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Review Article

Response Challenges to Cancer Immunotherapies

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Abstract

Humans have an exquisite immune system that enables them to not only identify and eliminate foreign antigens but also their own cells if they go awry. Cancer cells, through acquiring alterations in their genomes, can harbor slightly modified proteins and expression patterns. These changes can be detected and eliminated by a competent immune system. The immune system can be further assisted in killing rogue cancer cells through the use of immunotherapies. However, despite immunotherapies showing great promise in certain cancers and a subset of patients, these treatments are characterized by high rates of response resistance. Here, a narrative review is presented of the possible mechanisms underpinning resistance to immunotherapies, together with strategies to improve their response rates.

Keywords: Cancer immunotherapies, immunotherapy resistance mechanisms, resistance to immunotherapies, treatment of resistance to immunotherapies.

تحديات الاستجابه للعلاجات المناعيه للسرطان

الخلاصة

يتمتع البشر بجهاز مناعي رائع يمكنهم ليس فقط من التعرف على المستضدات الاجنبيه والقضاء عليها، ولكن ايضا خلاياهم اذا انحرفت عن مسارها. يمكن للخلايا السرطانيه، من خلال اكتساب تغيرات في موروثاتها، ان تأوي بروتينات وانماط تعبير معدله بشكلٍ طفيف. يستطيع جهاز المناعه من اكتشاف هذه التغيرات والقضاء عليها من خلال وظيفه مناعيه مختصه. يمكن كذلك زياده مساعده الجهاز المناعي لقتل الخلايا السرطانيه المارقه من خلال استخدام العلاجات المناعيه. ومع ذلك، وعلى الرغم من ان العلاجات المناعيه تبشر بالخير في بعض انواع السرطانات ومع مجموعه فرعيه من المرضى، الا ان هذه العلاجات تتميز بمعدلات عاليه من مقاومه الاستجابه. هنا، يتم تقديم مراجعه سردية للآليات المحتمله التي تدعم مقاومه العلاجات المناعيه جنبًا الى جنب مع استراتيجيات لتحسين معدل استجابتها.

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INTRODUCTION

There have been several accounts, dating back to ancient Egypt, of tumors shrinking or disappearing after an infection or high fever [1]. However, the modern roots connecting the immune system with cancer can be traced back to the early work of two German physicians, Wilhelm Busch and Friedrich Fehleisen, during the second half of the nineteenth century C.E. [2]. These two scientists independently observed the regression of tumors in patients following an accidental erysipelas infection, which is a superficial skin infection caused most often by *Streptococcus pyogens.* This represents the first description of an epidemiological association between the immune response and cancer and is supported by a parallel

observation by Rudolf Virchow, who noticed an increased prevalence of leukocytes in tumors [2-4]. Wilhelm Busch was the first to intentionally infect cancer patients with erysipelas and notice tumor shrinkage [1]. Decades later, William Coley and his colleagues carried out experiments in which cancer patients were injected with heat-inactivated bacteria (Coley's toxin) and reported significant successes in curing some patients with tumors, mostly sarcomas [1]. The observed cure was attributed incorrectly by Coley to the bacteria rather than the immune response as we understand it now. However, the development of radiotherapies and chemotherapies together with the failure of Coley's toxins to give consistent results led to the decline of this form of "immunotherapy." The century that followed these preliminary early efforts showed no substantial development in harnessing the power of the immune system to fight cancer. The renaissance of deploying the immune response came in the past 25 years with the demonstration of the key role of the adaptive arm of the system and the importance of the tumor microenvironment (TME) [5]. Before these developments was the proposition of the concept of immunosurveillance in the 1950s by Paul Ehrlich, which was later built on by Burnett and Thomas [6,7]. Immunosurveillance states that the emergence of cancer cells is a frequent event but is normally suppressed by the host's natural immunity. The lymphocytes are responsible for this process. This idea was further refined into cancer immunoediting by Schreiber and his co-workers [8]. Cancer immunoediting involves three sequential phases: elimination, equilibrium, and escape, whereby the immune system can both constrain and promote the development of cancer (Figure 1) [9]. This was soon followed by the development of novel immune checkpoint inhibitors to counteract cancer and the carrying out of a large number of clinical trials to assess their feasibility, which led to the selection of this field as the "2013 Breakthrough of the Year" by *Science* Journal [10,11]. The field was later crowned by the Nobel prize award to James P. Allison and Tasuko Honjo for their pioneering work on the inhibition of the negative immune regulation of T cells and how this could be exploited in the fight against cancer [5]. Researchers were focusing on the two main approaches to using the immune response to help cancer patients. The first approach involves removing some of the patient's immune cells, genetically modifying and expanding them *in vitro,* and then re-infusing them back into the patient (personalized treatment) [12]. The second approach, which is more widely used, involves employing drugs to remove the inhibition mechanisms (called checkpoints) by which the body restrains the immune system from over-reacting [12]. The new immunotherapies showed great promise and yielded excellent results in terms of longer survival rates and even cures in some cases. However, for all the promise and excitement, immunotherapies have worked for only a minority of patients and cancer types. In addition, subsequent treatment failures following an initial success are quite frequently encountered [13-15]. This review highlights the reasons and mechanisms involved in patients that either initially showed no response or became subsequently refractive to the treatment.

The Immune Response to Cancer

The human immune system is separated into two distinct components: the innate and adaptive immune systems. Innate immunity serves as the initial line of defence against foreign antigens, generating rapid, non-specific, and transient responses, whereas adaptive immunity generates long-lasting, specific responses [11,16]. Despite their disparate properties, both arms work together to form an immunity network, with certain of their components acting as linkers between the two types of responses [16]. Through both of these arms, the immune system is capable of detecting and eliminating not only foreign materials but also cancer cells via intricate pathways involving the cooperation of several cells. The tumor-associated antigens (TAAs) produced by cancer cells as a result of genetic and epigenetic DNA modifications are critical for the immune response's eradication of malignancies [7,17]. The innate arm of the

immune system consists of both soluble components such as cytokines, complement proteins, and chemokines and cells such as neutrophils, macrophages, dendritic cells, basophils, mast cells, and natural killer (NK) cells [6].

