

Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead

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How to cite this article: Oiseth SJ, Aziz MS. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. *J Cancer Metastasis Treat* 2017;3:250-61.

ABSTRACT

Article history:

Received: 12 Jun 2017

Accepted: 2 Oct 2017

Published: 31 Oct 2017

Key words:

Cancer immunotherapy, immune checkpoint inhibitors, PD-1, programmed death-ligand 1, cytotoxic T-lymphocyte-associated antigen-4, adoptive cell therapy, cancer vaccines, oncolytic viruses, history of cancer immunology

The knowledge that the body possesses natural defenses to combat cancer existed long before the modern period, with multiple anecdotal reports of tumors miraculously disappearing, sometimes spontaneously or after a febrile or infectious episode. Spontaneous tumor regression of untreated malignant tumors is currently a well-accepted albeit rare phenomenon, and it is recognized that immunosuppression is associated with a higher cancer risk. The treatment of bladder carcinoma by intravesical administration of live attenuated *Bacillus Calmette-Guérin* bacteria was shown to be very effective in 1976 and is now standard treatment. Effective immunity against cancer involves complex interactions between the tumor, the host, and the environment. Cancer immunotherapy uses various strategies to augment tumor immunity and represents a paradigm shift in treating cancer, since attention has become more focused on the “biologic passport” of the individual tumor rather than the site of origin of the tumor. The different types of cancer immunotherapies discussed here include biologic modifiers, such as cytokines and vaccines, adoptive cell therapies, oncolytic viruses, and antibodies against immune checkpoint inhibitors, such as the co-inhibitory T-cell receptor PD-1 and one of its ligands, programmed death-ligand 1.

INTRODUCTION

Cancer immunotherapy (CI) is rapidly advancing and can now be considered to be the “fifth pillar” of cancer therapy, joining the ranks of surgery, cytotoxic chemotherapy, radiation, and targeted therapy. The CI which has sparked the most interest involves antibodies to inhibitory immune checkpoint molecules. Although they have produced dramatic results in only a subset of

some malignancies to date^[1], it is difficult to not be very excited about their potential. In a recent meta-analysis of ipilimumab, a monoclonal antibody that targets cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), a type of immune checkpoint receptor or negative regulator of T-cell immune function, more than 20% of patients with metastatic melanoma who received a single round of treatment were alive 10 years later with no evidence of disease^[2]. Before this treatment, the 10-year survival



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rate was less than 10%^[3]. Combination therapy with another immunotherapeutic agent shows even greater promise, as seen when the addition of another checkpoint inhibitor antibody, nivolumab, a monoclonal antibody (mAb) which targets the PD-1 receptor on T-cells, produced a 50% response rate in metastatic melanoma^[4,5]. CI has also shown effectiveness in other types of malignances, and combinations with different treatment modalities (“immuno-oncology”) are also showing remarkable benefits^[6,7].

HISTORY OF OUR KNOWLEDGE OF THE IMMUNE SYSTEM’S ROLE IN CANCER

It has been known for many years that the immune system plays a major role in neoplastic development and control, since patients who are immunosuppressed have a higher risk of cancer, and spontaneous regression of many types of malignant tumors is a rare but well-recognized phenomenon-occurring in approximately 1 in every 60,000 to 100,000 cancer cases^[8-10].

Throughout history there are multiple accounts about tumorous growths regressing or disappearing after an infectious and/or high febrile episode, having been reported from ancient Egypt up to the early 18th century in Europe, but the scientific basis for attempts at modulating the immune system to treat cancer can find its modern roots only in the second half of the 18th century, when histologic confirmation of a malignancy became possible. More than 135 years ago the German physicians Busch^[11] and Fehleisen^[12] independently noticed regression of tumors in cancer patients after accidental infections by erysipelas. In 1868, Busch was the first to intentionally infect a cancer patient with erysipelas and he noticed shrinkage of the malignancy. Fehleisen^[12] repeated this treatment in 1882 and he also eventually identified *Streptococcus pyogenes* as the causative agent of erysipelas^[12,13]. In 1891, an American surgeon, William Coley, of the Bone Tumor Service at Memorial Hospital in New York, followed up on his own independent observation of a long-term regression of a sarcoma after an erysipelas infection by starting a 43-year-old project involving the injection of heat-inactivated bacteria (“Coley’s toxins”) into patients with inoperable cancers^[14]. He reported a significant number of regressions and cures in more than 1,000 patients, many or most with sarcomas, and the method started gaining wide acceptance and notoriety^[15] [Figure 1]. His toxins gradually disappeared from use because of several factors, including his failure to follow good scientific protocols and inability to consistently obtain reproducible results. The development of radiation therapy and chemotherapy also contributed to the loss of interest in using this type of therapy to treat

