma. Yervoy® (ipilimumab) was the first drug of any kind to show improved survival in a phase III trial in melanoma patients. It was approved by the FDA in 2011 for the treatment of advanced melanoma.

The existence of braking molecules on immune cells may help to explain why previous attempts to stimulate the immune system using vaccines may have failed. The vaccines may have started an immune response, but the braking molecules prevented it from taking off. Adding checkpoint inhibitors to vaccines may be an effective way to overcome this hurdle, and clinical trials are under way to test this possibility.

### FIGURE 17. CHECKPOINT INHIBITORS TAKE THE BRAKES OFF T CELLS

T cells have checkpoint proteins that act like brakes. Checkpoint inhibitors bind to these proteins and “take the brakes off” the immune response, enabling a stronger attack against cancer.



If releasing these immune system brakes is beneficial to health, why does the immune system have such a braking system at all? Scientists believe that these inhibitory pathways are necessary to keep the immune system from attacking the body's own tissues (called autoimmunity).

Scientists are learning that there are multiple brakes placed on the immune system, all of which provide potential targets for immunotherapy. Another immune checkpoint being studied is called PD-1. Like CTLA-4, PD-1 is a brake molecule found on T cells. Cancer cells often make a protein called PD-L1, which can bind to PD-1 and engage this brake molecule. In effect, cancer cells possess a “power-down” switch for T cells, stopping them in their tracks. By targeting PD-1 on T cells or PD-L1 on cancer cells, scientists hope to disable this switch and restore the T cells’ ability to attack cancer. Two new PD-1 targeting drugs, Keytruda® (pembrolizumab) and Opdivo® (nivolumab) were approved by the FDA in 2014 for the treatment of advanced melanoma. In 2015, Opdivo and Keytruda were approved for the treatment of advanced non-small cell lung cancer, and Opdivo was approved for kidney cancer.

Scientists are also learning that other immune cells, besides T cells, have checkpoints, and drugs targeting these molecules are in development as well.

Checkpoint inhibitors can also be combined for a greater effect. Several clinical trials, spearheaded by CRI associate director Jedd D. Wolchok, M.D., Ph.D., have shown that the combination of Yervoy and Opdivo in patients with metastatic melanoma results in higher overall response rates and improved survival. In one study, the 2-year survival rate was 88 percent, compared to about 15 percent for chemotherapy. The combination was approved by the FDA in 2015 (Figure 18).

As impressive as the early experience with checkpoint blockade has been, there is still much more work that needs to be done. There is an urgent need to understand why only a subset of patients responds to these therapies, and to generate alternative treatments that work for the non-responders (Table 8).

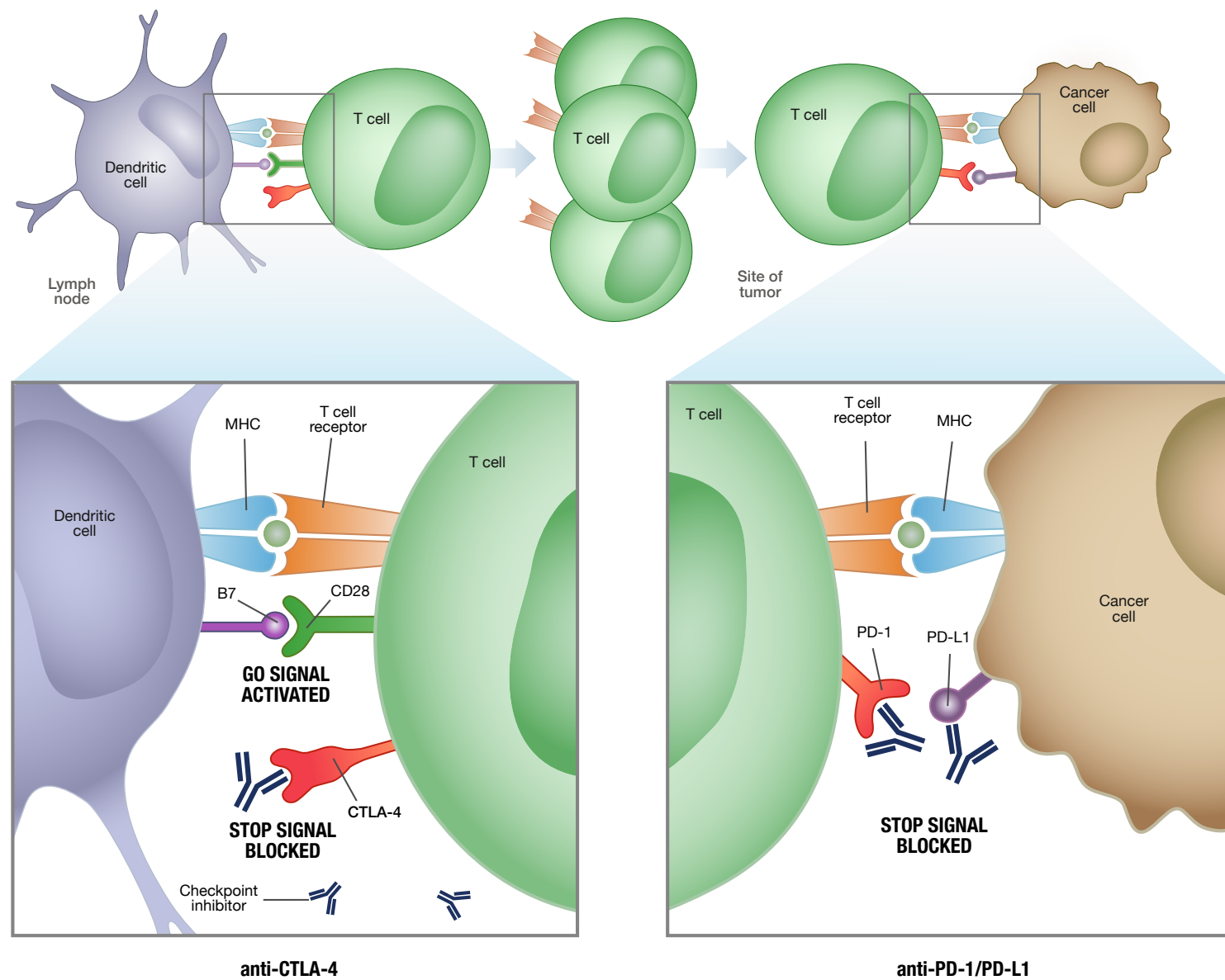
Another class of molecules, called **immune activators**, act in an opposite manner as checkpoint inhibitors—targeting stimulatory