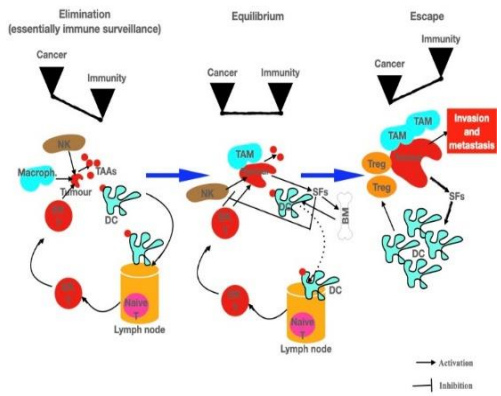

Figure 1: Cancer immunoediting. NK: natural killer cell; TAAs: tumour-associated antigens; DC: dendritic cell; TAM: tumour-associated macrophages; SFs: soluble factors, BM: bone marrow.

The soluble proteins of the innate system, particularly the cytokines, have a variety of activities depending on the milieu in which they are produced, the location of the receptor to which they bind, and the signalling pathway they follow after binding [18]. Once activated, the complement group of soluble proteins performs opsonisation by acting as a chemoattractant for other immune cells and inducing cell death through the creation of a membrane assault complex and lysis. Phagocytes (neutrophils, monocytes, macrophages, and dendritic cells) and natural killer (NK) cells are involved in the cell-mediated innate response. Phagocytosis enables immediate host protection by engulfing and killing cells that express non-self and altered-self antigens. NK cells, on the other hand, confer immune protection by recognizing major histocompatibility complex 1 (MHCI) molecules (in humans, the MHC groups of proteins are also referred to as HLA proteins and are ubiquitously expressed on the surface of all nucleated cells) and secreting perforin and granzymes to induce apoptosis in cells with abnormal MHC1 expression [19]. Other innate immune cells, such as eosinophils, basophils, and mast cells, emit inflammatory signals and contribute to the inflamed site's recruitment of additional immune cells [6,20]. The adaptive immune system is mostly composed of B cells, T cells, and NKT cells. Antibodies are produced when B cells are activated and matured (often referred to as immunoglobulins, or Igs) [21]. Antibodies neutralize antigens by initiating antibody-dependent complement cytotoxicity and attaching to specific cell receptors to activate their effector activities [6]. Except when it is pertinent to the subject of this review, the accompanying discussion will not go into depth about the role of B cells. NKT cells are a mix of NK and T cells; they exhibit the NK surface marker NK 1.1 as well as T cell receptors (TCRs) and are capable of recognizing and binding to lipids and glycolipids of both self and non-self-origin and secreting cytokines to activate additional immune responses [6,19]. The major cell type in the adaptive immune response to cancer is the T cell. There are two types of T cells present in the immune system that are distinguishable by their receptor type: αβT cells and γδT cells [6,22,23]. The latter subtype of T cells is a minority of cells that can recognize non-self molecules by pattern recognition and hence do not require MHC-mediated presentation. The major subtype that is of concern here is the αβT cells (often referred to as just T cells, a term that will be used in this review), which are further broken down into two subsets known as CD^+4 T cells and CD^+8 T cells, where CD stands for cluster of differentiation. For the naïve CD^+4 T cells to mature into effector CD^+4 T cells, they require stimulation through interactions between MHCII (only present on antigen-presenting cells (APCs) such as B cells, macrophages, and dendritic cells) and the T cell receptor (TCR) on the naïve CD^+4 T cells. Depending on the microenvironment, CD^+4 T cells can differentiate into several subsets of CD^+4 effector cells such as Th1, Th2 (T helper cells 1 and 2), and Treg (T regulatory cells) [24]. Each of these subsets can secrete cytokines that modulate the immune response.

Th1 cells produce interferon-gamma (IFN-γ) and interleukin-2 (IL-2) and play a role in autoimmunity, while Th2 cells produce interleukins 4,5,10,13 and 31 (IL-4, IL-5, IL-10, IL-13 and IL-31) and regulate the immune response to pathogens and allergic diseases. The Tregs help reduce inflammation via the production of transforming growth factor-beta (TGF-β) and IL-10 and IL-35. Naïve CD^+8 T cells, similar to NK cells, rely on MHCI for maturation into effector cells (cytotoxic T cells). The CD^+8 T cells, through the binding of their specific TCRs with antigen/MHCI presented by the target cell, will mature into effector T-cells (Teffs), releasing perforin and granzymes to kill and eliminate the target cell [25]. Both CD^+4 T cells and CD^+8 T cells express a multitude of other surface receptors. The immune system's two components, innate and adaptive responses, work in concert to kill cancer cells [6,26]. Collectively, these responses serve as the foundation for the ideas of immunosurveillance and cancer cell immunoediting. However, as the prevalence of cancer cases in humans demonstrates, the immune system's response to eradicate malignant cells is not always successful. As cancer progresses and its cells acquire additional oncogenic mutations, the microenvironment is reshaped to cancer's advantage [9]. Immunosurveillance, or the detection and removal of precancerous cells, is only one facet of the complicated connection between cancer and the immune system [27]. Later, the term "immunoediting" was coined to refer to the immune system's capacity to accelerate cancer progression in specific conditions [27,28]. Immunoediting is a notion that encompasses three states: elimination, equilibrium, and escape, or the three Es (Figure 1) [27]. During the elimination phase, innate and adaptive immunity work in concert to eradicate precancerous cells. The removed cells enter an equilibrium phase, during which adaptive immunity controls and restrains cancer cell proliferation and modifies their immunogenicity. This stage of immunoediting is expected to be the longest, possibly lasting years [27]. Cancer's dormancy may be abruptly disrupted by the appearance of tumor cells with low immunogenicity, which evade the immune system's regulation. These escaping cells may begin to proliferate and multiply, eventually invading neighbouring tissues and metastasizing. Cancer cells that have escaped may do so by diminishing their MHCI expression and/or producing fewer antigens. Additionally, they may defend against T cell attacks by expressing immunological checkpoint molecules on their surfaces [1]. Within the central premise of cancer immunity, there exist several factors that act as immune checkpoints, mediating the response to malignancy [29]. For instance, during the first encounter with antigen/MHCII (Figure 2), it is critical to have a co-stimulatory signal to initiate competent T cell activation [30,31].

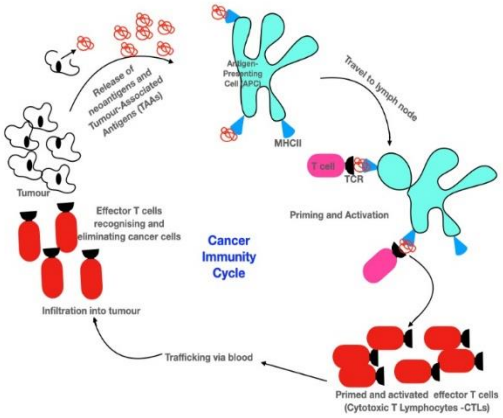

Figure 2: The cancer immunity cycle. TCR: T cell receptor; MHCII: major histocompatibility complex 2.