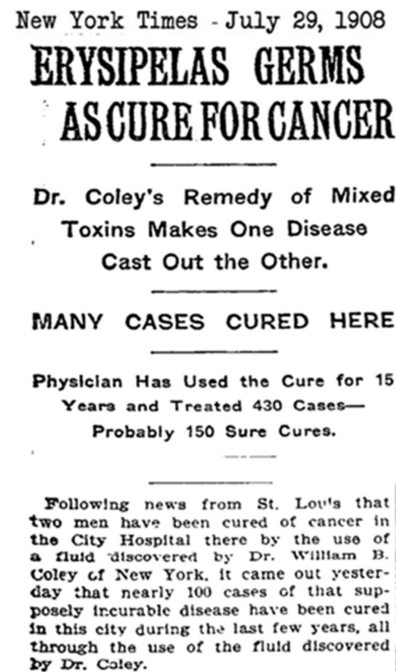


Figure 1: An article in a major U.S. newspaper printed in 1908 reflects the widespread attention given to Coley’s toxins

cancer. Nonetheless, after no fewer than five marked shifts in attitude toward CI since the 1890s^[16], Coley’s principles have been shown to be correct, and the use of bacteria finally found sound justification in 1976 when Morales *et al.*^[17] established the effectiveness of the bacterium *Bacillus Calmette-Guérin* (BCG) in the treatment of superficial bladder cancer. The underpinnings for this clinical trial include a 1959 study by Old *et al.*^[18] showing the anti-tumor effects of BCG in a mouse model. Besides his work on BCG, Old also performed extensive research on other CI-related topics, and was a discoverer of tumor necrosis factor in 1975^[19]. Due to their foundational discoveries and lifelong dedication to the field, Coley and Old have each been referred to as the “Father of Immunotherapy”, a title which is perhaps best shared.

Even viral infections were believed to have a cancer-suppressive effect as far back as 1904 when George Dock at the University of Michigan described a 42-year-old woman with acute leukemia who experienced a temporary remission after a presumed infection with influenza in 1896^[20]. At the same time, a better understanding of the immune system was being developed, including discoveries about cellular and humoral immunity [Table 1].