TABLE 8. SELECT CHECKPOINT INHIBITORS FDA APPROVED OR IN CLINICAL TESTING

TRADE/GENERIC NAME	TARGET	MANUFACTURER	PHASE
Yervoy (ipilimumab)	CTLA-4	Bristol-Myers Squibb	FDA approved for melanoma
Opdivo (nivolumab)	PD-1	Bristol-Myers Squibb	FDA approved for melanoma, lung cancer, and kidney cancer; phase III for esophageal, gastric, head and neck, and brain cancer
Keytruda (pembrolizumab)	PD-1	Merck	FDA approved for melanoma and lung cancer; phase III for bladder breast, colorectal, esophageal, gastric, and head and neck cancer, and multiple myeloma
atezolizumab (MPDL3280A)	PD-L1	Genentech/Roche	Phase III for bladder, kidney, breast, and lung cancer
avelumab (MSB0010718C)	PD-L1	Merck KGaA/Pfizer	Phase III for bladder, gastric, lung, and ovarian cancer
durvalumab (MEDI4736)	PD-L1	MedImmune/AstraZeneca	Phase III for bladder, head and neck, and lung cancer
tremelimumab	CTLA-4	MedImmune/AstraZeneca	Phase III for bladder, head and neck, and lung cancer
lirilumab	KIR	Bristol-Myers Squibb	Phase II for leukemia
BMS-986016	LAG-3	Bristol-Myers Squibb	Phase I for brain cancer

# FIGURE 18. CHECKPOINT INHIBITORS CAN BE COMBINED FOR GREATER EFFECT

T cells have multiple checkpoints. Using more than one checkpoint inhibitor can enable a more effective anti-cancer response.





rather than braking molecules. If checkpoint inhibitors release the brakes of the immune response, then immune activators step on the gas. Several immune activators, targeting different stimulatory receptors on immune cells, are currently in clinical testing, including CD40, OX40, and GITR.

## 3.6 ADOPTIVE CELL THERAPY

**Adoptive cell therapy** involves removing immune cells from a patient, expanding them outside the body, and then reinfusing them into the patient. Most often, the cells are manipulated in some way in the lab before giving them back to a patient. There are several approaches currently in use.

### TUMOR-INFILTRATING LYMPHOCYTES

In patients with cancer, immune cells will often be found associated with the tumor. Among these immune cells are tumor-infiltrating