The recognition of TCR-peptide/MHC interaction represents the first signal, and the interaction of co-stimulatory molecules between the T cell and the antigen-presenting cell (APC) is the second signal. Two of these co-stimulatory signals are mentioned here: CD28 and ICOS (inducible T cell co-stimulator). The CD28 protein on T cells interacts with CD80/CD86 on APCs while the ICOS ligand-protein on APCs interacts with the ICOS receptor on T cells [29]. The interaction of either or both of these co-stimulatory signals leads to the activation of T cells [31]. There are several of these co-stimulatory signals, and more are continually being discovered. The absence of co-

stimulatory signals means that T cells will not differentiate or proliferate and will ultimately result in a state of "T cell anergy" and immune tolerance to cancer-associated antigens [32]. Under this scenario, the immune response to the malignancy is shut down and the cancer progresses. Immune tolerance could also be initiated by the binding of CTLA4 (cytotoxic T lymphocyte-associated protein 4), an inhibitor protein of T cell function and proliferation, on T cells to CD80/CD86 proteins on APCs. Contrary to the binding of CD28 with these proteins, the interaction of CTLA4 with T cell inhibitors results in T cell inhibition and down-regulation of immune responses [2]. The subsequent discovery of Programmed Death 1 protein (PD1), a cell-surface receptor expressed on multiple immune cells including T cells, B cells, NK cells, monocytes, DCs, and Tregs, facilitated progress in the field of immunotherapies [2,30]. The ligands for this receptor, PDL1 and PDL2, are also expressed by various types of cancer cells. PD1 and PDL1/PDL2 interactions lead to the inhibition of the immune response. PDL1 is the major ligand and the focus of consideration in the subsequent writing. The interactions of PD1 and PL1 point to an exploitable mechanism by which cancer cells escape immunity [33-36].

Cancer Immunotherapies

Based on the immune response to cancer, there are several broad categories of immunotherapies for the treatment of cancer. These categories are: monoclonal antibodies (mAbs), autologous T cells, recombinant cytokines, small molecules, and vaccines.

Monoclonal antibodies (mAbs)

The identification of tumor associated antigens (TAAs) and the high specificity of antibodies to these antigens have fuelled intense study in this area of cancer treatment in recent decades [11]. Monoclonal antibodies are very specific, with the term "monoclonal" referring to the fact that they can only detect one epitope of the antigen, with tiny alterations in that epitope causing the antibody to lose recognition. Furthermore, the antigen must be present on the cell's surface, as antibodies are unable to cross through the cell's plasma membrane [37]. Rituximab was the first monoclonal antibody to be developed for the treatment of non-lymphoma Hodgkin's based on CD20 expression on the surface of B cells. This was followed by the anti-human epidermal growth factor receptor-2 (HER-2) antibody Trastuzumab for breast cancer, the anti-vascular endothelial growth factor (anti-VEGF) antibody Bevacizumab for colorectal cancers, and the anti-epidermal growth factor receptor (anti-EGFR) antibody Cetuximab for colorectal cancers [38,39]. Over 30 monoclonal antibodies (mAbs) have been licensed for cancer treatment in various countries to date [11]. The idea of mAb treatments is that they target a specific antigen found on cancer cells and can be employed alone (unconjugated) or in combination with a medicine known to be toxic to cancer cells [37]. For example, Zevalin is an Yttrium-90 combination with Rituximab used to treat non-lymphoma Hodgkin's [40], while Kadcyla is a DM1 and Trastuzumab combo used to treat HER-2 positive breast cancer [41,42]. Monoclonal antibodies directed against two distinct proteins have also been studied. Blincyto (Blinatumomab) is a monoclonal antibody (mAb) that functions as a bispecific T cell engager (BiTE), with one portion adhering to CD19 on target B cells and the other part interacting with CD3 on T cells, allowing for increased interaction and elimination of malignant B cells [43,44]. Monoclonal antibodies targeting inhibitory immune checkpoints, such as CTLA4 and PD1, are a subset of this category that deserves detailed attention in this review because they are the molecules that manipulate the immune response and have shown clinical activity in several cancers [7,26,33]. Immune checkpoint inhibitors (CPIs, also known as immune checkpoint blockers, ICBs) have proven particularly effective in melanomas, for which approved treatments now include the anti-PD1 antibodies Pembrolizumab (Keytruda) and Nivolumab (Opvido), as well as the anti-CTLA4 antibody Ipilimumab (Yervoy), as well as combinations of anti-PD1/anti-

