Cancer Immunotherapy, Part 1: Current Strategies and Agents

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This is the first in a series of three articles about cancer immunotherapy. The second article will discuss efficacy, safety, and other clinical considerations. The third article will discuss challenges and future trends.

INTRODUCTION

Cancer is a leading cause of mortality worldwide, accounting for one in seven deaths globally.[1–3] Progress has been made in treating cancer, but a significant proportion of patients still die despite treatment, indicating that new, more effective targets for cancer therapy are needed.[2,4] Cancer immunotherapy is providing promise for revolutionizing cancer treatment through the discovery and development of new approaches that enhance the body's antitumor immune functions.[1,5] Although cancer progression involves a wide variety of methods to overcome host immunity, cancer immunotherapy can potentially revive the patient's suppressed immune system, ideally resulting in the eradication of the disease.[6] A range of cancer immunotherapy approaches have proven effective in many patients, including: monoclonal antibodies, immune checkpoint blockers, cancer vaccines, and cell-based therapies.[4,6–8] This article introduces cancer immunology, cancer immunotherapy strategies, and each of these classes of anticancer therapeutic agents.

CANCER MORTALITY

Cancer is a leading cause of mortality worldwide, accounting for one in seven deaths globally—more than malaria, tuberculosis, and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) combined.[1–3] In 2012, 14.1 million new cases and 8.2 million cancer-related deaths were reported internationally, according to GLOBOCAN data.[1–3] Lung, breast, colorectal (CRC), and prostate cancer are the four types most frequently diagnosed worldwide, with lung cancer being the leader with respect to both incidence and mortality.[1,2] The global incidence of cancer is growing rapidly; it has been estimated that in 2030, new cases will surpass 21.7 million worldwide, and 13 million cancer deaths will occur each year simply due to aging and population growth.[2,3] This trend is expected to be even more dramatic in low- to middle-income countries, where 60% of cancer deaths now occur.[2,3]

Cancer is also a common cause of mortality in the U.S., accounting for one in four deaths—exceeded only by heart disease.[3] In 2016, an estimated 1.69 million new cancer cases and 595,690 cancer deaths (1,630 per day) occurred nationwide.[3,4] Nonetheless, since 1971, the cancer survival rate has improved significantly.[4] In the U.S., advances in early diagnosis, improved therapies, and cancer prevention have translated into countless saved lives yearly.[4] Progress has been made in the war against cancer, but much work remains.[4] A high rate of patients still die in the U.S. despite treatment, demonstrating that research focusing on new, more effective targets in cancer therapy is needed.[2,4]

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HISTORICAL TRENDS IN CANCER IMMUNOTHERAPY

Interest in cancer immunology and use of the immune system as a tool to destroy cancer cells was evident as early as the late 1800s.[7] In 1893, William B. Coley, MD, considered the father of cancer immunotherapy, observed cases in which cancer went away after a patient contracted erysipelas, a bacterial infection.[8,9] Dr. Coley wrongly suspected that bacteria were destroying the tumors; however, researchers today think the infection triggered an intense immune response that destroyed the cancer.[9] Based on his observation, Dr. Coley prepared a mixture of killed *Serratia marcescens* and *Streptococcus pyogenes*, known as "Coley's toxins," and administered them to patients who had different types of inoperable carcinomas.[8]

Coley's toxins were effective in curing some types of tumors, particularly sarcomas, and were used as a cancer treatment for many decades.[8] Parke-Davis and Company began producing Coley's toxins in 1899 and continued doing so for many years.[9] Various European and American hospitals, including the Mayo Clinic, used the toxins, but they observed inconsistent results.[9] Enthusiasm for Coley's toxins moderated due to the limitations in clinical efficacy, and radiation became the preferred cancer treatment with more predictable results.[5,9] In 1915, even the institution that Dr. Coley was associated with, Memorial Hospital (now Memorial Sloan Kettering Cancer Center in New York City), established a policy stating that cancer patients were to be given radiation, not Coley's toxins.[9]

Chemotherapy was developed during World War II, further diminishing interest in Coley's toxins or any other cancer immunotherapy.[3] In 1965, the American Cancer Society classified Coley's toxins as an "unproven" treatment (a categorization that has since been reversed).[1] After his death in 1936, Dr. Coley's daughter, Helen Coley Nauts, studied many of her father's case records and became convinced that her father's work was significant.[9] Although she tried to rekindle interest in his work, physicians still opposed it, including the cancer experts at Memorial Sloan Kettering.[9] Undeterred, Helen Coley Nauts founded the Cancer Research Institute in New York City in 1953. This nonprofit organization has become a significant source of support for cancer immunotherapy research, awarding more than $29.4 million in scientific grants in 2015.[9]

Another major step in cancer immunotherapy occurred in 1957 when E. Donnall Thomas, MD, explored stem cell transplantation.[8] Initially, Dr. Thomas administered bone marrow from healthy individuals to patients with advanced leukemia and achieved some success.[8] However, the therapy (now known as allogeneic hematopoietic stem cell transplantation [allo-HSCT]) caused significant toxicity.[8] Still, allo-HSCT

ABBREVIATIONS

ADCC—antibody-dependent cellular cytotoxicity
Allo-HSCT—allogeneic hematopoietic stem cell transplantation
APC—antigen-presenting cell
CAR—chimeric antigen receptor
CD—cluster of differentiation
CRC—colorectal cancer
CTL—cytotoxic T lymphocyte
CTLA-4—cytotoxic T-lymphocyte–associated antigen 4
DC—dendritic cell
HLA—human leukocyte antigen
HSCT—hematopoietic stem cell transplantation
IC—immune checkpoint
ICB—immune checkpoint blocking
IFN—interferon
IL—interleukin
mAb—monoclonal antibody
MHC—major histocompatibility complex
NK cell—natural killer cell
NSCLC—non–small-cell lung cancer
PD-1—programmed death-1
PD-L1—programmed death ligand-1
RCC—renal cell carcinoma
rhIL—recombinant interleukin
TAA—tumor-associated antigen
TCR—T-cell receptor
Th1—T cell helper 1
Th2—T cell helper 2
TIL—tumor-infiltrating lymphocyte
Treg—regulatory T cell

has been used continuously as a treatment for hematologic malignancies since its conception.[8] The next significant achievement in cancer immunotherapy occurred in the late 1980s, when Steven A. Rosenberg, MD, administered activated immune cells and cytokines to patients with melanoma.[8] Dr. Rosenberg has reported cures in many patients with advanced melanoma and continues to contribute to the field of cancer immunotherapy, primarily in the study of melanoma and renal cell carcinoma (RCC).[8]