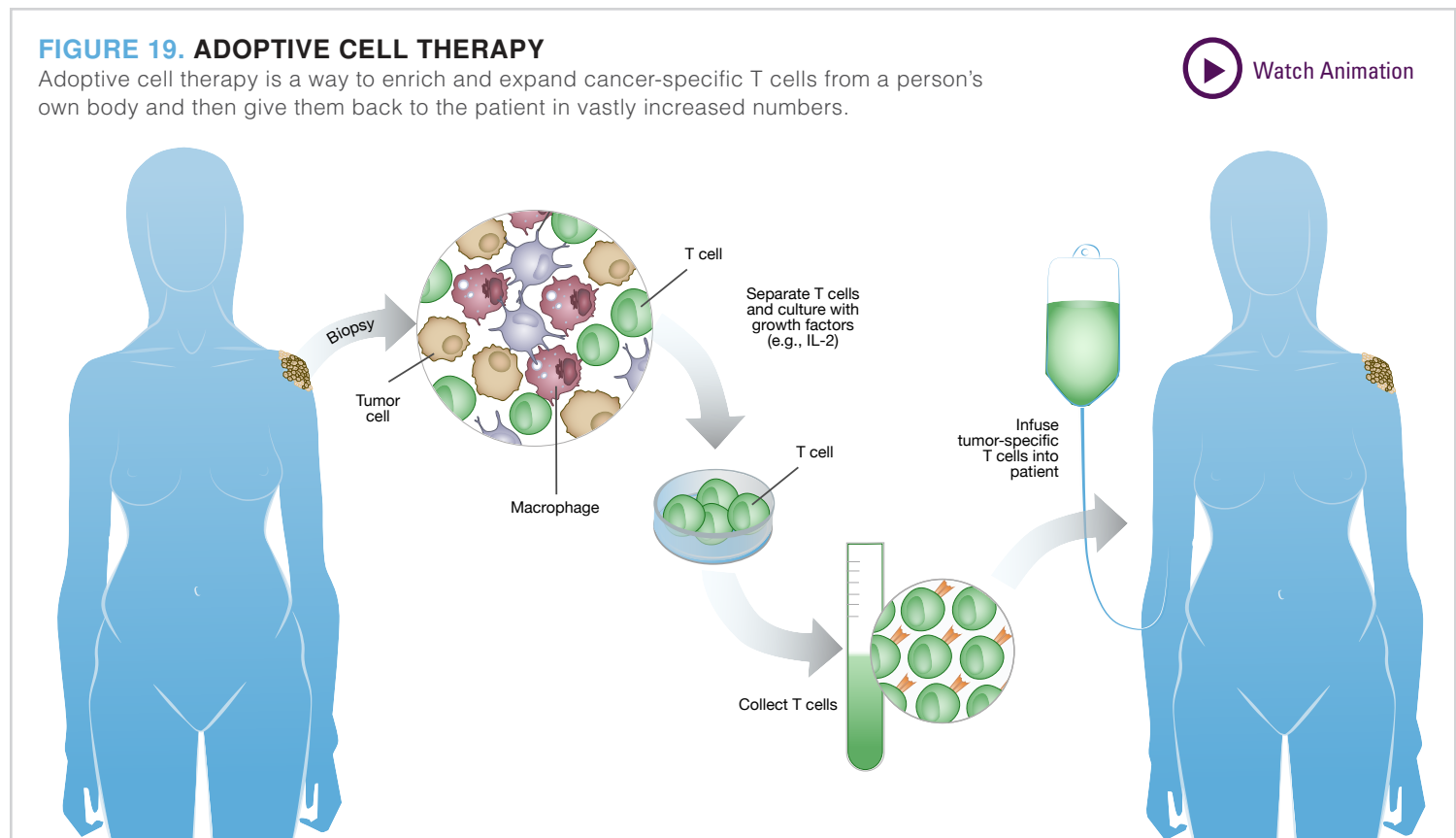
lymphocytes (TILs) that recognize cancer. TILs can be isolated from a tumor and expanded in the lab by treating them with the T cell growth factor IL-2. These pretreated TILs can then be infused back into the patient (**Figure 19**).

In a 2011 study, 20 of 93 melanoma patients (22 percent) treated with TILs achieved complete tumor regression, and 19 of these 20 (93 percent) were in complete remission nearly 5 years later. Care must be taken when interpreting such results since the trial was not randomized, but still the results show that the approach can work very well for some patients.

Importantly, for this approach to work, it appears necessary to first ablate, or kill off, the patient's immune system with high dose chemotherapy or full-body radiation—called lymphodepletion. This somewhat drastic-seeming step is necessary to “make room” for the new cells and also to remove suppressive cells (e.g.,

**FIGURE 19. ADOPTIVE CELL THERAPY**

Adoptive cell therapy is a way to enrich and expand cancer-specific T cells from a person's own body and then give them back to the patient in vastly increased numbers.



regulatory T cells) and molecules in the immune system that might interfere with the TILs' ability to combat cancer. Though this step is not without risks, studies have shown that lymphodepletion significantly increases the effectiveness of adoptive T cell therapy in patients.

## ENGINEERED T CELLS

T cells can also be genetically engineered in the lab before they are infused back into a patient. Special viruses are used as vectors to deliver genes encoding specific proteins to T cells. Typically, these genes code for T cell receptors (TCRs) with known specificity for distinct tumor antigens—for example, NY-ESO-1.

Through such genetic engineering techniques, T cells can be generated that will attack any known tumor antigen. These techniques greatly expand the number of cancer types, beyond melanoma, that can be treated with adoptive cell therapy, including neuroblastoma, synovial cell sarcoma, leukemia, and lymphoma.