CTLA4 regimens such as Nivolumab. The CPIs have revolutionized the treatment of cancer by making the immune response a target for therapeutic intervention [15]. T cell depletion in animal models abolishes the tumoricidal activity of CPIs, which is important for the therapeutic benefits of drugs targeting these checkpoints [2]. The basic mechanism of action of anti-CTLA4 antibodies is to block the CTLA4 immunological checkpoint, resulting in a stronger immune response. Another effect of anti-CTLA4 therapy, as seen in animal models, is the depletion of Tregs in the tumor microenvironment, changing the balance away from immunosuppression [47,48]. The manner of action of the latter arm of anti-CTLA4 treatments, on the other hand, remains equivocal and requires more research. In general, the ratio of effector T cells to Treg cells in the tumor microenvironment is the most important determinant in predicting anti-CTLA4 therapy outcomes (TME). Anti-CTLA4 antibodies have been shown to be ineffective in cancers that are less immunogenic, such as breast and skin cancers [49,50]. CTLA4 blockade has mixed results, depending on the tissue and tumor burden [46]. The role of the PD1 axis in T cell negative regulation has sparked renewed interest in this system for cancer treatment and the use of its molecules as diagnostics [2]. Pembrolizumab and Nivolumab, both humanised and completely human monoclonal antibodies, were approved as the first PD1–targeted treatments for melanomas in 2014. Pembrolizumab was the first medicine to be approved based on a molecular biomarker rather than on the location of the tumour. However, because different tissues have suppressive TME, it's difficult to say which patient will benefit the most [51,52]. Pembrolizumab had a superior 6-month progression-free survival rate and gave an overall benefit when compared to Ipilimumab [53,54]. For unknown reasons, PD1 blockage has shown to be more effective in the clinic than anti-CTLA4 medications. The fact that the PD1 axis is typically hijacked by tumors via ligand expression, but CTLA4 represents a larger immune regulatory circuit [55,56] is one explanation for the disparity. The other partner in this axis, PDL1, is also targeted by monoclonal antibodies and has proven effective in the treatment of multiple types of cancers. In 2016, the first PDL1-targeted humanized monoclonal antibody, Atezolizumab (Tecentriq), was approved for the treatment of urothelial carcinoma expressing PDL1 with a modest response rate of only 15%, but was still deemed statistically significant [57]. Additional trials using Atezolizumab have failed to demonstrate better clinical efficacy beyond standard care, although it is less toxic when compared to traditional chemotherapy [58]. Further anti-PDL1 antibodies entered the market in 2017, such as Avelumab and Durvalumab. A list of the currently approved immune checkpoint blockers (ICBs) in the USA and Europe is given in Table 1, together with more molecules licensed in other countries including China, such as the anti-PD1 antibodies Toripalimab (for melanoma), Sintilimab, Camrilizumb, and Tislelizumab, the last three being for the treatment of Hodgkin's lymphoma [59]. Other agonist and antagonist antibodies are being investigated for their potential therapeutic value in various cancers [60]. PD1 blockade therapy has the same immune-related side effects as anti-CTLA4 therapy, but they happen less often. This could be because the PD1 checkpoint doesn't show up until later in the T cell response, which limits the T cell reactivity to cancer cells [61]. CTLA4 and PD1 antibodies have distinct mechanisms of action and can be used in tandem therapy [62,63]. Clinical testing of this combination showed up to 60% improved clinical response in melanoma but with increased toxicity [61]. By blocking a natural immune checkpoint, a powerful response may be unleashed that may overcome the normal tolerance to self-tissues [64]. The common feature of toxicity with the use of ICBs is the loss of naïve T cells and the accumulation of overactive memory T cells that cause inflammation and damage. When compared to those targeting the PD1 axis, anti-CTLA4 therapeutics are associated with a higher risk of severe autoimmune complications [1,65].

Table 1: American and European approved immune checkpoint inhibitors.

Immune checkpoint inhibitor	Target	Indication
Ipilimumab	CTLA4	Melanoma, renal cell carcinoma and CRC*
Pembrolizumab	PD1	Melanoma, NSCLC, HNC, Hodgkin's lymphoma, urothelial carcinoma, CRC*, gastric cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma, renal cell carcinoma, SCLC, oesophageal carcinoma and endometrial cancer
Nivolumab	PD1	Melanoma, NSCLC, HNC, Hodgkin's lymphoma, urothelial carcinoma, CRC*, hepatocellular carcinoma, SCLC and renal cell carcinoma
Atezolizumab	PDL1	Urothelial cancer, breast cancer, SCLC and NSCLC.
Avelumab	PDL1	Merkel cell carcinoma, urothelial carcinoma and renal cell carcinoma
Durvalumab	PDL1	Urothelial carcinoma and NSCLC
Cemiplimab	PD1	Cutaneous squamous cell carcinoma

* Showing high microsatellite instability or being deficient in mismatch repair. NSCLC = non-small cell lung cancer; SCLC: small cell lung cancer; CRC: colorectal cancer; HNC: head and neck cancer; CTLA4: cytotoxic T-lymphocyte associated protein 4; PD1: programmed death 1; PDL1: programmed death ligand 1.

Autologous T cells

In this group of therapeutics (sometimes also referred to as adoptive cell therapy, ACT), T cells in their natural role of eliminating cancers are used and manipulated in various ways. T-cells are collected from the cancer patient's blood or tumor tissue and manipulated ex vivo before re-infusing them back into the patient [66-68]. In tumor-infiltrating lymphocyte therapy (TIL therapy), which constitutes a subcategory of autologous T-cell therapy, the T cells that have already infiltrated the tumor are collected and simply expanded, usually using IL-2, to provide a sufficient number before injecting them back [2]. However, for TIL therapy to work, effector T cells must be present in the tumor, which is not often the case [69]. For this reason, genetically engineered T cell receptors (TCRs), usually via a retroviral gene transfer or more recently through CRISPR-Cas9 technology, have been developed [66,70]. This approach not only activates the T cells but also enables them to target specific cancer antigens [11]. In both TIL and engineered TCR therapies, the T cells can only recognize cancer cells presenting their antigens in the context of MHC molecules, hence both approaches are MHC-dependent [69]. Unfortunately, cancer cells can downregulate their MHC expression, which could render these therapies ineffective. As a result, a new approach that can recognize cancer cells in an MHC-independent manner and overcome the weak immunogenic nature of most spontaneous cancers was developed [71,72]. Therefore, this recent therapy can circumvent immune evasion by cancer cells if they lose their MHC expression [73]. This new approach is called "chimeric antigen receptor therapy" or CAR-T, representing a form of personalized medicine and was comprehensively reviewed by Sadelain [74]. In CAR-T, a patient's T cells are transfected with a construct encoding the binding domain of an antibody against a tumor-specific antigen fused to the T cell-signaling domain [23]. A typical example of a tumor-specific antigen is CD19, which is expressed by all B cells, yielding CAR-T that has been successful in the treatment of B cell malignancies [2]. Other targets apart from CD19, such as neoantigens, are currently being investigated for hematological cancers that do not express CD19 as well as solid tumors [75,76]. A recently identified target across several types of cancer is the B7-H3 (CD276) protein, which has shown success in

multiple pediatric solid tumor models [77]. A required feature for efficacy is the incorporation into the CAR of a signaling domain of either CD28, CD40, or CD137 and other positive regulators of T cell activation to potentiate their cytotoxicity [78-81]. This removes the dependence of the transduced T cells on the usual checks during the regulation of the immune response [82]. Combining the variable regions (Fvs) of antibodies with the constant regions of TCRs results in chimeric genes conferring the necessary specificity to the T cells against cancers that was not previously possible. Two CAR-T medicines are currently widely approved globally for lymphomas: Kymriah and Yescarta [11]. CAR-T has shown promising results against hematological cancers such as lymphomas and B cell leukemia, but not against solid tumors due to the difficulty in identifying good targets on the surface of their cells [82]. T cell therapy necessitates a patient-specific design, which can have prohibitive costs and access to treatment facilities.