Cancer treatments involving the immune system were being developed throughout the 20th century.[10] However, even in 1971, when President Richard Nixon declared a war on cancer, there was still very little understanding of cancer etiology, oncogenes, and environmental influences, and even less knowledge concerning cancer-relevant functions of the immune system.[4] In fact, B and T cells had not even been identified.[4] Still, due to earlier discoveries, most investigators were aware that the immune system was involved in cancer detection and elimination, and they viewed the cause of cancer development to be immunodeficiency.[4]

During the past few decades, tremendous progress has been made in understanding how cancer is detected, eliminated by, and avoids the immune system.[5] Particularly during the past 15 years, advances in molecular and tumor biology have significantly changed cancer-treatment paradigms.[1] Previously, cancers were classified and treated solely according to histomorphological features and organ of origin.[1] Recognition has also grown that the broad use of cytotoxic chemotherapy drugs has reached a therapeutic plateau and that treatments should instead be targeted based on specific identifiable molecular alterations.[1] Figure 1 presents a timeline that summarizes some of the significant events in cancer immunotherapy.

Currently, two major revolutions in cancer research and treatment are fulfilling the need for targeted treatments.[1] One of these trends is based on significant advancements in cancer immunotherapy, allowing new treatments that enhance the body's antitumor immunity functions.[1] Because this approach is so promising, "cancer immunotherapy" was named "Breakthrough of the Year" by *Science* in 2013.[5] It has been encouraging that clinical trials with immune checkpoint-blocking (ICB) therapies or chimeric antigen receptor (CAR) T-cell–based therapy have proven to be potentially lifesaving.[5] In some patients, cancer immunotherapy treatments have caused tumors to disappear and terminal cancer to go into remission for years.[9] Such success, supported by solid clinical data, has inspired major interest and investment in the growing field of cancer immunotherapy by pharmaceutical companies, governments, and philanthropists.[9] Consequently, there were nearly 1,700 clinical trials related to cancer immunotherapy listed on ClinicalTrials.gov as of May 2017.[11]

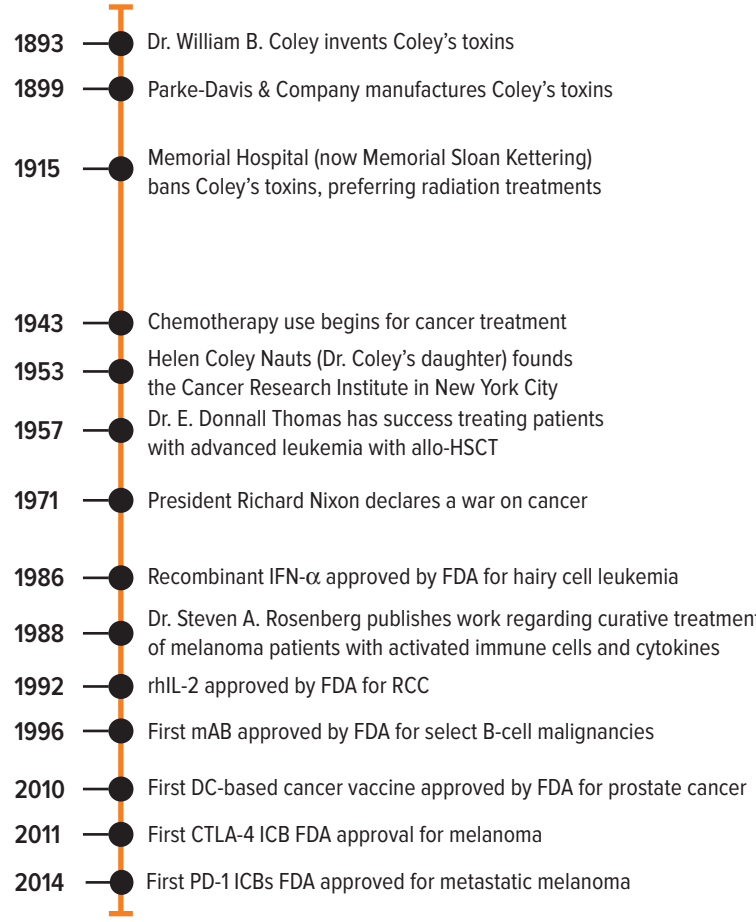
A second revolution now under way focuses on the identification and targeting of actionable genetic alterations in oncogene-driven cancers.[1] New technology can profile and identify molecular targets that are integral to the efficacy of some cancer immunotherapies.[1] This allows genotype-directed therapies to be tailored to subsets of patients who have specific genomic abnormalities across different tumor types.[1] Theoretically, treatments that target tumor-specific molecular abnormalities are likely to be more effective and less toxic.[1]

NORMAL HOST IMMUNE DEFENSES

The immune system is typically able to detect both internal threats (e.g., malignant cells) and external threats (e.g., viruses, bacteria, fungi, parasites).[4] The capacity of the immune system to identify, reject, and remember external threats has been well established, dating to the development of the first vaccine by Edward Jenner in 1796.[4] Since then, vaccines directed against many infectious diseases have significantly diminished human misery and saved countless lives.[4] The success of vaccines in preventing disease provides one indication that the immune system has "host protective memory," formed by innate and adaptive immunity.[4]

Innate immunity has been present throughout vertebrate evolution.[4] Although it is a primitive system, it is capable of a rapid response that occurs within minutes to hours.[4] This is accomplished through the use of protein products of germline genes that do not have to undergo receptor rearrangements, which provide B and T cells with their specificity.[4] Thus, the innate immune response is not capable of immunological memory and is not directed against any particular target or organism.[4] Instead, the innate immune response has recognition properties with low specificity that are based on the molecular patterns displayed by membrane proteins called

Figure 1 Significant Events in the History of Cancer Immunotherapy[3,5–9]



allo-HSCT = allogeneic hematopoietic stem cell transplantation; CTLA-4 = cytotoxic T-lymphocyte–associated antigen 4; DC = dendritic cell; FDA = Food and Drug Administration; ICB = immune checkpoint blocker; IFN-α = interferon alpha; mAb = monoclonal antibody; PD-1 = programmed death-1; RCC = renal cell carcinoma; rhIL-2 = recombinant interleukin-2