## CAR T CELLS

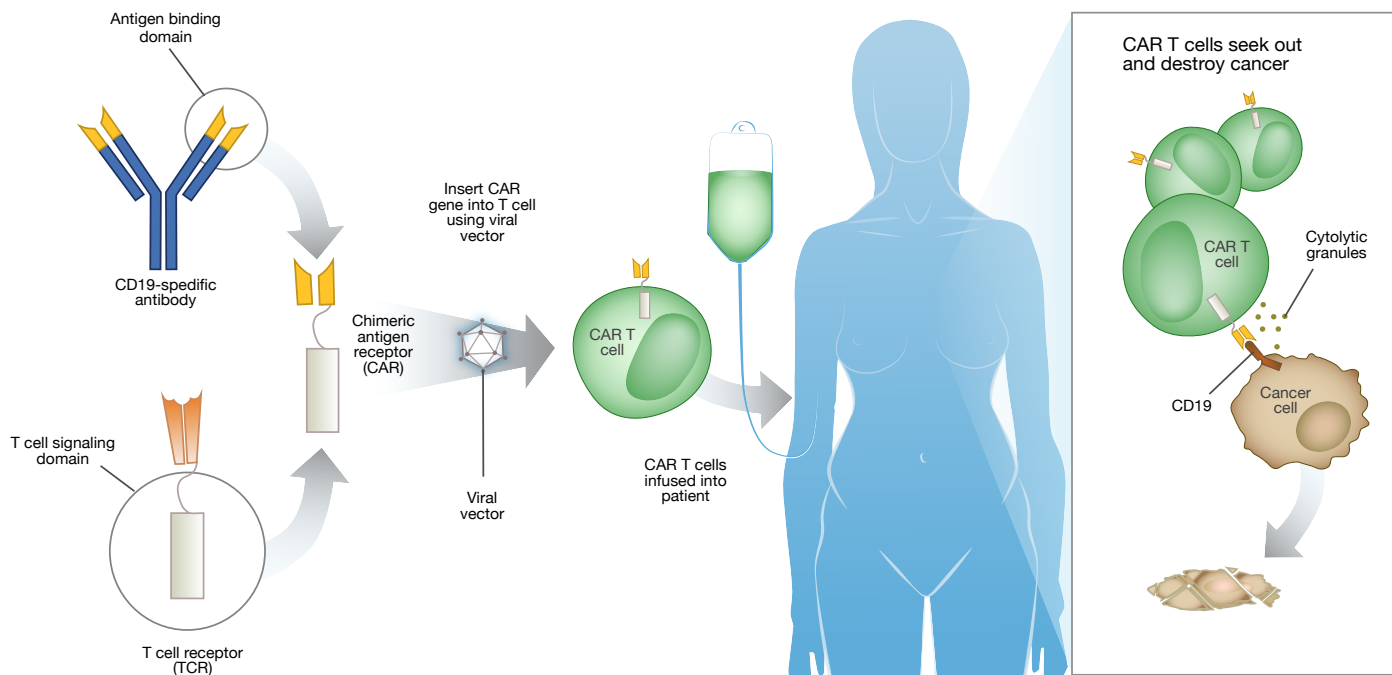
Like all T cells, engineered T cells recognize antigens only when bound to MHC. The downside of this approach is that many cancers decrease their expression of MHC, thus making them invisible to T cells.

An alternative genetic engineering approach involves equipping a T cell with a new kind of receptor, derived from the antigen-binding portion of an antibody and the cell signaling portion of a TCR. This hybrid receptor is called a chimeric antigen receptor (CAR). Binding of a CAR to a tumor antigen triggers the T cell to kill the cancer cell (**Figure 20**).

CAR T cells have several advantages over regular T cells, mostly because they bring together the best part of an antibody with the best part of a T cell. Because antibodies bind much more strongly to their target antigens than do normal TCRs, they are extremely powerful. Also, because antibodies do not require co-binding with

### FIGURE 20. CAR T CELL THERAPY

Chimeric antigen receptors (CARs) are genetically engineered proteins made up of an antibody binding domain linked to a T cell signaling domain. They turn a person's own T cells into cancer fighting weapons.



MHC, they can be used to target any antigen on the surface of cancer cells, including antigens on cancer cells that may lack MHC. But unlike antibodies, CAR T cells can actually carry out direct cell killing. Moreover, CAR T cells can reproduce and persist for years in a patient. Scientists have shown that CAR T cells injected into a patient can expand more than 1,000-fold and kill many pounds' worth of cancer cells. Each CAR T cell becomes a kind of immunological assassin—able to find and recognize a cancer cell, kill it, and then disengage and kill another one, and then another, and another. This is similar to what happens when T cells attack a viral infection in the body.

Like TIL therapy, CAR T cell therapy usually includes a lymphodepletion step to remove the suppressive immune cells that might blunt the effectiveness of the transferred CAR T cells.

Most CAR T cells have been designed to recognize one particular antigen, called CD19, found on B cells. Acute lymphoblastic leukemia (ALL) results from uncontrolled proliferation of B cells. Since these leukemia cells express CD19, they can be selectively targeted by the CAR T cells that recognize this antigen. This method has been used to treat leukemia and lymphoma, achieving dramatic total remissions—or “cures”—in a high percentage of cases. In one trial conducted at the University of Pennsylvania, complete responses were seen in 27 of 30 patients (90 percent). In another trial at Seattle Children's Hospital, 11 out of 13 patients (85 percent) achieved a complete remission. Several clinical trials of CAR T therapies are ongoing at major cancer centers in the U.S. and beyond.

Going forward, the challenge will be to develop CARs for other antigens found in other cancer types. CD19 is in some ways a perfect target, since all cells with this antigen—including normal B cells—can be eliminated from the body without undo harm. (Patients receive infusions of immunoglobulin to help them compensate for the lack of B cells.) Many other cancer antigens are also found on normal cells that cannot safely be eliminated. Therefore, an ongoing challenge is to identify cancer antigens that can be targeted by CAR T cell therapy without causing toxicity in the body by the destruction of normal cells that also display these antigens.