Recombinant cytokines

Cytokines are the major proteins that modulate (enhance or inhibit) the immune response depending on the context [37]. Employing specific cytokines that enhance the immune response can constitute another category of immunotherapies. Interleukin-2 (IL-2) is an FDA-approved recombinant cytokine "Proleukin" for the treatment of melanoma and renal cell cancers [6,66,83]. Its ability to promote T cell activation as well as other immune cells expressing IL-2 receptors is the mechanism of action [84,85]. Another member of the recombinant cytokines that are FDA-approved is IFN-α2β (Syltron), which is used as adjuvant therapy in melanomas. This product consists of the cytokine IFN-α2β conjugated to polyethylene glycol, which functions to conceal the cytokine from being detected and attacked by the immune system until it reaches its target tissue to activate dendritic cells and promote antigen presentation [86]. The recombinant cytokine G-CSF (granulocyte colony-stimulating factor), known as Filgrastim, has also been approved and is on the market for the treatment of certain forms of leukemia. This cytokine can bind to its corresponding receptors on the surface of neutrophil progenitor cells to stimulate their differentiation [87]. This, in turn, will lead to the increased production of neutrophils to mediate the elimination of cancer cells through phagocytosis and the release of cytokines to attract other immune cells. However, care must be exercised in the use of Filgrastim as the neutrophils can have a dual role in the pathogenesis of cancer, facilitating metastasis under certain conditions [88]. Filgrastim is often employed in combination with other immunotherapies. Leukine is a recombinant GM-CSF (granulocyte-macrophage colony-stimulating factor) cytokine similar to Filgrastim that functions to elevate the levels of myeloid cells (any white blood cell that is not from the B or T lineage) and is used in patients with leukemia and individuals undergoing bone marrow transplantation [87]. The patient will require a robust immune system for the recombinant cytokines to be effective, which consequently contributes to variable immune responses among different patients [37].

Small molecules immunotherapies

The biologics mentioned so far above are characterized by being large molecules that are often difficult and expensive to produce [3,89]. Small molecule immunotherapies have the advantages of greater penetration into the tumor and the ability to cross cell membranes to access intracellular targets [90]. This includes their possible refinements to cross the blood-brain barrier and access tumors previously inaccessible with larger molecules. Furthermore, they are more amenable to fine-tuning their bioavailability to improve their effectiveness as well as reduce some of the immune-associated side effects often associated with biologics [90]. These small molecules can act as immune checkpoint inhibitors, innate immunity activators, cytotoxic lymphocyte activators, blockers of immunosuppression or inducers of immunogenic cell death (Figure 3) [91]. Small molecules offer the advantage of retaining the success of targeting the PD1-PDL1 axis and

being more amenable to fine-tuning to minimize the side effects. Targeting two immune checkpoints, VISTA (CA-170, V-domain Ig suppressor of T cell activation) [92] and TIM3 (CA-137, T-cell immunoglobulin and mucin domain 3) [93,94], has resulted in more recent efforts in this field.

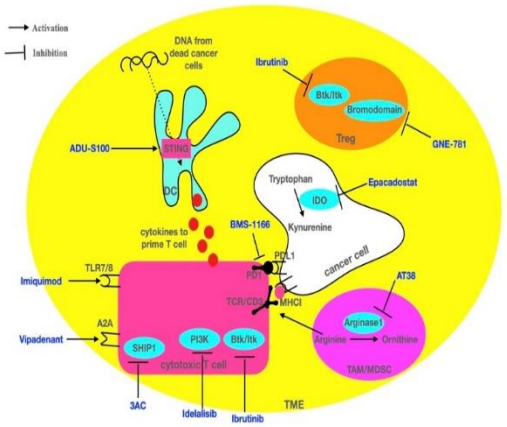

Figure 3: Small immunotherapy molecules and their targets. Blue texts represent the small molecules employed. For targets of these molecules please refer to the manuscript. DC: dendritic cell; Treg: regulatory T cell; TAM; tumour-associated macrophages; MDSC: myeloid-derived suppressor cell; TME: tumour microenvironment.

Small molecules can also act as agonists of pattern recognition receptors and be employed as potential immunotherapies or adjuvants for cancer vaccines [95]. The small molecule Ibrutinib was found effective in inhibiting two kinases: Bruton tyrosine kinase (Btk) and inducible T cell kinase (Itk). The inhibition of these two signaling molecules creates conditions that promote an immune response to tumors as well as reduce Treg cell numbers [98]. Toll-like receptor (TLR) agonists have gained the most research interest as they can induce the secretion of proinflammatory cytokines, suppress Tregs and promote Th1 cell-mediated activation of NK cells to eradicate cancer [6,96]. The best-characterized group of TLR agonists is the imidazoquinolines, such as Imiquimod and its derivatives. Imiquimod itself is a TLR7 agonist that has been approved for topical use in basal cell carcinoma [3,89]. However, in addition to the imidazoquinolines' potential systemic toxicity in the form of cytokine storm, they can, under certain conditions, promote cancer growth [89,97]. Ibrutinib (Imbruvica) is currently approved as a monotherapy for the treatment of mantle cell lymphoma. The inhibition of the immunosuppressant PI3K (phosphoinositide 3-kinase) by Idelalisib has led to the approval of this small molecule for the treatment of various B cell cancers [97,99]. Small molecules are also in various stages of clinical evaluation, including those targeting the enzyme IDO (indole amine 2,3 dioxygenase), which is involved in the breakdown of Tryptophan to Kynurenine as the latter has several immunosuppressive effects [3,100]. Inhibiting arginine catabolism is also being considered as a potential approach to alleviating immune suppression in TME, and the compound AT-38 has shown good anticancer activity in vivo [101]. Adenosine binds to A2A receptors on lymphocytes in the tumor and suppresses their activity. Adenosine can also amplify the immunosuppressive effect of Tregs by binding to A2A receptors on their surfaces [102]. Thus, small molecules targeting A2A could serve as potential targets to reduce the immunosuppressive milieu present in the tumor. Several antagonists of A2A receptors such as CPI-444, Vipadenant, Preladenant, PBF509, and AZD4635 are in various stages of development. TGF-β (transforming growth factor-beta) is well known for promoting immunosuppressive signaling, and its inhibition can cause immune activation [103-105]. The TGF-βR1 kinase/Alk5 inhibitor, Galuniseritib, is currently under clinical assessment. The Bromodomains enable transcription factors and proteins that regulate epigenetic markers to bind selectively to acetylated histones and alter the accessibility of genes. Small molecule inhibitors of these domains have been identified [106-108] that might reduce Treg cell function in tumors while making tumors more visible to killer immune cells.