THE IMMUNE SYSTEM’S ROLE IN ELIMINATING OR CONTROLLING MALIGNANT CELLS

As a brief background review, the immune system is

Table 1: Timeline of selected key events in modern cancer immunology, 1868-2017

Year	Event
1868	First report of an intentional infection of a cancer patient with erysipelas by Wilhelm Busch, with notable shrinkage of the tumor ^[11]
1883	Elie Metchnikoff publishes a key paper describing phagocytic cells (macrophages) in frogs ^[102] ; awarded Nobel Prize in 1908
1890	Discovery of antibodies (diphtheria and tetanus) by Emil von Behring and Kitasato Shibasaburō ^[103] ; Nobel Prize awarded to von Behring in 1901
1891	William Coley injected his first of many cancer patients with bacteria, reporting tumor regressions in many of them ^[14]
1895	Discovery of complement, by Jules Bordet ^[104] ; awarded Nobel Prize 1919
1897-1901	Paul Ehrlich "Side-chain" theory of antibody specificity (adaptive immunity, autoimmunity) ^[105] ; awarded Nobel Prize in 1908
1901	Serological discovery of blood groups, by Karl Landsteiner ^[106] ; awarded Nobel Prize in 1930
1901-08	Rejection of transplanted tumors in mice, reported by Carl Jensen & Leo Loeb ^[107,108]
1914	Genetic basis for the rejection of transplantable tumors, reported by Clarence Little ^[109]
1909-20	Establishment of inbred strains of mice by Leonell Strong and Clarence Little ^[110]
1948	First report of histocompatibility antigens being the basis for transplant rejection, by P. Gorer, S. Lyman, & G. Snell ^[111] . 1908 Nobel Prize awarded jointly to G. Snell, B. Benacerraf, & J. Dausset in 1980
1955	Natural-Selection Theory of Antibody Formation, first formulated by N. Jerne ^[112] ; awarded Nobel Prize in 1984
1956	Discovery of acquired immunological tolerance by R. Billingham, L. Brent & P. Medawar. Nobel Prize awarded to Medawar & F. Burnet in 1960 ^[113,114]
1957	Immune rejection of transplanted syngeneic tumors (i.e., each tumor is antigenically unique) ^[115] . Reported by Richmond Prehn & Joan Main.
1957	Interferon discovered, described as a factor that conferred the property of viral interference ^[116] , reported by Alick Isaacs and Jean Lindenmann. Its anti-leukemic effect is reported in 1984.
1959	Immune surveillance of cancer theory by Lewis Thomas & F. Macfarlane Burnet ^[30-32]
1959	Chemical structure of antibodies, by Gerald Edelman & Rodney Porter ^[117-120] ; Nobel Prize awarded to both in 1972.
1959	BCG shown to have anti-tumor effects in a mouse model, reported by Lloyd Old, Donald Clark, & Baruj Benacerraf ^[118]
1973	First description of dendritic cells, by Ralph Steinman & Zanvil Cohn ^[121] ; Steinman awarded Nobel Prize in 2011 for discovery of the dendritic cell and its role in adaptive immunity
1974	First reports of the specificity of cell-mediated immunity by Peter Doherty and Rolf Zinkernagel ^[122,123] ; Nobel Prize awarded to both in 1996
1975	Monoclonal antibodies manufactured by George Koehler & Caesar Milstein ^[124,125] ; Nobel Prize awarded to Koehler, Milstein & N. Jerne (for his theoretical contributions) in 1984
1975	Discovery of tumor necrosis factor, reported by Lloyd Old, with Elizabeth Carswell, Robert Kassel, S. Green, N. Fiore, & B. Williamson ^[19]
1975	First description of NK cells on a functional basis according to their ability to lyse tumor cells in the absence of prior stimulation ^[126] . Reported by Ronald Herberman, Myrthel Nunn, Howard Holden, & David H. Lavrin.
1976	Discovery of the genetic principle for generation of antibody diversity, by Susumu Tonegawa ^[127,128] ; awarded Nobel Prize in 1987.
1982	Discovery of the T-cell receptor in 1982, reported by James Allison, B. McIntyre, & D. Bloch ^[101]
1984	First report of interferon response in patients with hairy cell leukemia ^[129]
1991	First report of a human tumor antigen recognized by T-cells, reported by Pierre van der Bruggen, C. Traversari, P. Chomez, <i>et al.</i> ^[36]
1996	Discovery that CTLA-4 blocking antibodies could treat tumors in animal models, reported by Dana Leach, Matthew Krummel & James Allison ^[72]
1998	Discoveries regarding the activation of innate immunity, by R. Medzhitov, P. Preston-Hurlburt, C. Janeway; & B. Beutler; Beutler awarded Nobel Prize in 2011 ^[130,131]
2001	Rag2 -/- immunodeficient mice, with no B or T cells, show increased susceptibility to spontaneous and carcinogen-induced tumors, reported by V. Shankaran, with L.J. Old, R. Schreiber, <i>et al.</i> ^[132]
2005	Memory T-cells in colorectal tumors shown to predict clinical outcome, reported by F. Pagès, A. Berger, M. Camus <i>et al.</i> ^[133]
2010	First autologous cell-based cancer vaccine (sipuleucel-T) is approved by the FDA for the treatment of metastatic, asymptomatic stage IV prostate cancer ^[43,134]
2010	First successful use of gene-edited T-cells for the treatment of CD19+ hematologic malignancies in humans, reported by W. Qasim, H. Zhan, S. Samarasinghe <i>et al.</i> ^[135]
2011	Anti-CTLA-4 (ipilimumab), is the first inhibitory checkpoint inhibitor (ICI) approved by the FDA for treatment of stage IV melanoma ^[136]
2012	Discovery of the CRISPR/Cas9 system, a simpler and more efficient method of genome editing, reported by J.A. Doudna & E. Charpentier, with M. Jinek, K. Chylinski, I. Fonfara, & M. Hauer ^[49]
2013	First use of CRISPR/Cas9 technique in eukaryotic cells, reported by F. Zhang, with L. Cong, F. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. Hsu, X. Wu, W. Jiang, & L. Marraffini ^[50]
2016	A second class of ICIs, anti-PD-1 (pembrolizumab), is approved for the treatment of melanoma ^[137]
2016	First characterization of the role of dendritic cell CTLA-4 in Th-1 immunity, reported by M. Halpert, V. Konduri, D. Liang <i>et al.</i> ^[138]
2016	A third class of ICIs, PD-L1 (atezolizumab), is approved for treatment of bladder cancer ^[139]
2016	First test in humans of CRISPR gene-editing technique for CAR T-cell therapy ^[51]
2017	Phase I/IIa study of an inhibitor of indoleamine 2,3-dioxygenase (IDO1), a non-membrane-attached enzyme with a checkpoint inhibitor function, shows promise ^[95]