Another cancer antigen that may be suitable for CAR T cell therapy is mesothelin. This is a protein found in a thin layer of cells in the body's inner surface (mesothelium) that is also abundantly expressed in several cancers, including mesothelioma, pancreatic cancer, and ovarian cancer. Because this protein is strongly associated with cancer—nearly 100 percent of mesotheliomas and pancreatic cancers and 70 percent of ovarian cancers express this protein—yet is not widely expressed elsewhere in the body, it may be possible to target cells bearing this marker without causing life-threatening toxicity. CAR T cells engineered to target mesothelin are currently being tested as a treatment for pancreatic cancer in a phase I study by researchers at the University of Pennsylvania, with support from the Cancer Research Institute and the Lustgarten Foundation.



Emily Whitehead was treated with CAR T cell therapy  
(Credit: Kari Whitehead)

## 3.7 COMBINATIONS: THE FUTURE OF CANCER IMMUNOTHERAPY

A great advantage of immunotherapy is that it targets the immune system rather than the tumor itself. Because cancer cells develop new mutations quickly, they can grow resistant to conventional chemotherapies. A first round of chemotherapy might wipe out nearly all cancer cells, but a few cells will usually remain; these survivors will likely be the source of a cancer recurrence.

The immune system, by contrast, can adapt to the changing tumor, evolving with it. Even if new mutations occur, the immune system may be able to develop new cancer-specific T cells. The result is that immunotherapy has the potential to offer patients long-term, durable remissions—possibly even cures. Some of the earliest melanoma patients treated with CTLA-4 checkpoint inhibitors, for example, are now more than 10 years out and still free of disease.

Even more impressive are the results of clinical trials of combination immunotherapies. As we saw earlier, in one trial, nearly 90 percent of patients with advanced melanoma treated with a combination of ipilimumab and nivolumab were still alive 2 years later—an impressive result when you consider that the 2-year survival rate for these patients treated with conventional therapies is 15 percent. It is because of such impressive results that analysts predict that immunotherapy will become the backbone of treatment for cancer over the next decade.

Adding immunotherapies to conventional therapies like chemotherapy and radiation may also be a powerful combination. These conventional methods may complement immunotherapy by, for example, increasing the availability of tumor antigens. When tumor cells are killed by chemotherapy or radiation, they release their genetic contents into the body and the cells of our immune system clean up the fragments; this could be a good way to trigger the process of antigen presentation.

A few scientists have even gone so far as to suggest that all successful cancer therapy is immunotherapy. What they mean is that when chemotherapy and radiation work to eliminate cancer completely, it's likely because these therapies have triggered an

immune response that has killed off the remaining cancer cells in the body, preventing recurrence.

Immunotherapy is increasingly recognized as a promising and powerful way to treat cancer. Yet more work needs to be done to bring the benefits of immunotherapy to more types of cancer and more patients. The Cancer Research Institute is committed to supporting the research necessary to realize this goal.

# GLOSSARY

**ADAPTIVE IMMUNE SYSTEM:** Arm of the immune system that learns to identify and respond to disease-causing pathogens and cancer; must be primed over time to become fully active.

**ADOPTIVE CELL THERAPY:** Removing immune cells from a patient, expanding them outside the body, and then reinfusing them into the patient.

**ANGIOGENESIS:** Growth of new blood vessels, which tumors need to obtain nutrients.

**ANTIGEN:** A specific molecule or part of a molecule to which an antibody or T cell receptor binds.

**ANTIGEN PRESENTATION:** Process in which innate immune cells digest proteins from dangerous cells and post fragments of them to the cell surface where they are encountered by adaptive immune cells (T cells).

**ANTIGEN-PRESENTING CELL (APC):** Cells such as macrophages or dendritic cells that obtain antigens from pathogens and cancer and present them to T cells.

**ANTIBODY:** A protein that binds to a specific antigen on a pathogen or cancer cell, marking it for destruction; produced by mature B cells called plasma cells.

**AUTOIMMUNITY:** Pathological condition in which one's immune system attacks a part of one's own body.

**B CELL:** One of the two types of lymphocyte; responsible for making antibodies.

**BISPECIFIC ANTIBODY:** An engineered antibody that binds to two different antigens.

**BONE MARROW:** Spongy substance in the center of bones that is responsible for making blood cells.

**CANCER IMMUNOTHERAPY:** A type of cancer treatment that uses the patient's own immune system to fight the cancer.

**CARCINOGEN:** A chemical or physical insult that can cause cancer.

**CARCINOMA:** Cancers that arise in the epithelium—the layer of cells covering the surface of the body and lining the internal organs and glands.

**CELL DIVISION:** The process of cell reproduction, during which the contents of a cell are duplicated (including its DNA) and one cell cleaves into two daughter cells.

**CHECKPOINT BLOCKADE:** An immunotherapy approach that uses antibodies to target “braking molecules” on T cells, making them more active against cancer.

**CHECKPOINT INHIBITOR:** Drug (usually an antibody) targeted to proteins on T cells that act as “brakes” on the immune system. By “taking the brakes off” T cells, these drugs enable a more powerful anti-cancer response.