Cancer vaccines

Cancer vaccines fall into two major classes: prophylactic and therapeutic. For example, human papillomavirus and hepatitis B vaccines, for example, have been enormously successful in reducing the incidence of cervical and liver cancers, respectively [2,23]. Therapeutic vaccines, on the other hand, are designed to activate the immune response to eliminate (or prevent relapse of) existing cancer, as in the case of using the tuberculosis BCG vaccine (Bacillus Calmette-Guerin vaccine) as a repurposed vaccine for bladder cancer [109]. The early attempts, five decades ago, to produce therapeutic cancer vaccines involved the use of a patient's tumor cells together with adjuvants or viruses to elicit a polyclonal immune response. However, this approach suffers from the difficulty of obtaining patient-derived tumor cells from certain cancer types [110]. Sipuleucel-T (Provenge) was the first commercially approved cancer vaccine and is a dendritic cell-based vaccine developed for the treatment of prostate cancer [111] (Figure 4).

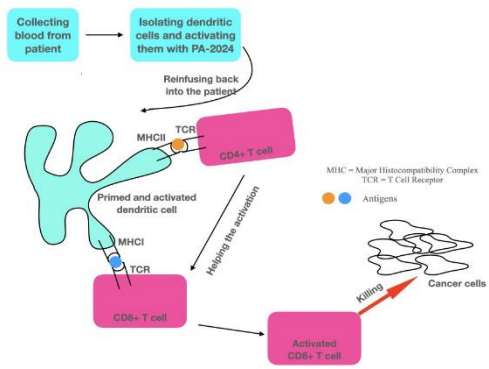

Figure 4: The mode of action of dendritic cell-based cancer vaccines.

The manufacture of this vaccine involves the extraction of dendritic cells from the patient's blood, activating these cells using a fusion protein called PA-2024 (made up of prostatic acid phosphatase (PPA), which is expressed in 95% of prostate cancers, and GM-CSF (granulocyte-macrophage colony-stimulating factor) to help the maturation of DCs) before being reinfused into the patient as a vaccine [37,112]. Talimogene laherparepvec (T-VEC) is an approved vaccine for the treatment of melanoma and is an oncolytic herpes simplex virus [113,114]. Advances in genomic DNA sequencing have led to an improved selection of neoantigens and the development of personalized recombinant cancer vaccines. Neoantigens are considered more appropriate, as opposed to TAA, for the development of this class of vaccines because the T cells for these antigens are not deleted by the central tolerance mechanism [115,116]. These vaccines should induce a more robust immune response and cause fewer autoimmune-related toxicities [2]. The choice of neoantigens and the cost/time associated with their development and production are some of the major challenges facing this form of immunotherapy, which remains under intense research.

Resistance to Cancer Immunotherapies

The availability of cancer immunotherapies has bolstered our armaments in the fight against this disease. Currently, seven immune checkpoint inhibitors are approved by the FDA for the treatment of 19 different cancer types in addition to the other forms of immunotherapy [21,29]. Cancer immunotherapies have revolutionized the way we treat cancer by prolonging the survival of patients. However, despite their promising overall successes, the response varies greatly, with only a small subset of cancers and a small percentage of patients within these subsets being responsive to ICBs, and even fewer achieving a durable response [34,117-120]. Given that immunotherapies are involved in the activation of the individual's immune response, it is perhaps understandable to see different response rates reflecting different patients' immune competencies and diversity [37]. This secondary resistance may appear in as little as two weeks following treatment initiation, despite the continuation of the immunotherapy. Furthermore, the resistance to immunotherapies can either be due to factors operating within the tumor cells, leading to what is called intrinsic resistance, or factors operating outside the tumor cells, usually in the TME, giving rise to extrinsic resistance (Figure 5). It should be noted that

the evolution of resistance to immunotherapies is a dynamic process and can exhibit overlap between intrinsic and extrinsic factors. The mechanisms of cancer resistance to immunotherapies are very complex and continue to be the subject of intense research [128].

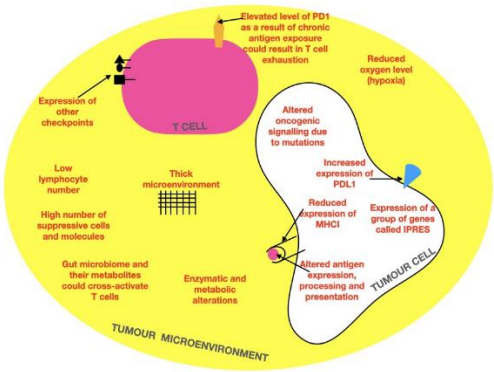

Figure 5: Depiction of the intrinsic and extrinsic factors in resistance to cancer immunotherapies. PD1: programmed death 1 protein.

Intrinsic Resistance

Many tumor-intrinsic factors have been identified that preclude response to immunotherapies, and they include; a) alterations in antigen expression, processing and presentation, b) loss of MHCI expression, c) alteration in oncogenic signaling pathways, d) upregulated expression of the ligands for immune checkpoints such as PDL1, e) resistance to TNF-α and INF-mediated cell-killing, and f) the expression of a group of proteins known as IPRES [118,129].