Not all of the events listed are discussed in the text, but all are referenced to the pertinent literature

classically considered to be comprised of the innate and adaptive arms, although this is a simplification since these arms have overlapping functions and are intimately related. The innate immune system includes dendritic cells, natural killer cells (NK), macrophages, neutrophils, eosinophils, basophils, and mast cells. Innate immune cells do not require prior stimulation by antigens and act as a first line of defense against foreign antigens. The adaptive immune system includes B lymphocytes, CD4⁺ helper T lymphocytes, and CD8⁺ cytotoxic T lymphocytes (CTLs), and requires formal presentation by antigen-presenting cells (APCs) for its activation^[21]. The adaptive immune system generates antigen-specific T- and B-cell lymphocytes. The immune system is highly variable between individuals but relatively stable over time within a given person^[22].

Each cell is estimated to experience over 20,000 DNA damaging events each day^[23], which are normally repaired by specific DNA repair pathways with no lasting effects^[24]. Cells which are not repaired and which acquire malignant or potentially malignant changes are then usually recognized and killed by the tumor immunosurveillance system. This involves predominantly cell-mediated mechanisms that can differentiate between self and non-self antigens. Since a malignant cell can have more than 11,000 genomic mutations, many new tumor-associated antigens (TAAs) may be expressed^[25]. TAAs include products of mutated proto-oncogenes, tumor suppressor genes, overexpressed or aberrantly expressed proteins, tumor antigens produced by oncogenic viruses, oncofetal antigens, altered glycolipids and glycoproteins, and cell type-specific differentiation antigens. These new TAAs, or fragments thereof, are presented on the cell surfaces with their major histocompatibility complex (MHC) molecules. However, recognition of an antigen-MHC complex by a T-cell antigen receptor is insufficient for the initial activation of naive T-cells, requiring additional costimulatory signals that are provided by the engagement of the CD28 receptor on the T-cell surface with B7 ligand molecules (two of which are CD80 and CD86) on the APCs. This CD28 receptor/B7 ligand combination or “immunological synapse” stimulates the proliferation and function of the T-cells. Many other receptor/ligand combinations are possible between activated T-cells and other cells, including tumor cells, and some of these interactions are inhibitory, such as PD-1/PD-L1 and CTLA-4/B7^[26-29], and are discussed later in this monograph.

Some malignant cells are able to evade the tumor immunosurveillance system by manipulating their own characteristics as well as the cells in their microenvironment to become “successful” tumors;

these evasive mechanisms represent the major area of interest in current CI research. The concept that the immune system is capable of detecting and killing nascent “non-self” malignant cells was first developed by Burnet^[30,31] and Thomas^[32] in their cancer immunosurveillance hypothesis. The concept was not accepted initially but it is now considered a component of cancer immunoeediting, whereby the surveillance system can determine or “shape” the immunogenicity of the tumor cells which are not eliminated initially^[33]. The immunoeediting process has been formally divided into three main phases: elimination, equilibrium, and escape. The elimination phase refers to the initial damage and possible destruction of tumor cells by the innate immune system, followed by presentation of the tumor antigens in the cellular debris to dendritic cells which then present them to T-cells and thereby create tumor-specific CD4⁺ and CD8⁺ T-cells. These help destroy the remaining tumor cells if elimination is complete. The equilibrium phase occurs when any tumor cells survive the initial elimination attempt but are not able to progress, being maintained in a state of equilibrium with the immune cells. In the escape phase, cancer cells grow and metastasize due to loss of control by the immune system. The cancer cells which are not eliminated and which escape may do this by expressing fewer antigens on their surfaces or even by losing their MHC class I expression^[34]. They may also show the ability to protect themselves from T-cell attack by expressing immune checkpoint (IC) molecules on their surfaces like normal cells; these IC molecules are upregulated by cytokines produced by activated T-cells and are part of a normal negative feedback loop to control excessive tissue damage from inflammation by downregulating or suppressing T-cells^[35].

The dynamic that exists between the immune system and tumor antigens is a phenomenon recognized relatively recently, since it was only in 1991 that van der Bruggen and colleagues first reported the existence of a human tumor antigen recognized by T-cells^[36]. They were able to clone the melanoma antigen-encoding gene (MAGE), which encodes an antigen recognized by cytotoxic T-cells. This provided not only proof that the immune system was capable of seeking and destroying tumor cells but also provided the first identification of a molecular target.