**CHEMOTHERAPY:** Chemicals that kill rapidly dividing cells.

**COMPLEMENT:** Proteins found in the blood that punch holes in target cells and cause them to lyse, or burst.

**CO-STIMULATION:** Secondary signal required to activate a T cell and trigger cell killing.

**CYTOKINE:** Chemical that immune cells use to communicate with one another.

**GENE:** A segment of DNA that provides instructions for making a protein.

**“HELPER” T CELL:** A type of T cell that provides crucial activation signals to other immune cells, including B cells and killer T cells. Helper T cells are identified by a CD4 marker on their surface.

**HUMORAL IMMUNE RESPONSE:** Cascade of events that produces antibodies and leads to the destruction of invading pathogens and cancer found circulating in the blood.

**INFLAMMATION:** Redness, heat, swelling, and pain that occur at a site of injury as a result of the accumulation of immune cells and fluid; a normal process that participates in both immune defense and in wound healing.

**IMMUNE ACTIVATOR:** A drug that “steps on the gas” of immune cells.

**IMMUNE SYSTEM:** The defense system of the body consisting of lymph nodes, thymus, tonsils, spleen, and white blood cells.

**IMMUNITY:** The ability of the body to resist disease upon exposure to a particular pathogen or cancer.

**INNATE IMMUNE SYSTEM:** Arm of the immune system that is active from birth and responds immediately to well-recognized pathogens and cancer.



**“KILLER” T CELL:** A type of T cell responsible for direct cell killing; the targets of killer T cells include virally-infected and cancer cells. Killer T cells are identified by a CD8 marker on their cell surface.

**LEUKEMIA:** Cancer of the white blood cells.

**LYMPHOCYTE:** A white blood cell, such as a B or T cell, that plays a part in adaptive immunity.

**LYMPHOMA:** Cancer of the lymph glands.

**LYMPH NODE:** Small, bean-shaped structures located throughout the body that filter lymph fluid; where immune cells are alerted to the presence of pathogens or cancer.

**MACROPHAGE:** White blood cell that can swallow up (phagocytose) and kill bacteria, virus-infected cells, and cancer cells.

**MAJOR HISTOCOMPATIBILITY COMPLEX (MHC):** Protein found on cells that cradles an antigen and identifies a cell as self or non-self.

**MELANOMA:** A cancer that originates in the melanin-producing cells called melanocytes present in the skin.

**MEMORY CELL:** T cells and B cells that remain in the body after an infection or cancer has been cleared and remembers the specific pathogen or cancer cell that originally caused disease.

**METASTASIS:** Spread of cancer cells from a local to a distant site through the blood or lymph fluid.

**MONOCLONAL ANTIBODY:** Antibody produced in the lab that is specific for one particular antigen; many immunotherapy drugs are monoclonal antibodies.

**MUTATION:** A change in the nucleotide sequence of DNA. Cancer is caused by the accumulation of mutations in DNA.

**NATURAL KILLER CELL:** Innate immune cell that kills virally-infected and cancer cells.

**NEOANTIGEN:** An antigen that is unique to an individual patient’s tumor.

**NEUTROPHIL:** Innate immune cell that is often the first phagocytic cell to appear at the site of infection.

**ONCOLYTIC VIRUS:** A type of virus that preferentially infects cancer cells and causes them to lyse, or burst.

**ONCOGENE:** A mutated gene that can lead to cancer.

**PATHOGEN:** Bacterium, virus, or parasite that can cause disease.

**PATHOGEN-ASSOCIATED MOLECULAR PATTERN (PAMP):** Distinct molecular “fingerprints” found on all bacteria, viruses, and parasites.

**PATTERN RECOGNITION RECEPTOR (PRR):** Proteins found on cells of the innate immune system that recognize PAMPs on pathogens.

**PHAGOCYTOSIS:** The engulfment of one cell (or cell part) by another cell.

**PLASMA CELL:** A mature B cell that serves as a factory of antibody production.

**PREVENTIVE CANCER VACCINE:** A vaccine that can prevent cancer, usually by preventing viral infections.

**PROTO-ONCOGENE:** A gene that, when mutated, can promote cancer.

**RADIATION:** Treatment that involves directing a beam of high-energy electrons to a tumor, causing rampant DNA damage and cell death.

**REGULATORY T CELL (TREG):** A type of T cell that guards against autoimmunity by shutting down activated T cells.

**SARCOMA:** Cancer of the supporting tissues of the body such as bone, muscle, and blood vessels.

**T CELL:** One of two types of lymphocyte; T cells can be subdivided into killer T cells, helper T cells, memory T cells, and regulatory T cells.

**T CELL RECEPTOR (TCR):** The protein on T cells that binds to antigens sitting in an MHC molecule; how T cells “see” their targets.

**TARGETED THERAPY:** Treatment that interrupts the actions of a specific molecule or cellular pathway; often the targets are oncogenes.

**THERAPEUTIC CANCER VACCINE:** A vaccine that is used to treat cancer that already exists.

**THYMUS:** Organ in the chest where T cells develop.

**TOLERANCE:** Condition that occurs when the immune system ignores the presence of certain antigens and does not mount an immune response.

**TUMOR:** A solid mass of cancerous cells.

**TUMOR SUPPRESSOR GENE:** A gene that normally protects against cancer.

**VACCINATION:** Process of priming the immune system to recognize a pathogen (or cancer) when it next encounters it in order to prevent full-blown disease.