Toll-like receptors.[4] Among the components of the innate immune system are macrophages, dendritic cells (DCs), neutrophils, and mononuclear phagocytes, as well as natural killer (NK) cells and their cellular products, which may include a large number of chemokines and cytokines.[4]

In contrast, adaptive immunity is based upon the ability to rearrange antigen receptors on B and T cells in the immune system.[4] This allows the recognition of specific structures on antigens, called epitopes, that trigger immune responses.[4] B cells and the antibodies they secrete recognize both linear (i.e., peptide sequences) and conformational epitopes with high affinity and specificity.[4] Antibodies produced by B cells specifically bind to native (unaltered and unprocessed) antigens on the cell surface that are readily accessible and are typically hydrophilic sequences of six to seven amino acids.[4] Immunoglobulin M antibodies are pentavalent structures that are released early in the adaptive immune response, subsequently followed by immunoglobulin G antibodies.[4] These antibodies are most relevant to cancer immunity because they remove pathogens; clear circulating antigens, and manipulate complement fixation, antibody-dependent cellular cytotoxicity (ADCC), and target cell signaling.[4]

The receptors on T cells play a different role than those on B cells. T-cell receptors (TCRs) recognize defined linear peptide sequences expressed on antigen-presenting cells (APCs), such as DCs, when these fragments are presented in the grooves of major histocompatibility complex (MHC) loci.[4,7] MHC class I loci typically present short peptide fragments to cluster of differentiation 8-positive (CD8+) T cells with cytotoxic properties, whereas MHC class II loci present longer peptide fragments to CD4+ T cells, which regulate B cell function, secrete immunoregulatory cytokines, and can be cytotoxic.[4] It is important to note that T cells must be "turned off" after they have been activated and the immune response is completed to prevent autoimmunity.[4] To achieve this, T cells express increased levels of checkpoint inhibitors and induce regulatory T cells (Tregs) to shut down the proliferative phase of the activated T-cell response.[4]

CANCER IMMUNITY

Cancers induce immune and inflammatory responses as they invade healthy tissue and metastasize.[7] Sometimes these responses can eliminate a tumor through "immune surveillance."[1,7] The immune surveillance hypothesis proposes that a major role for the immune system is to survey the body for malignant cells and tumors (as it does for pathogens), recognizing and eliminating them based on the tumor-associated antigens (TAAs) they express.[7] A competent immune system then responds to a tumor by a process called "immunoediting."[5,7] Therefore, successful immune surveillance and response based on the recognition of tumor-specific antigens should eliminate tumors at early stages.[7]

The principle of tumor immune surveillance presumes that most premalignant cells and early malignancies can be eliminated (or controlled) by the immune system.[6] However, a critical feature of advanced tumors compared to early malignant lesions is their capability to evade adaptive immune responses.[6] During malignant transformation, non-self TAAs or "neoepitopes" resulting from gene mutations are created that can be recognized by the immune system.[1] Initially, adaptive tumor antigen-specific T-cell responses are generated, leading to cancer-cell elimination.[1] To survive, developing tumors must adapt to their immunological environment in a manner that turns off immune responses that are potentially harmful to the tumor and/or creates a local microenvironment that inhibits immune cell tumoricidal activity.[7] These processes are called immune tolerance induction and immune evasion, respectively.[7]

As the principle effectors of adaptive anticancer immunity, cytotoxic T cells specifically recognize TAAs displayed in complex with human leukocyte antigen (HLA) class I molecules on the surface of tumor cells.[1,7] APCs (namely DCs) and macrophages, plasma cells, cytokines, helper T cells, antibodies, and complements all function in a coordinated way to prevent the nascent tumors from maturing.[6,10] To elicit antitumor responses, tumor antigens are presented by DCs in the context of major MHC class I to activate CD8+ cytotoxic T lymphocytes (CTLs) and MHC class II to activate CD4+ helper T cells.[8] Human MHC molecules act as designated HLAs.[8] Two types of helper T cells are involved: Th1 and Th2.[8] Th1 cells have multiple functions, including interleukin (IL)-2 and interferon (IFN) production, which promote a CTL-mediated immune response that activates other CTLs to recognize and kill tumor cells.[8] Cytokines that are commonly associated with antibody-mediated immune responses and suppressor immune functions are produced by Th2 cells.[8] Tumor antigen recognition by CTLs is HLA restricted, meaning that to be recognized, the tumor cell must share the same HLA type with the CTL.[8] Early malignant lesions, which have downregulated or deleted genes for HLA class I molecules, therefore may become invisible to cytotoxic cells, but they can still be eliminated by macrophages, NK cells, and gamma-delta T cells.[6]

Mechanisms Tumors Use to Evade Host Immunity

Even if immune system mechanisms are functional, tumors can escape from immune attack.[1,7] Developing cancers use a variety of methods to overcome host immunity; these methods allow them to overwhelm, hide from, subvert, shield, defend against, and outlast the host immune response.[4] Major mechanisms by which tumors suppress the immune system and evade destruction include downmodulation of components of antigen presentation and processing; upregulation of checkpoint receptor ligands that downmodulate tumor-infiltrating lymphocyte (TIL) activity; recruitment of suppressor immune cells, such as Tregs, tumor-associated macrophages, and myeloid-derived suppressor cells; and the production of soluble factors associated with immunosuppression, such as IL-10 and transforming growth factor-beta.[1,5]

Tumors that become clinically evident are poorly immunogenic, meaning that they avoid recognition and elimination by the immune system despite displaying antigens that are capable of being recognized.[5–7] One way a tumor can evade antitumor immune responses is by developing mechanisms that induce immune tolerance to its antigens.[7] Some cancers can adapt to immune selection pressures by reducing or losing target antigen expression, through diminished expression of MHC class I or class II on malignant cells or APCs.[1,4] Malignant cell signaling can also degrade tumor-related T-cell signaling molecules and transcription factors, and generate immunosuppressive small molecules.[4] Cancer cells can also evade an immune response by upregulating membrane receptors, such as FasL and tumor necrosis factor-related apoptosis-inducing ligand, which induce apoptotic signals in T cells.[6] A tumor can also recruit Tregs to suppress the activation of T-cell anticancer immunity.[4]