The ability of cancer cells to evade immune destruction has been proposed as the eighth hallmark of cancer^[37]. As noted above, a tumor is able to do this not only by modulating its own cellular characteristics but also by creating its own “tumor microenvironment” by recruiting apparently normal immune cells to help shield it from attack by the immune system. Through the production of various cytokines and chemokines, successful

cancers and their metastatic derivatives are able to generate an immunosuppressive, protumorigenic, and prometastatic microenvironment by recruiting and “training” immune cells, including macrophages, regulatory T-cells, immature myeloid cells (which become “myeloid-derived suppressor cells”), T helper 17 cells, regulatory B cells, and leukocytes^[38]. Even before they metastasize, tumors can influence the systemic environment by altering hematopoiesis as well as the tissue parenchyma of organs at distant sites, thereby forming “pre-metastatic niches”^[39]. While some cancer immunotherapies have had marked successes in manipulating these tumor microenvironments, the loss of MHC class I expression by a tumor represents a major immunotherapy treatment challenge^[40].

The intrinsic immunological ability of an individual to combat cancer has been called the “cancer-immune set point”, and is influenced by a complex set of factors involving the tumor, the host, and environmental factors^[41]. Clinical studies are trying to better characterize these factors to help predict a person’s response to immunotherapy, as discussed in the following paragraphs.

BIOLOGIC MODIFIERS: CYTOKINES AND VACCINES

The term “immunotherapy” encompasses a wide variety of concepts and methods. Older and non-specific immunotherapies include immunostimulatory cytokines such as interleukin-2 (IL-2) and interferon (IFN). L-MTP is a synthetic analogue of a bacterial cell wall that is capable of activating monocytes and macrophages and has had limited success in cancer treatment as reported in other countries; it is not approved by the Food and Drug Administration (FDA) of the USA^[42]. The only vaccine for cancer which has received approval by the FDA is sipuleucel-T for metastatic castrate-resistant prostate carcinoma. Dendritic cells from the patient are exposed to prostatic acid phosphatase and granulocyte-macrophage colony-stimulating factor (GM-CSF) and reinfused into the patient. Treatment results in a 4-month increase in median survival^[43]. Sipuleucel-T is a dendritic cell vaccine, while other types of vaccines employ killed tumor cells or selected tumor antigens, and various vaccines may use microorganisms as vectors for delivery. Vaccine trials using multiple neoantigens specific to an individual patient’s tumor have shown promise in two small early trials^[44]. The goal of all of these tumor vaccines is try to expose patients to those tumor antigens which can provoke an antitumor immune response via the generation of tumor specific antibodies and/or T-cells. Vaccines are one type of biologic response modifier, and BCG was

the first one to be used in cancer therapy, for treatment of bladder carcinoma^[45], where it indirectly increases the expression of tumor antigens after the tumor cells internalize the bacteria. This induces an intense and complex coordinated release of multiple cytokines, including those from T helper 1 cells (IL-2, IL-12, IFN- γ , tumor necrosis factor), as well as those from T helper 2 cells (IL-4, IL-5, IL-6, IL-10). Macrophages, epithelial cells, and fibroblasts contribute IL-8, and T helper 17 cells release IL-17^[46]. This wide array of cytokines then induces antitumor activity mediated by cytotoxic T lymphocytes, natural killer cells, neutrophils, and macrophages.

ONCOLYTIC VIRUSES

Oncolytic viruses are an emerging class of cancer therapeutics which lie at the junction of biologic therapy and immunotherapy. These viruses are genetically modified to lack virulence against normal cells but are able to invade and lyse cancer cells which have sacrificed many of their normal anti-viral cellular defenses in order to amplify their growth potential. Lysis is only one of multiple mechanisms involved in the viral-induced destruction of cancer cells, which undergo further attack by an immune system stimulated by a plethora of tumor antigens released by lytic destruction^[47]. The oncolytic virus which was approved by the FDA in 2015 to treat advanced melanoma is a herpes simplex-1 virus (HSV-1) named “T-VEC”, modified to express GM-CSF which further stimulates proliferation of immune cells. T-VEC is injected directly into areas of melanoma that a surgeon cannot remove. Clinical trials are underway with other oncolytic viruses for treatment of different types of cancer, with some of these trials combined with other types of cancer therapies.

ADOPTIVE CELL THERAPY

Adoptive cell therapy (ACT) is another type of immunotherapy which mostly involves the isolation and *in-vitro* expansion of tumor-specific T-cells, followed by infusion back into the cancer patient. These efforts have also extended to using natural killer cells, since they display rapid and potent immunity to solid tumor metastasis and hematological cancers^[48].