**VACCINE:** Antigens from a pathogen or cancer, used to induce an adaptive immune response.

# REFERENCES

- Allison, J. P., & Lanier, L. L. (1987). Structure, function, and serology of the T-cell antigen receptor complex. *Annual Review of Immunology*, 5(1), 503-540.
- American Cancer Society (2016). Cancer Facts and Figures 2016. Atlanta: American Cancer Society. <<http://www.cancer.org/Research/CancerFactsStatistics/cancerfactsfigures2016/cancer-facts-and-figures-2016>>
- Bjorkman, P. J., Saper, M. A., Samraoui, B., et al. (1987). Structure of the human class I histocompatibility antigen, HLA-A 2. *Nature*, 329(6139), 506-512.
- Brahmer, J. R. (2013). Harnessing the immune system for the treatment of non-small-cell lung cancer. *Journal of Clinical Oncology*, 31(8), 1021-1028.
- Brahmer, J. R., Tykodi, S. S., Chow, L. Q., et al. (2012). Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *New England Journal of Medicine*, 366(26), 2455-2465.
- Cann, S. H., Van Netten, J. P., & Van Netten, C. (2003). Dr. William Coley and tumour regression: a place in history or in the future. *Postgraduate Medical Journal*, 79(938), 672-680.
- Carswell, E. A., Old, L. J., Kassel, R. L., et al. (1975). An endotoxin-induced serum factor that causes necrosis of tumors. *Proceedings of the National Academy of Sciences*, 72(9), 3666-3670.
- Chambers, C. A., Kuhns, M. S., Egen, J. G., & Allison, J. P. (2001). CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. *Annual Review of Immunology*, 19(1), 565-594.
- Cheever, M. A., & Higano, C. S. (2011). PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. *Clinical Cancer Research*, 17(11), 3520-3526.
- Chen, L., & Flies, D. B. (2013). Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nature Reviews Immunology*, 13(4), 227-242.
- Christiansen, A., & Detmar, M. (2011). Lymphangiogenesis and cancer. *Genes Cancer*, 2(12), 1146-58.
- Colditz, G. A., & Wei, E. K. (2012). Preventability of cancer: the relative contributions of biologic and social and physical environmental determinants of cancer mortality. *Annual Review of Public Health*, 33, 137.
- Coley, W. B. (1893). The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. *The American Journal of the Medical Sciences*, 105(5), 487-510.
- Delves, P. J., & Roitt, I. M. (2000). The immune system. First of two parts. *New England Journal of Medicine*, 343, 37-49.
- Dranoff, G., Jaffee, E., Lazenby, A., et al. (1993). Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proceedings of the National Academy of Sciences*, 90(8), 3539-3543.
- Duan, F., Duitama, J., Al Seesi, S., et al. (2014). Genomic and bioinformatic profiling of mutational neoepitopes reveals new rules to predict anticancer immunogenicity. *The Journal of Experimental Medicine*, 211(11), 2231-2248.

- Dunn, G. P., Old, L. J., & Schreiber, R. D. (2004). The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*, 21(2), 137-148.
- Dunn, G. P., Old, L. J., & Schreiber, R. D. (2004). The three Es of cancer immunoediting. *Annual Review Of Immunology*, 22, 329-360.
- Dvorak, H. F. (1986). Tumors: wounds that do not heal: similarities between tumor stroma generation and wound healing. *New England Journal of Medicine*, 315(26), 1650-1659.
- Gardner, R., et al. (2014). American Society of Hematology (ASH) abstract.
- Gayed, P. M. (2011). Bicentennial: Toward a Modern Synthesis of Immunity: Charles A. Janeway Jr. and the Immunologist's Dirty Little Secret. *The Yale Journal of Biology and Medicine*, 84(2), 131.
- Gnjatic, S., Nishikawa, H., Jungbluth, A. A., et al. (2006). NY-ESO-1: Review of an Immunogenic Tumor Antigen. *Advances in Cancer Research*, 95, 1-30.
- Gregory, A. D., & Houghton, A. M. (2011). Tumor-associated neutrophils: new targets for cancer therapy. *Cancer Research*, 71(7), 2411-2416.
- Gubin, M. M., Zhang, X., Schuster, H., et al. (2014). Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*, 515(7528), 577-581.
- Hall, S. S. (1997). *A Commotion in the Blood: Life, Death, and the Immune System*. New York: Henry Holt.
- Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, 144(5), 646-674.
- Haskins, K., Kubo, R., White, J., et al. (1983). The major histocompatibility complex-restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *The Journal of Experimental Medicine*, 157(4), 1149-1169.
- Haudley, K., et al. (2014). Multiplatform Analysis of 12 Cancer Types Reveals Molecular Classification within and across Tissues of Origin. *Cell*, 158, 929-944.
- Heemskerk, B., Kvistborg, P., & Schumacher, T. N. (2013). The cancer antigenome. *The EMBO Journal*, 32(2), 194-203.
- Herr, H. W., & Morales, A. (2008). History of bacillus Calmette-Guerin and bladder cancer: an immunotherapy success story. *The Journal of Urology*, 179(1), 53-56.
- Janeway, C. A., Travers, P., Walport, M., & Shlomchik, M. J. (2001). *Immunobiology: The Immune System in Health and Disease*, 5th edition. New York: Garland Science.
- Kalos, M., Levine, B. L., Porter, D. L., Katz, S., Grupp, S. A., Bagg, A., & June, C. H. (2011). T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Science Translational Medicine*, 3(95).
- Kappler, J., Kubo, R., Haskins, K., et al. (1983). The major histocompatibility complex-restricted antigen receptor on T cells in mouse and man: identification of constant and variable peptides. *Cell*, 35(1), 295-302.
- Kelly, E., & Russell, S. J. (2007). History of oncolytic viruses: genesis to genetic engineering. *Molecular Therapy*, 15(4), 651-659.
- Kelly, R. J., Sharon, E., Pastan, I., & Hassan, R. (2012). Mesothelin-targeted agents in clinical trials and in preclinical development. *Molecular Cancer Therapeutics*, 11(3), 517-525.
- Kindt, T. J., Goldsby, R. A., Osborne, B. A., & Kuby, J. (2007). *Kuby Immunology*. New York: W.H. Freeman.
- Leach, D. R., Krummel, M. F., & Allison, J. P. (1996). Enhancement of antitumor immunity by CTLA-4 blockade. *Science*, 271(5256), 1734-1736.
- Lodish, H., Berk, A., Kaiser, C. A., et al. (2012) *Molecular Cell Biology*, 7th edition. New York: W.H. Freeman.
- Maude, S. L., Frey, N., Shaw, P. A., et al. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *New England Journal of Medicine*, 371(16), 1507-1517.
- Maus, M. V., Grupp, S. A., Porter, D. L., & June, C. H. (2014). Antibody-modified T cells: CARs take the front seat for hematologic malignancies. *Blood*, 123(17), 2625-2635.