Immune checkpoints (ICs) are receptor and ligand pairs that are involved in the modulation of immune responses. After antigen recognition and activation by T cells, ICs mediate the balance between inhibitory and costimulatory signals.[12] Their role is to modulate the duration and intensity of the immune response to maintain self-tolerance so that autoimmunity does not develop.[12] However, cancers can also use ICs to evade the immune system by deactivating TILs that penetrate tumor defenses to attack malignant cells.[3] For example, when the IC ligand known as programmed death ligand-1 (PD-L1), expressed by malignant cells, engages its IC receptor, programmed death-1 (PD-1), on the surfaces of activated T cells, the T cells adopt an "exhausted" phenotype and become ineffective.[4] It is important to note that there are many other IC receptor/ligand pairs in addition to PD-L1/PD-1.[4]

In order to exist within the context of a competent immune system, developing tumors need to create a "microenvironment" that diminishes the efficacy of tumoricidal immune cells.[7] Immune tolerance to tumor antigens begins with events that take place in the tumor microenvironment that influence tumor initiation, progression, and treatment response.[7,13] Incomplete elimination of the tumor by the immune system is followed by an equilibrium phase, during which cancer cells initiate complex mechanisms of immune evasion that will allow immune escape and tumor progression.[1,7,13] To accomplish this, the tumor not only organizes the immunological components of the microenvironment in a fashion that protects against antitumor immune responses, but also shifts immune responses to those that promote and support tumor growth.[7] As a result, T cells that do manage to "home in" on the tumor reach a tumor microenvironment that is dominated by tumor-associated immunosuppressive leukocytes (myeloid-derived suppressor cells, Tregs) and soluble immunosuppressive molecules (transforming growth factor-beta, IL-10, adenosine, indoleamine 2,3-dioxygenase, and many others).[1] Malignant cells can also create an immunosuppressive microenvironment sometimes referred to as a "Th2 milieu" by secreting cytokines and chemokines.[4]

Cancer tissue consists of tumor cells (parenchyma) and nonmalignant cells, as well as the cancer stroma, which is the subcellular matrix of the tumor microenvironment.[6] Cancers can physically hide from the immune system by generating dense collagenous stroma, profound hypoxia, and disordered angiogenesis.[4] In some malignancies, such as Hodgkin's lymphoma, the nonmalignant stroma often comprises the vast majority of the tumor bulk.[6] Tumor tissue can also induce physical changes, such as the creation of new vessels (through neovascularization or angiogenesis), in order to invade surrounding tissues and spread, or to cope with the deregulation of cellular energetics in a chronically hypoxic microenvironment.[6]

Inflammation can play either a positive or negative role in cancer development.[5] Chronic tumor-related inflammation can promote an immunosuppressive tumor microenvironment, whereas acute inflammation can enhance antitumor immunity by promoting T-cell priming, as well as DC maturation and function.[5] A further understanding of how inflammation influences cancer development and progression may lead to novel cancer immunotherapy strategies that target chronic inflammation.[5]

CANCER IMMUNOTHERAPY APPROACHES

The principle goal of cancer immunotherapy is to resurrect the patient's suppressed immune system so that it is again

capable of launching sustained attacks against tumor cells, ideally resulting in the eradication of cancer.[6] The principles of evolutionary biology suggest that a malignant cell population need not employ all possible immunosuppressive mechanisms to survive in a particular host; instead, that population would do only what is necessary.[4] Therefore, the dominant mechanism of immune evasion taken by a tumor likely represents a potential Achilles' heel that can be attacked therapeutically to restore immune control.[4] More than one of these mechanisms may be present in a particular patient, but it is likely that many cancer types employ similar defense mechanisms.[4] This has been the focus of much of the work conducted in cancer immunotherapy over the past decade, which has been remarkably productive and promising.[4,8]

A wide range of cancer immunotherapy approaches have proven effective.[6,8] A number of therapeutic monoclonal antibody medications have displayed significant antitumor activity in diseases such as malignant melanoma, RCC, non–small-cell lung cancer (NSCLC), bladder cancer, Hodgkin's lymphoma, breast cancer, and CRC.[4] ICB medications that prevent the binding of IC ligands to receptors can liberate T cells to attack relevant antigen-expressing tumor cells.[4] Vaccines can be administered to patients to induce host immunity against existing or newly identified tumor antigens.[4] Numerous adoptive cell transfer studies have also demonstrated the potent capacity of T cells to kill growing tumors, either directly through CTL activity or indirectly through multiple CD4 T-cell–dependent effector mechanisms.[7] Some of these approaches cause broad activation of the immune system, while others have a narrower range of activity.[8] Some immunotherapies can be "personalized" through genetic engineering, while others, such as monoclonal antibodies, are widely available commercially.[8]

A more detailed discussion of current cancer immunotherapy approaches follows.

Monoclonal Antibodies

During the past 20 years, monoclonal antibodies (mAbs) have been a major treatment for diverse cancers, including breast, lymphoma, and CRC malignancies.[4] The mAbs are artificial versions of large proteins produced by a particular B-cell clone, which have unique antigen specificity that allows them to bind to epitopes on the cancer cell or in its plasma.[6,10] Therapeutic mAbs are typically of the immunoglobulin G class and are composed of a fragment antibody-binding and a fragment constant component.[6] An mAb can be "naked," meaning it is not combined with any other drug, or conjugated.[10] Conjugated mAbs are joined with chemotherapy drugs, radioactive particles, or toxins so that they can act as a tool to lead these agents into cancer cells.[10]

The primary mechanisms of action for most naked mAbs are ADCC and complement-dependent cytotoxicity.[6] However, additional mAb mechanisms include triggering direct cell death or blocking prosurvival signaling, angiogenesis, or immune checkpoints.[6] Alternatively, conjugated mAbs serve as a critical part of targeted drug delivery systems that include mAb–drug conjugates, mAb–radionuclide conjugates, or even mAbs conjugated to nanoparticles, liposomes, or biodegradable polymers.[6] The antitumor efficacy of these mAb conjugates, however, is no longer mediated by ADCC and complement-dependent cytotoxicity actions, but by the radionuclides, toxins, or other anticancer agents that are specifically targeted toward the tumor or malignant cells—a strategy that limits toxicity in normal tissue.[6]