There are many forms of ACT, including those using techniques such as culturing tumor-infiltrating lymphocytes obtained directly from the tumor; isolating and expanding one particular T-cell or clone; or using T-cells that have been engineered *in vitro* to potentially recognize and attack tumors, which technique is known as chimeric antigen receptor T-cell (CAR T-cell) therapy. The revolutionary CRISPR/Cas9 (or “CRISPR” for

short) technique is a much simpler and more efficient method of editing genes than previous methods, and was first reported in 2012^[49]. The acronym stands for “Clustered Regularly Interspaced Short Palindromic Repeats”, which refers to a method normally used by bacteria and archaea for protection against the invading nucleic acids of viruses and plasmids. In 2013, the method was adapted for use in eukaryotic cells^[50], and in late 2016 a group at Szechuan University became the first to use CRISPR-edited cells in humans^[51]. Other similar trials are scheduled to start in 2017 in the United States. Prior vaccination with a cancer vaccine can also be used, in an attempt to “prime” rare tumor-specific T-cells^[52]. Although ACT has produced remarkable results in clinical trials with melanoma and hematologic malignancies as well as with solid cancers, some deaths have occurred in the trial phases secondary to marked cytokine release (“cytokine storm”, or “cytokine release syndrome”) and cerebral edema^[53]. Researchers are still studying other ways of modifying T-cells to treat cancer. Relapsed and refractory B-cell acute lymphoblastic leukemia in pediatric and young adult patients is the first disease to receive approval from the FDA for CAR T-cell therapy, outside of clinical trials^[54].

IMMUNE CHECKPOINTS

In order to ensure that an immune inflammatory response is not constantly activated once foreign or tumor antigens have stimulated a response, multiple controls or “checkpoints” are in place or activated. These checkpoints are mostly represented by T-cell receptor binding to ligands on cells in the surrounding microenvironment, forming immunological synapses which then regulate the functions of the T-cell, which become specialized, or “polarized”, to perform different activities. As noted earlier, initial T-cell activation involves antigen presentation by the MHC molecules on the antigen-presenting cells (APCs) to the corresponding T-cell receptor (TCR) on naive T-cells. The interaction of the costimulatory T-cell receptor CD28 with the B7 ligand is required for full activation, which is tightly regulated or suppressed by inhibitory checkpoint receptor/ligand pairs to avoid collateral damage from autoimmunity^[35].

This type of suppression or induced dysfunctionality of T-cells is also referred to as “T-cell exhaustion” and is different from anergy or senescence. Although it is a mostly reversible physiologic protective mechanism against autoimmunity, the first observation of it was made in mice infected with a chronically persistent strain of lymphocytic choriomeningitis virus^[55]. This T-cell dysfunction was later discovered to exist in multiple other conditions involving persistent antigen exposure by other viruses such as HIV, hepatitis B, and hepatitis

C, or by cancer, thereby allowing these agents to avoid detection and destruction by immune cells^[35,56-59].

More than twenty checkpoint molecule pairs, both co-stimulatory and co-inhibitory, have been discovered, including TIGIT/CD155, LAG-3/MHCII, and TIM3/Gal-9, which are variably expressed not only by T-cells but also by other cells of both myeloid and lymphoid derivation^[56,60]. Some of these molecules are similar to more common or better-known membrane moieties but with important differences: for example, lymphocyte-activated gene-3 (LAG-3) is structurally homologous to CD4 but has a higher binding affinity to MHC class II antigens than CD4. Since these checkpoint molecules are upregulated in suppressed T-cells, they can also be used as markers of “T-cell exhaustion”. The two pairs of inhibitory receptor/ligands which have received the most attention in recent years are cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) receptor with B7 ligand, and programmed cell death protein 1 (PD1) receptor with PD1-L1 ligand. The expression of CTLA-4 receptors on activated effector T-cells and regulatory T-cells was reported in 1987^[61]. CTLA-4 has very high homology to CD28, with a higher competitive binding affinity to B7, causing inhibition of proliferation and IL-2 secretion by T-cells^[62]. PD-1 was cloned in 1992^[63], and its ligands PD-L1 and PD-L2, which are members of the B7 ligand family, were later characterized^[64-66]. Unlike CTLA-4, PD-1 does not interfere with costimulation, but generates signals that prevent phosphorylation of key signaling intermediates in the T-cell, which reduces their activation^[67]. While B7 ligands are expressed by professional APCs (includes dendritic cells, macrophages and B cells), PD-L1 can be expressed on many cell types, including T-cells, epithelial cells, endothelial cells, and tumor cells after exposure to interferon-gamma, produced later in the immune response by activated T cells. PD-L2 is primarily expressed on dendritic cells and monocytes, but can be induced in a wide variety of other immune cells and nonimmune cells^[68].