Alterations in antigen expression, processing and presentation

The presentation of antigen to naïve T cells plays a crucial role in the presence and durability of the immune response against cancer due to the stimulation of anticancer specific T cells. Cancer cells displaying a large number of novel antigens are usually more immunogenic and are thus better targets of immunotherapies [130]. Colon cancers, for example, with mutations in the DNA repair genes causing them to accumulate more genetic errors, can have 10-50 times more neoantigens compared to colon cancers without such mutations [131]. The increase in neoantigen expression is associated with significantly higher T cell infiltration into the tumor and, consequently, better prognosis. Cancers that inherently express low antigen levels are characterized by having a primary resistance to immunotherapies although some cancer types can develop secondary resistance through this mechanism by reducing the expression of the neoantigens. When this happens, the immune system will selectively eliminate cancer cells presenting a high level of neoantigens sparing the variants, and their progeny, with low neoantigen expression [132]. Mechanisms leading to the loss of neoantigens by cancer cells may result in resistance to immunotherapies. A recent study showed that the relapse of NSCLC (non-small cell lung cancer) after treatment with PD1/PDL1 or CTLA4 inhibitors could be due to the loss of 7-18 putative neoantigens [133]. The expression of high levels of tumor-associated antigens (TAAs) and neoantigens is directly correlated with the tumor mutational burden (TMB) [17,134-137]. Genetic instability due to alterations in DNA repair genes (such as BRCA1 and BRCA2) can increase TMB, rendering the cancers more susceptible to immunotherapies [138]. The TMB is defined as the number of mutations per megabase (Mb) of DNA. Cancers with high TMB tend to be more immunogenic and show better response across some cancer types [15,17,134,135,139]. High TMB cancers (TMBH cancers) are those with TMB $\geq$ 10. Levels of TMB higher than 20 were demonstrated to be more sensitive to PD1 blockade in melanoma, renal cell carcinoma, and non-small cell carcinoma (NSCC) [140]. Low levels of TMB (less than 10) result in poor immunogenicity, as in pancreatic and prostate cancers [140]. Van Allen demonstrated that TMB is significantly associated with anti-CTLA4 therapy [134]. However, the correlation of TMB with ICB response is not consistent across or within cancer types, so it is important to continue to seek additional factors that influence response and resistance to IC therapies [137,141]. Several proteins are involved in the

processing and presenting of antigens on the surfaces of cells, including MHC, beta-2 microglobulin (B2M), large multifunctional protein (LMP), and transporter-associated with antigen processing (TAP). Alterations of these proteins, through genetic and epigenetic modifications of their corresponding genes, can lead to resistance to immunotherapies and contribute to the heterogeneity of cancer [15,130,142,143]. It is well documented that subjects who initially respond to cancer immunotherapies with IL-2 or TIL therapy might develop acquired resistance through loss of B2M protein, which is an essential component of MHCI processing and presentation machinery [142]. Multiple other proteins are expressed by tumor cells (as well as normal cells) to regulate cell lysis. Tumor cell-expressed proteins such as PDL1 inhibit both T cells and NK cells [144]. Cancer cells lacking the expression of PDL1 have shown inferior clinical outcomes to ICB compared to those with higher levels of this ligand [145]. PDL1 positivity is determined by a 5% PDL1 positive expression threshold (the percentage of cells in a tumor that express PDL1) [139,146]. However, cancers with absent PDL1 can still respond to ICB as PDL1 expression can be induced upon activation of the interferon response pathway. However, unlike PDL1, tissues that lack TILs are unlikely to respond to ICBs [29,147,148]. The importance of two major factors, PDL1 expression at the surface of cancer cells and TILs, has led to an empirical system for the classification of tumours according to their anticancer immunity [59,149]. This system is called tumor immunity in the microenvironment (TIME) (Figure 6). Four distinct tumor subtypes can be described according to TIME and these are T1 ($TIL^-/PDL1^-$), T2 ($TIL^+/PDL1^+$), T3 ($TIL^+/PDL1^-$) and T4 ($TIL^-/PDL1^+$). Tumor subtypes T1 and T4 suggest no cancer immunity, as there are no TILs, and ICBs may not work. The absence of PDL1 in T3, despite the presence of TILs, indicates that targeting another axis other than PD1/PDL1, such as the CTLA4 checkpoint, might be more appropriate. Subtype T2, according to this classification, would be the only cancer that is likely to respond to anti-PD1/PDL1 immunotherapies.

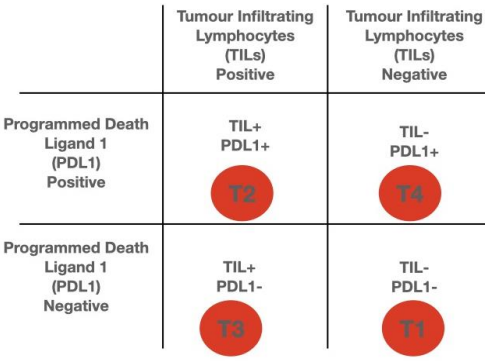

Figure 6: The TIME classification of cancer. TIL: tumour-infiltrating lymphocytes; PDL1: programmed death ligand 1.

A significant proportion of cancers, estimated to be 15%, can be traced back to viral infections such as hepatitis B virus (HBV), hepatitis C virus (HCV), human papillomavirus (HPV), Epstein-Barr virus (EBV), human lymphotropic cell leukemia virus 1 (HTLV1), human herpesvirus 8 (HHV8), and Merkel cell polyomavirus (MCV). Antigens derived from these viruses and expressed on cancer cells are widely acknowledged to be more immunogenic, highly expressed, and important targets for T cell responses [15]. Several studies found improvements in response when using virus-specific T cells for the treatment of many cancer types [152–154]. Furthermore, cancer-testis antigens (CTAs), which are encoded by 276 genes, are frequently found in some cancers, such as esophageal cancer [155,156]. These CTAs are immunogenic enough to be used in antigen-based vaccines against cancer [157]. Mutations in the genes encoding the tumor antigens can result in changes in these antigens after the initial response to ICB immunotherapies, resulting in acquired resistance [17]. Therapy with CAR-T cells is also antigen-specific, although it relies on the whole protein (as in the case of CD19) being expressed on the surface of cancer cells. However, the epitope that is being recognized by the CAR can be selectively deleted, leading to acquired resistance similar to the loss of neoepitope expression after ACT therapy

[158,159]. The epigenetic state of cancer cells and agents that influence the epigenetic marks on the DNA, such as DNA-methyl transferase inhibitors and histone modifiers, can also determine the expression of various components of the antigen-presenting machinery [160]. Chromatin remodeling is involved in the resistance to ICB and some chromatin remodeler complexes are frequently altered in a variety of cancers [161].

Loss of MHCI expression

Multiple studies show that downregulation of MHCI allows cancer cells to resist immunosurveillance [162,163]. Loss of function of B2M, an indispensable component of MHC proteins, results in the disruption of MHCI folding and transport to the cell surface [164-166]. Truncating mutations in B2M lead to loss of MHCI expression and acquisition of resistance to ICBs in patients with melanoma [142]. Loss of MHCI only partially explains the lack of immunogenicity of certain cancers but does not fully account for resistance to immunotherapies due to the actions of other mechanisms that are independent of MHCI expression, such as those mediated by NK cells [130]. A greater diversity of MHCI molecules is associated with an increased number of cancer antigens that could be presented, leading to a better therapeutic response to immunotherapies [37,167].