The Food and Drug Administration (FDA) has approved many therapeutic mAbs to treat different types of cancers.[10] In 1997, rituximab (Rituxan, Genentech) became the first mAb approved for clinical use, indicated in patients with select B-cell malignancies.[6] Numerous other mAbs have been approved since then, among them trastuzumab (Herceptin, Genentech), alemtuzumab (Campath, Genzyme), ibritumomab tiuxetan (Zevalin, Spectrum Pharmaceuticals), cetuximab (Erbitux, Lilly), bevacizumab (Avastin, Genentech), panitumumab (Vectibix, Amgen), ofatumumab (Arzerra, Novartis), ipilimumab (Yervoy, Bristol-Myers Squibb), brentuximab vedotin (Adcetris, Seattle Genetics), nivolumab (Opdivo, Bristol-Myers Squibb), and pembrolizumab (Keytruda, Merck Sharp & Dohme Corp.).[10] Many other mAbs are undergoing regulatory review at the FDA or are in phase 3 clinical trials.[6]

Immune Checkpoint Blockers

Immunomodulatory mAbs that block IC receptors are emerging as promising treatments for various cancers because they cause a remarkable and long-lasting treatment response in some patients.[12] Unlike chemotherapy or even some targeted therapies, they can provide durable, long-term survival benefits and are well tolerated.[1,2,12] A notable case is the success of the ICB drug pembrolizumab, which, combined with surgery and radiation, has reportedly eradicated all evidence of advanced melanoma in former President Jimmy Carter even though it had metastasized to his liver and brain.[9]

The mechanism of action for ICBs signifies a true shift in oncology—rather than being directed at destroying tumor cells, as is the case with chemotherapy and radiation treatments, they target the immunosuppression induced by the cancer.[12] As noted, tumors can exploit immunosuppressive checkpoints to impede T-cell activity and evade the body's immune system.[8] ICBs overcome this mechanism by blocking the checkpoint receptors on T cells that act as brakes to the immune response; this results in prolonged antitumor responses.[8,9,12] Checkpoint ligand/receptor interactions can be stopped by immunomodulatory mAbs that block IC receptors, such as cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and PD-1, or IC ligands, such as PD-L1.[5,6,8,9] These are the ICBs that have been studied the most in clinical trials, but many other checkpoints have been identified that may lead to the development of new ICB therapeutics.[1,7,8,9,12]

The FDA has approved several ICBs that have been shown to produce significant clinical benefits in treating several tumor types. In 2011, the FDA approved the anti–CTLA-4 ICB ipilimumab for the treatment of melanoma, marking the beginning of the new era for cancer immunotherapy.[5,6] The PD-1 blockers pembrolizumab and nivolumab were granted accelerated approval by the FDA in September and December 2014, respectively, for patients with unresectable or metastatic malignant melanoma.[6] The FDA subsequently added indications for both pembrolizumab and nivolumab for NSCLC, head-and-neck squamous cell carcinoma, and Hodgkin's lymphoma, and for nivolumab for bladder cancer and RCC.[12,14] Atezolizumab, an anti–PD-L1 ICB, was approved for metastatic bladder cancer in May 2016, followed by an indication for NSCLC several months later and expansion of its bladder-cancer indication in April

Table 1 Anti-CTLA-4 and Anti-PD-1/PD-L1 Immune Checkpoint Blockers Approved for Use in the U.S. or in Development[12,14]

Target	Drug *(Brand Name [if applicable], Company)*	Indication(s)*	Development Stage (Tumor Type)
CTLA-4	Ipilimumab *(Yervoy, Bristol-Myers Squibb)*	Melanoma	Phase 1–3 (multiple)
	Tremelimumab *(AstraZeneca/MedImmune)*	—	Phase 1–3 (multiple)
PD-1	Nivolumab *(Opdivo, Bristol-Myers Squibb)*	Melanoma, NSCLC, RCC, bladder, HNSCC, cHL	Phase 3 (HNSCC, gastric)
	Pembrolizumab *(Keytruda, Merck Sharp & Dohme)*	Melanoma, NSCLC, HNSCC, cHL	Phase 3 (bladder, gastric/GE)
	Pidilizumab *(Pfizer)*	—	Phase 2 (CNS, CRC, pancreatic, prostate, RCC)
PD-L1	Atezolizumab *(Tecentriq, Genentech)*	Bladder, NSCLC	Phase 3 (RCC, TNBC)
	Durvalumab *(Imfinzi, AstraZeneca)*	Bladder	Phase 3 (NSCLC, HNSCC)
	Avelumab *(Bavencio, EMD Serono, Inc.)*	MCC, bladder	Phase 3 (NSCLC)

* See full prescribing information for detailed listing of FDA-approved indications.

cHL = classical Hodgkin's lymphoma; CNS = central nervous system; CRC = colorectal cancer; CTLA-4 = cytotoxic T-lymphocyte–associated antigen 4; FDA = Food and Drug Administration; GE = gastroesophageal; HNSCC = head-and-neck squamous cell carcinoma; MCC = Merkel cell carcinoma; NSCLC = non–small-cell lung cancer; PD-1 = programmed death-1; PD-L1 = programmed death ligand-1; RCC = renal cell carcinoma; TNBC = triple-negative breast cancer.

2017.[14] Additional CTLA-4 and PD-1 ICBs and indications are being studied in clinical trials (Table 1).[7,12] Additional details follow regarding CTLA-4 and PD-1 blockade and ICB.

CTLA-4 Blockade

CTLA-4, the first IC receptor to be clinically targeted, is expressed exclusively on the surface of T cells, where its main function is to regulate the amplitude of early-stage T-cell activation.[7] The ligands CD80 and CD86 bind to CD28 antigen expressed on T cells, promoting T-cell activation by amplifying signals from the TCR.[7] CTLA-4, which has a much higher affinity for CD80 and CD86 than CD28 does, inhibits T-cell activation by delivering inhibitory signals and competing for binding to CD80 and CD86.[7,8,12]

The specific molecular pathways by which CTLA-4 blocks T-cell activation remain under investigation, although many studies suggest that it disrupts kinase signals that are triggered by CD28 and the TCR.[7] Direct CTLA-4 signaling may also involve a mechanism that includes protein phosphatase and is unrelated to CD80 and CD86 binding.[8] Specifically, CTLA-4 is thought to activate the phosphatases SHP2 and PP2A, which counteract the phosphorylation cascade initiated by the TCR and CD28 activation, ultimately attenuating T-cell activation.[8]