Since the CTLA-4/B7 synapse acts earlier than the PD1/PDL-1 synapse in the immune response, CTLA-4 is considered the “leader” of the immune checkpoint inhibitors, because it stops potentially autoreactive T-cells at the initial or priming stage of naive T-cell activation, occurring chiefly within lymph nodes^[62,69]. The PD-1/PD-L1 pathway functions during the later effector phase in the periphery and protects the cells found there from T-cell attack, including tumor cells which express PD-L1^[70]. The PD1/PD-L1 pathway represents an adaptive immune resistance mechanism that is exerted by tumor cells in response to endogenous anti-tumor activity^[71].

ANTIBODIES TO IMMUNE CHECKPOINT MOLECULES

In 1996, Leach, Krummel and Allison reported that CTLA-4-blocking monoclonal antibodies (Mabs) could treat tumors in animal models^[72]. These MAb became known as “immune checkpoint inhibitors” (ICIs), although they should really be called “anti-immune checkpoint inhibitors” if traditional usage in nomenclature could be easily changed. They are receiving much attention recently because they are much less toxic than conventional cancer therapies, are easier to prepare and administer than other types of cancer immunotherapeutics, and have great potential for widespread application.

MAbs that have been approved for clinical use target either PD-1, PD-L1, or CTLA-4, which “block the negative blocking” of the T-cells, with a consequent boost of the immune response against cancer cells. Assays of PD-L1 protein expression by immunohistochemistry are used to determine which tumors would best be treated with an anti-PD-L1 antibody, but it is an imperfect measurement practice because there is lack of standardization of methods and it can sometimes be difficult to differentiate PD-L1-positive tumor cells from the other PD-L1-positive cells in the tumor microenvironment^[73]. Moreover, immunohistochemistry has a lower sensitivity compared to studies measuring PD-L1 mRNA expression^[74].

Anti-PD-1 and anti-PD-L1 antibody treatments are currently the most investigated ICIs because they have shown less severe toxicity, or high-grade “immune-related adverse effects” (irAEs), than anti-CTLA-4 antibody treatments (5-20% compared to 10-40% respectively)^[75-79]. The wide ranges in the percentages of adverse effects reported reflect the variabilities associated with single or multiple drug regimens, dosage levels, and types of malignancies treated. The more common side effects are fatigue (with or without associated endocrinopathies), dermatologic and mucosal toxicities, diarrhea/colitis, and hepatotoxicity. Corticosteroids or other immunomodulators can reverse nearly all of the toxic manifestations of these drugs^[75-78]. Pneumonitis is an uncommon but potentially severe complication, and rarely deaths have occurred^[80]. As the authors noted in one comprehensive review article about management of immunotherapy toxicities, “This new family of dysimmune toxicities remains largely unknown to the broad oncology community^[77]”.

These drugs have powerful effects, as seen when a Phase I trial using an antibody to the CD28 ligand nearly cost the lives of all six healthy volunteers in a British

study when a cytokine storm was provoked, associated with multiorgan failure and resuscitation in the intensive care unit^[81]. Since this reaction occurred after the very first infusion of a dose 500 times smaller than that found safe in animal studies, this study raised awareness of the need to develop better animal models which more closely mimic drug behavior in humans. In addition, there was increased appreciation of the wisdom of restricting the initial testing of a new pharmaceutical to only a few human subjects^[82].

The less toxic antibodies to checkpoint inhibitors have shown a great deal of promise and are now approved by the FDA for six malignancies which are in advanced stages - melanoma, lung cancer, renal cell carcinoma, head and neck cancer, urothelial cancer, and Hodgkin's lymphoma - with many other tumor types being investigated in clinical trials^[83,84]. Some of these trials are using specific antibodies to modulate the function of the more recently discovered inhibitory and co-stimulatory checkpoint molecules.

The immunotherapy/immuno-oncology field has shown such exponential gains in recent times, associated with an accumulation of a dizzying array of complex results arriving from numerous clinical trials, that mechanistic patient studies are necessary to best advance understanding. This is essentially “reverse translational research”, or the opposite of the usual “bench to bedside” philosophy, requiring genetic, phenotypic, functional, and immunohistochemical studies of pre-treatment, on-treatment, and post-treatment tissues. These are necessary in order to generate hypotheses that can then be tested in animal models and thereby provide more precise biologic pathways about tumor immunity and rejection^[85].