Mellman, I., Coukos, G., & Dranoff, G. (2011). Cancer immunotherapy comes of age. *Nature*, 480(7378), 480-489.

Moore, P. S., & Chang, Y. (2010). Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nature Reviews Cancer*, 10(12), 878-889.

Restifo, N. P., Dudley, M. E., & Rosenberg, S. A. (2012). Adoptive immunotherapy for cancer: harnessing the T cell response. *Nature Reviews Immunology*, 12(4), 269-281.

Riedel, S. (2005). Edward Jenner and the history of smallpox and vaccination. *Proceedings (Baylor University Medical Center)*, 18(1), 21.

Rosenberg, S. A., et al. (2011). Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clinical Cancer Research*, 17, 4550-4557.

Schreiber, R. D., Old, L. J., & Smyth, M. J. (2011). Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science*, 331(6024), 1565-1570.

Sharpe, A. H., & Abbas, A. K. (2006). T-cell costimulation—biology, therapeutic potential, and challenges. *New England Journal of Medicine*, 355(10), 973-975.

Shuster, M., Vigna, J., Sinha, G., Tontono, M. (2014) *Biology for a Changing World*, 2nd edition. New York: Scientific American/W.H. Freeman.

Sinkovics, J. G., & Horvath, J. C. (2008). Natural and genetically engineered viral agents for oncolysis and gene therapy of human cancers. *Archivum Immunologiae et Therapiae Experimentalis*, 56(1), 1-59.

Slingluff, C. (2011). The Present and Future of Peptide Vaccines for Cancer: Single or Multiple, Long or Short, Alone or in Combination? *Cancer Journal*, 17(5), 343-350.

Steinman, R. M. (2012). Decisions about dendritic cells: past, present, and future. *Annual Review of Immunology*, 30, 1-22.

Stern, A. M., & Markel, H. (2005). The history of vaccines and immunization: familiar patterns, new challenges. *Health Affairs*, 24(3), 611-621.

Sznol, M., & Chen, L. (2013). Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. *Clinical Cancer Research*, 19(5), 1021-1034.

Topalian, S. L., Hodi, F. S., Brahmer, J. R., et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *New England Journal of Medicine*, 366(26), 2443-2454.

van der Bruggen, P., Traversari, C., Chomez, P., et al. (1991). A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science*, 254(5038), 1643-1647.

Vogelstein, B., Papadopoulos, N., Velculescu, V. E., et al. (2013). Cancer genome landscapes. *Science*, 339(6127), 1546-1558.

Weinberg, R. (1996). *Racing to the Beginning of the Road: The Search for the Origin of Cancer*. New York: W.H. Freeman.

Wiemann, B., & Starnes, C. O. (1994). Coley's toxins, tumor necrosis factor and cancer research: a historical perspective. *Pharmacology & Therapeutics*, 64(3), 529-564.

Wolchok, J. D., Kluger, H., Callahan, M. K., et al. (2013). Nivolumab plus ipilimumab in advanced melanoma. *New England Journal of Medicine*, 369(2), 122-133.

zur Hausen, H. (2009). Papillomaviruses in the causation of human cancers—a brief historical account. *Virology*, 384(2), 260-265.

## ACKNOWLEDGMENTS

The authors wish to thank Ellen Puré, Pramod Srivastava, Vanessa Lucey, Emily Helck, Michelle Liew, Alexandra Mulvey, and Brian Brewer for helpful comments and suggestions. The artwork in the chapter was created by Carl Grauer, and we are grateful to him as well for his fine work. Page layout and design were completed by Lucy Dalmeida, with art direction by Michelle Liew.