Activation of CTLA-4 also enhances the suppressive action of Tregs while decreasing IL-2 production and IL-2 receptor expression.[12] The mechanism by which CTLA-4 amplifies the inhibitory function of Tregs is unknown, but ICBs have been shown to significantly reduce the ability of Tregs to control both the antitumor immune response and autoimmunity.[7] Thus, the mechanism of action for CTLA-4 ICBs involves both enhancement of T-cell activity and inhibition or elimination of Treg activity.[7]

PD-1 Blockade

The PD-1 receptor present on activated T cells has also emerged as a promising immunotherapy target.[7,8] The main role of PD-1 is to limit T-cell activity in peripheral tissues in order to prevent autoimmunity during an inflammatory response to infection.[7,8,12] The binding of PD-1 to its ligands, PD-L1 or PD-L2, on CD8 T cells leads to apoptosis, as well as decreased T-cell proliferation and cytokine production.[8] Similar to CTLA-4, PD-1 is highly expressed on Tregs, where it induces Treg proliferation when it binds to its ligands, causing suppression of CD4 and CD8 T-cell effector functions.[7,8,12] The PD-1 pathway can also cause a shift from T-cell activation to immune tolerance in secondary lymphoid tissues at early stages in the immune response.[7] PD-1 is more broadly expressed within the body than CTLA-4, including on other activated non-T lymphocyte subsets, including B cells and NK cells, limiting their lytic activity.[7] PD-1 regulates T-cell activation in part through phosphate kinase inhibition.[8] When PD-1 is bound to its ligands, it is thought to inhibit the phosphatase SHP2, which works to dephosphorylate TCR signaling molecules.[7,8]

Unlike the CTLA-4 ligands CD80 and CD86, PD-L1 and PD-L2 are also overexpressed on cancer cells and in the tumor microenvironment.[7,8,12] Consequently, PD-L1 expression is described in many cancer types, including solid tumors.[7] It is also expressed on a large proportion of TILs from many different tumor types—a finding that correlates with low cytokine production resulting in reduced antitumor activity.[7,8] PD-L1 is also commonly expressed on myeloid cells in the tumor microenvironment.[7] Therefore, it is expected that blocking the PD-1/PD-L1 pathway can lead to a more active and prolonged antitumor immune response.[12]

PD-1 blockade with ICBs induces and enhances T-cell activation, expansion, and effector functions.[6] PD-1 blockade may also enhance antitumor responses by diminishing the number and/or suppressive activity of Tregs that have infiltrated tumors.[7] PD-1 blockade is also thought to enhance NK activity in tumors and tissues, as well as antibody production either indirectly or through direct affects on PD-1–positive B cells.[7] The state of exhaustion among cognate antigen-specific T cells that is induced by persistently high levels of PD-1 expression also appears to be partially reversible by PD-1 pathway blockade.[7] Taken together, these findings suggest that the mechanisms of action for PD-1 pathway blockade and its role in restoring the antitumor function of the immune response are complex.[7]

Cytokines

Cytokines, such as interferons, interleukins, lymphokines, monokines, chemokines, and growth factors, are immune modulators that are produced naturally by numerous cell types.[8] They are small-protein molecules that have variable activities—most importantly, regulation of immunity and inflammation.[8] These messenger molecules, which are secreted by cells of the immune system (such as lymphocytes, monocytes, and macrophages), regulate leukocyte differentiation, migration, activation, and suppression.[6] Certain cytokines can directly enhance or suppress T-cell response against cancer cells, so it is not surprising that the systemic administration of cytokines (initially interferons and interleukins) was among the first approaches to cancer immunotherapy.[6,8] Early cytokine-based treatments were made possible by the development of recombinant DNA technology using genetically engineered *Escherichia coli* strains.[6] This enabled the large-scale production of purified recombinant human cytokines that are suitable for systemic administration to patients.[6] Although IFN-α and IL-2 have been best characterized and used for cancer treatment, many additional cytokines are being investigated for use in cancer immunotherapy.[8]

Interferons

Interferons are cytokines that exert context-dependent pleiotropic effects on immunodulatory, virostatic, and/or antiproliferative activities.[6] IFN-α, discovered 45 years ago, is a member of the I IFN family, which also includes IFN-β, IFNA-Σ, IFN-κ, and IFN-ω.[8] It is an immunostimulant that uses several well-characterized molecular mechanisms to mediate its effects.[8] Its enhancement of DC activation and maturation improves antitumor immunity by enhancing antigen presentation to immune cells.[8] In addition, IFN-α promotes a Th1 immune response, increasing the activity of CTLs involved in tumor cell lysis, and it also enhances the cytotoxic activities of NK cells.[8] IFN-α activity also directly targets tumor cell growth and oncogene expression.[8] Importantly, IFNs also enhance molecular MHC expression, which makes them ideal to use as adjuvants in different cancer immunotherapy approaches, including cancer vaccines.[6]

IFN-α was the first cytokine produced with recombinant technology.[6,8] In 1986, it was approved for the treatment of hairy cell leukemia, and an indication for chronic myelogenous leukemia was added later.[6] Of the numerous IFN subtypes, IFN-α, specifically IFN-α2b, is the one used most widely in cancer immunotherapy.[8] IFN-α2b has received regulatory approval for the treatment of metastatic melanoma, follicular lymphoma, and AIDS-related Kaposi sarcoma.[6,8]

Interleukins

Interleukins are predominantly secreted by CD4+ helper T-cell subsets that are involved in the development, activation, and suppression of CD8+ cytotoxic T cells, macrophages, and NK cells.[6] Of all the interleukins, IL-2 has been the most thoroughly studied, with recombinant IL-2 (rhIL-2) being the most broadly used agent in cancer immunotherapy strategies.[6,8]

IL-2 is a critical cofactor that activates cytotoxic TILs; enhances the antitumor activity of NK cells; induces lymphokine-activated killer cells that mediate antitumor effects; and promotes the growth and proliferation of Tregs.[6,8] A key characteristic of IL-2 is its dose dependency, particularly with respect to activation of different immune-cell subsets.[8] At higher doses, IL-2 stimulates a Th1 immune response and promotes CTL antitumor activity; at lower doses, IL-2 exerts both immune-enhancing and immune-suppressing activities (by stimulating Tregs).[8] IL-2 was first characterized in the 1970s as a T-cell growth factor.[8] Because of the broad activity of IL-2, rhIL-2 was developed.[6,8] Similar to native IL-2, rhIL-2 broadly activates the immune system by promoting the proliferation and differentiation of T cells, B cells, and NK cells.[8] In addition, rhIL-2 enhances the cytolytic activity of lymphocyte subsets and promotes interaction between malignant cells and the immune system.[8]