Some of the complexities of interacting with the immune system include timing of administration of ICIs to coincide with a high inflammatory microenvironment in the tumor to ensure the presence of many potential tumor-fighting CD8⁺ T-cells^[86,87]. This often correlates with tumor necrosis provoked by prior conventional chemotherapy, and is also related to the number of mutations present in the tumor (“mutational burden”), which is associated with higher antigenicity and correlates positively with response to ICIs^[88]. Lung cancers occurring in smokers have a higher mutational burden and have shown more responsiveness to ICIs^[89]. Microsatellite instability-high colorectal cancers, which tend to have a high-mutation/high-neoantigen load based on owing their genesis to a deficiency in DNA repair, have also been proven to respond well to ICIs^[90,91]. Even the patient's intestinal bacteria needs to be considered, as noted in two recent reports: in one study of patients with metastatic melanoma treated with anti-PD-1 antibody, the diversity

and composition of the gut microbiome differed in responders versus non-responders, with the non-responders showing less diversity and higher abundance of Bacteroidales, while the responders had higher diversity and a higher abundance of Clostridiales^[92]. Another study of patients with metastatic renal cell carcinoma showed faster tumor progression in the patients who had received broad-spectrum antibiotics up to one month before treatment with ICIs^[93].

Not all immune checkpoint or immunomodulatory molecules take the form of a receptor or ligand. Some may be expressed by the cell in a free soluble form, such as indoleamine 2,3-dioxygenase (IDO1), an enzyme produced by some activated macrophages and also overexpressed by many tumors^[94]. The enzyme depletes tryptophan in the microenvironment, with production of the catabolite kynurenine, which harms the cytotoxic T-cells. Phase I/IIa studies of one IDO1 inhibitor show promise^[95].

CONCLUSION

With the development of the field of cancer immunotherapy, the focus of treatment has shifted from treating the disease site to treating the specific tumor biologic characteristics and its interaction with the intrinsic immunological ability or “cancer immune set-point” of the patient to combat the disease. Since the immune system has the capacity to remember and the ability to detect and destroy tumor variants as they emerge, immunotherapy will always possess inherent advantages over other therapies that lack these two key attributes. The challenges ahead are to discover why immunotherapy treatments work so dramatically well in some cancers and in some patients while not at all in others, and how tumors which were once sensitive to treatment can acquire resistance. Specifically, to be effective, cancer immunotherapy needs to find ways to manipulate the immune system in the (probable majority of) patients who show little or no immune response to their tumors, even to the point where the tumor microenvironment is an “immune desert” with no tumor-infiltrating T-cells^[86,87]. Breakthrough discoveries will be necessary to be able to consistently elevate a patient’s cancer immune set point and to recover MHC class I antigens in those tumors that downregulate them. One recent study with large therapeutic and prognostic implications used the new CRISPR technique to reveal multiple mutations in the tumor genes of individual patients who failed immunotherapy^[96]. Some of these identified genes may be associated with loss of tumor antigen expression, while others may involve disturbances in tumor cytokine production or T-cell co-stimulation.

The pharmacoeconomics of these treatments also needs to be considered. The cost of the typical treatment using antibodies to ICIs is near \$150,000 a year. A combination of ipilimumab and nivolumab, approved by the FDA for advanced or inoperable melanoma, has a cost of \$256,000 a year for patients who respond to the treatment^[97]. Some relief may be obtained by the entry of at least some less costly biosimilars, which are biological products that must be “highly similar” to the original reference product, per FDA regulations. Biosimilars are usually made by a different/competing company^[98].

Immunotherapy drugs are now approved for treatment of multiple cancer types either as first-line treatment or when standard first-line treatment has failed. The FDA has recently approved the anti-PD-1 antibody pembrolizumab for the treatment of any unresectable or microsatellite instability-high or mismatch repair-deficient solid tumors that have progressed after prior treatment and who have no other satisfactory treatment options^[99]. This is the first time the agency has approved a cancer treatment based on a common biomarker rather than the location of the body where the tumor originated.

Immunotherapies do not yet represent a panacea in cancer therapy since only a minor subset of some cancers respond to some of these treatments, and it is difficult or impossible to determine precisely who will benefit.

Before finishing this brief review, it is proper to recognize the work of Dr. James Allison of Houston’s MD Anderson Cancer Center in Texas, the winner of the 2015 Lasker Award, since he is the one who discovered the T-cell receptor in 1982 and went on to develop the field of checkpoint blockage, leading to the breakthrough drug ipilimumab^[100,101].

DECLARATIONS

Authors’ contributions

Both authors contributed equally to this review.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

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