In 1992, rhIL-2 (aldesleukin [Proleukin, Prometheus Laboratories]) received approval for the treatment of RCC, and in 1998, an indication was added for metastatic melanoma.[6] High-dose aldesleukin can be used as a single agent, as part of a multiagent chemoimmunotherapy regimen, or as part of a diverse cell-based cancer immunotherapy approach.[6] Several other recombinant interleukins are being studied in clinical trials, including rhIL-12, rhIL-21, rhIL-24, and others.[6] For example, mesenchymal stem cells transduced with secretable human IL-24 have been successful as an experimental therapy for lung cancer.[6] Diverse interleukin-containing fusion proteins, immunocytokines, or fragment constant fusion proteins targeting particular interleukins or chemokine receptors are also being used in cancer immunotherapy.[6]

Cancer Vaccines

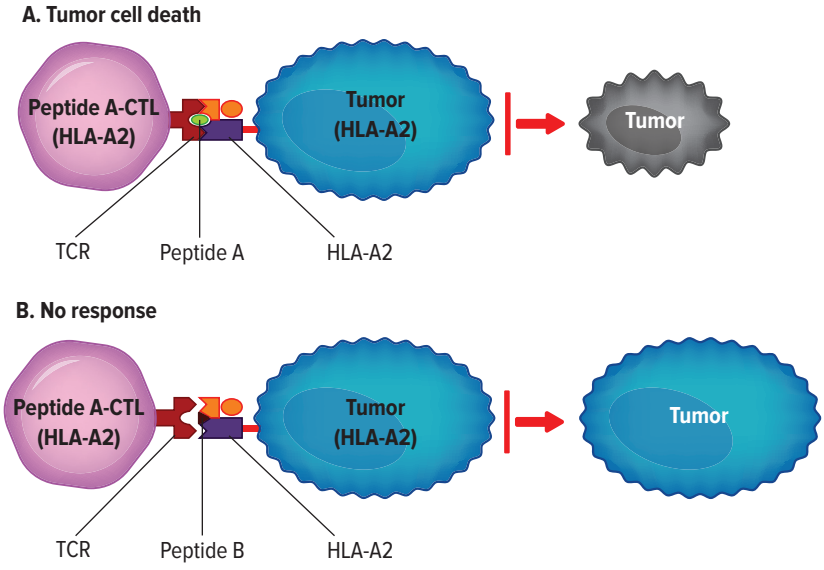
A more detailed understanding of tumor-specific immune responses has led to the search for an even more focused approach to cancer immunotherapy.[8,10] One such approach is the use of a vaccine to encourage the body to develop antibodies that target peptides or antigens that are present on the tumor.[8] Cancer antigens are often released into systemic circulation and the tumor microenvironment due to focal necroses caused by thrombotic or hemorrhagic events in the tumor vasculature, surgical procedures, tumor irradiation, or chemotherapy treatment.[6] Proper T-cell responses against these cancer antigens can, however, be compromised by low T-cell affinity to self-antigens; immune system impairment due to immunosuppressive therapy; tumor-induced immune response inhibition due to secretion of cytokines (IL-10, transforming growth factor-beta); or manipulation of immune cells through immunosuppressive factors (e.g., Tregs, tumor-associated macrophages, and myeloid-derived suppressor cells).[6]

One goal of cancer vaccines is to stimulate the immune system to attack and eradicate cancer cells.[10] To this end, cancer vaccines contain whole cancer cells, parts of cancer cells, or purified antigens that enhance the immune response against cancer cells.[10] Tumor vaccines can be peptide-based, immune cell- or DC-based, or tumor cell-based, each offering unique advantages and disadvantages.[8] Oncolytic virus, nanocarrier, and DNA-based vaccines are also being investigated.[6]

Peptide-Based Vaccines

Peptide-based vaccines elicit an immune response against a single tumor antigen expressed in association with HLA molecules on the surface of tumor cells.[8] These vaccines are less

Figure 2 Mechanism of Peptide Cancer Vaccines[8]



This diagram illustrates how the efficacy of peptide cancer vaccines is restricted by both HLA type and peptide sequence. (A) When both the vaccine HLA type and peptide sequence presented by the tumor cognate HLA molecule are matched, the antitumor cytotoxic effects of peptide-specific CTLs are activated. (B) However, when they are not matched, the peptide-specific CTLs will fail to lyse tumor cells, even in the presence of matched HLA type.

CTL = cytotoxic T lymphocyte; HLA = human leukocyte antigen; TCR = T-cell receptor.

likely to produce toxicity in normal cells and tissues; however, they have limitations with respect to the need to properly identify the tumor antigen peptide and patient HLA type (see Figure 2).[8] Still, because peptide vaccines have demonstrated the ability to stimulate antigen-specific immune responses while exhibiting a favorable safety profile, there is interest in further investigating these agents.[8]

Immune- or Dendritic-Cell–Based Vaccines

As discussed earlier, DCs are specialized antigen-presenting cells that play a major role in capturing, processing, and presenting tumor antigens to T cells and eliciting an immune response.[6] Among the earliest cancer vaccine approaches was the use of monocyte-derived DCs grown *ex vivo*, pulsed with purified TAAs or autologous tumor cell lysates.[6]

Sipuleucel-T (Provenge, Dendreon Corp.), the FDA-approved first-in-class DC-based cancer vaccine, represented a milestone for cancer immunotherapy when it was approved in 2010 for the treatment of castration-resistant prostate cancer.[6,8] It is a DC-based autologous vaccine that is designed to use the patient's own immune system to generate antitumor immunity.[8] Preparation of this vaccine involves the patient undergoing leukapheresis to obtain blood that naturally contains APCs, including DCs, that are key to this vaccine protocol.[8] The patient's APCs are then activated by exposure to the recombinant prostate tumor antigen PA2024 and granulocyte macrophage colony-stimulating factor, a potent immune activator.[8] This results is the development of APCs that, when administered to the patient in a vaccine, can activate the patient's T cells to specifically target the PA2024 antigen present on prostate cancer cells.[8] Another type of immune-based vaccine uses monoclonal anti-idiotypic mAbs against TAAs that it imitates.[6] For example, abagovomab is a monoclonal anti-idiotypic antibody that functionally imitates the TAA CA-125, a glycoprotein expressed on the surface of more than 95% of ovarian cancers.[6]

Second-generation DC-based vaccines use innovative *in vitro* culturing techniques with media enriched with critical cytokines, enhancing immunogenicity and improving DC function.[6] Recombinant technology has also created the ability to generate genetically engineered DCs that secrete growth factors or interleukins that activate T cells and NK cells, providing significant improvement in the anticancer immune response of these cancer vaccines.[6]

Tumor-Cell–Based Vaccines

Tumor-cell–based vaccines use whole tumor cells to provide a source of immunogenic material.[8] Unlike peptide-based vaccines, tumor-cell–based vaccines are not limited by HLA type restrictions, so they can be used to present a broad range of epitopes to the immune system to mount a defense.[8] These vaccines can be autologous, using tumor cells from the vaccine recipient, or allogeneic, using tumor cells from another patient.[8] Once the tumor cells are obtained, they can be prepared for immunization by irradiation.[8] They are then administered either alone or in combination with an adjuvant such as granulocyte macrophage colony-stimulating factor.[8] Tumor-cell–based vaccines such as M-Vax (AVAX Technologies) have demonstrated efficacy in targeting RCC, melanoma, and acute myeloid leukemia in clinical trials.[8]

Cell-Based Immunotherapy

Rather than provoking an immune response, cell-based immunotherapies, such as adoptive T-cell therapy, contain intrinsic antitumor properties.[1] Adoptive T-cell therapy is the transfer of natural or genetically modified T cells that have been expanded *ex vivo* into patients to treat metastatic cancers.[8,10] The infused cells can be allogenic or autologous. The efficacy of allogenic HSCT from a healthy donor by infusion into a cancer patient has been recognized for 50 years.[8] After the infusion, an immune response is elicited based on allogeneic differences in the expression of peptide/HLA complexes or minor histocompatibility antigens.[8] Cell-based immunotherapy treatments with infusions of genetically modified autologous or allogeneic T cells have also demonstrated impressive antitumor activity in some hematologic malignancies.[4,10]

Treatment using infusion of TILs is another cell-based cancer immunotherapy method that is supported by extensive clinical experience.[6] TILs react to epitopes and to shared anti-

gens and neoantigens created by tumor-specific mutations.[6] The tumor-specific antigens may include: mutant protein antigens, oncogenic viral antigens, tissue differentiation antigens, cancer testis antigens, and stromal-specific or vascular antigens.[10] However, identification of the tumor antigen is not required for TIL cell-based treatment because the TILs infiltrating a tumor are already antigen-specific T cells.[6] TIL-based therapy does, however, require a tumor biopsy to isolate sufficient numbers of TILs so that they can be cultured *ex vivo*.[6] TIL infusions (with concomitant systemic high-dose IL-2 treatment) have induced durable remissions of advanced metastatic melanoma.[6] IL-2 can also be used *in vitro* for the activation and *ex vivo* expansion of lymphocytes as part of diverse cell-based immunotherapy approaches.[6,10]

Genetic Engineering Approaches To Cell-Based Immunotherapy

The success of allogenic immune cell-based therapy has spurred novel genetic engineering approaches to maximize the efficacy and minimize the toxicity of this procedure.[8] Recently, a personalized method was developed for TIL immunotherapy based on the mutational analysis of tumors, which was considered impossible just 10 years ago.[6] This method is based on the identification of all mutated genes through exome sequencing of tumor samples.[6] Then, the amino-acid sequences present around the mutation sites are entered into prediction software to identify all potential epitopes.[6] These peptide sequences are used for *ex vivo* expansion of TILs that are targeted with known specificity.[6]

Genetic engineering-based approaches to cell-based cancer immunotherapy have also been investigated for the purpose of overcoming the limitations of generating autologous TILs.[8] The development of CAR therapy is one of the most promising of these approaches.[6,8] In this method, CARs are introduced by genetic engineering into autologous T cells *ex vivo* to enhance their activity and specificity against antigens expressed on the tumor cell surface.[6,8] The TCR is modified so that its antigen-binding portion is conjugated to an artificial signaling molecule that sends activation signals to T cells when it binds to the antigen/MHC complex.[6,8] After modification, the expanded CAR T cells are infused back into the patient, where they can specifically target and eliminate cancerous cells.[6] Signaling through CAR can fully substitute for endogenous TCR signaling, allowing a targeted cytotoxic immune response that is potent, swift, and non-HLA-restricted.[6] Consequently, CAR-based adoptive cell therapy approaches are insensitive to tumor escape mechanisms arising from HLA molecule loss.[6]

A large number of CARs targeting diverse tumors have been developed and have shown impressive clinical outcomes in treating patients with relapsed or refractory B-cell malignancies, such as acute and chronic lymphocytic leukemia.[5] However, targeting solid tumors with CAR T cells has yielded only modest results.[5] Currently, the major limitation to CAR treatment is the lack of sufficiently specific tumor surface antigens.[6] Attempts have been made to target antigens such as HER2/neu, CAIX, and CD33, but this has resulted in significant toxicity and damage to healthy tissues. However, the search for improved targets is ongoing.[6]

CONCLUSION

The success observed with cancer immunotherapy treatments emphasizes the importance of understanding tumor immunology—particularly the roles of tumor antigens and the immunosuppressive tumor microenvironment.[5,7] Since the 1980s, many novel immunotherapy agents have been developed that effectively fight cancer.[6] However, despite these accomplishments, further progress is needed.[10] While many cytokine-based approaches and numerous mAbs and their derivatives have become standard-of-care treatments for a variety of malignancies, other immunotherapy approaches, such as most cancer vaccines and cell-based approaches, remain experimental.[6] Additional suitable tumor antigens must also be defined for targeted immunotherapies.[10] Fortunately, many new immunotherapy strategies and agents are being researched and tested in clinical trials, which will hopefully provide new effective treatments for patients living with relapsed or refractory malignancies.[6]

The second article of this series will discuss the efficacy and safety of cancer immunotherapy treatments and other important clinical considerations.

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