Alterations in oncogenic signalling pathways

Alterations in pathways that are fundamental for the process of oncogenesis in cancer cells can prevent immune cells' infiltration and/or function in TME, rendering the tumor resistant to ICB. The mitogen-activated protein kinase (MAPK) signaling pathway is involved in the proliferation, apoptosis, and motility of cells. Abnormalities in this signaling pathway promote cancer [168]. Signaling through MAPK eventually leads to the production of VEGF (vascular and endothelial growth factor) and IL-8, which are known to possess an inhibitory effect on T cell recruitment and function [169–171]. Several studies have shown that MAPK inhibitors increase TILs, IFN-gamma signaling, MHCI expression, and PDL1 levels, thereby promoting tumor cell killing [172–174]. Loss of the tumor suppressor protein PTEN (phosphatase and tensin), an enhancer of the PI3K-AKT-mTOR signaling pathway, is common in many cancers, including 30% of melanomas, and has been linked to ICB resistance [7,139]. PTEN deficiency was also associated with significantly lower expression of genes encoding IFN- and granzymes, as well as B cell and CD8+ T cell infiltration in melanomas, according to cancer genome atlas data [175]. Peng observed that the loss of PTEN increased the expression of immunosuppressive cytokines, which in turn reduced the infiltration of T cells into the tumour and led to poorer outcomes in melanoma patients treated with ICBs [176]. PTEN-associated checkpoint therapy resistance has also been observed in patients with other cancers [177, 178]. Cancers lacking PTEN tend to be poorly immunogenic, and studies show that tissue specimens of glioblastoma are more effectively lysed by T cells if they possess the wild-type PTEN compared to the mutated version [179]. The WNT/β-catenin pathway plays a critical role in oncogenesis and contributes to the immune resistance of cancers. Increased signaling through WNT/β-catenin has the potential to induce T cell exclusion from cancers, partly due to the reduction in the levels of the chemokine CCL4 [180]. The latter is an attractant of NK cells, monocytes and other components of the immune system, which was shown to be associated with an improved response to immunotherapies in melanoma [181,182]. Melanomas lacking T cells and specific DCs in TME had significantly higher β-catenin expression [7]. Mutations in the JAK/STAT genes can lead to loss of function of the cytokine IFN-ϒ and are linked to resistance to PD1 therapy [183]. Multiple studies have demonstrated that loss of JAK/STAT signaling results in resistance to PD1 or CTLA4 blockade through the inability to upregulate MHCI and PDL1 expression [142,183-185]. However, on continuous exposure, IFN- can aid in the immune-editing of cancer cells, thereby protecting cancers

from immune system attack [185]. cells can escape the effects of this cytokine by downregulating the genes encoding proteins involved in its pathways, such as JAK1, JAK2 and STAT [186,187]. One study on the development of Pembrolizumab resistance discovered that two patients had mutations in the JAK1 and JAK2 genes, resulting in disruption of IFN- signaling [142].

The upregulated expression of ligands for immune checkpoints

Immune checkpoint ligands, such as PDL1, can be upregulated in cancers in response to intrinsic oncogenic signaling or cytokines released by Teff cells, such as IFN [45]. The expression of PDL1 by cancer cells is an important determinant of the response to PD1 blockade [188]. The upregulated expression of PDL1 can allow cancers to escape immunosurveillance and exhibit resistance to the blockade of this checkpoint [189].

Resistance to TNF-α and IFN-ɣ mediated cell killing

A CRISPR-based approach to identifying mechanisms by which cancers can avoid the killing of Teff cells and NK cells discovered deletions in genes involved in the TNF-α and IFN-γ signaling pathways [118,190]. Furthermore, the upregulation of the TNF receptor-2 gene (TNFR2) signaling pathway was found in non-responders to anti-CTLA4 therapy [183].

The expression of IPRES

Cancer cells innately resistant to PD1 blockades, such as pancreatic cancer, exhibit a transcriptional signature of genes, collectively called IPRES, involved in various stages of malignant progression [171]. Attenuating the biological processes of IPRES may lead to improved anti-PD1 responses [15].

Extrinsic Resistance

Tumours exist and are supported by an environment consisting of an extracellular matrix, blood vessels, various immune cells, fibroblasts and signaling soluble molecules. This environment, often referred to as the tumour microenvironment (TME), has a large influence on the progression of tumours and response to therapies, particularly immunotherapies.

Tumour infiltrating lymphocyte (TIL) density

Various studies have demonstrated that TILs can influence host immunity in a range of cancers [191,192]. The density of Teff cells and NK cells in the TME is often associated with clinical response [192] (Figure 3). Responses to ICB in melanoma were associated with intra-tumour $CD8^+$ T cell density [193,194]. Inflammation of the TME due to the presence of Teff cells has also been linked with clinical benefits in patients with melanoma upon treatment with mAbs targeting CTLA4 and with IL2 [193,195]. One of the main factors associated with ICB resistance is the lack of T cell infiltration in the tumour microenvironment, which is often referred to as a "non-inflammatory or cold tumour". The presence of a certain number of TILs in the tumour is the basis of judging the efficacy of checkpoint blockade [196]. More TILs lead to hot tumours and more effective immunotherapies. Several approaches have been experimented with to turn cold tumours into hot ones [15]. Tumour infiltrating B cells have also been found to play a role in anti-PD1 treatment, correlating with improved response [197].

The presence of immunosuppressive cells and molecules

Immunosuppressive cells such as Treg cells, myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), especially M2 macrophages, and cancer-associated fibroblasts (CAFs), also often infiltrate into the tumor microenvironment. Tregs are known to suppress T cell responses through the secretion of certain inhibitory cytokines such as IL-10, Il-35 and TGF-β and by direct contact [198,199]. Treg cell depletion has been shown to improve anti-tumor immune response [200, 201]. Tregs are known to facilitate self-tolerance through the

suppression of Teff function. The ratio Teff/Treg in murine models of cancer is associated with response to ICBs and the inability to increase Teffs or decrease Tregs may result in resistance to immunotherapies [202,203]. MDSC are a major regulator of the immune response against cancer, and reports suggest that their presence correlates with reduced survival and reduced efficacy of various immunotherapies [204-206]. The accumulation of MDSC in TME was detected in patients that developed secondary resistance after an initial response to ICB [207]. These MDSC were found to express PDL1 and galactin-9, ligands for PD1 and TIM3, respectively, endowing them with the power to inhibit anti-tumor T cell function. The TAMs are another subset of cells that can affect responses to immunotherapies. They include both M1 (involved in promoting antitumor immunity) and M2 (involved in promoting pro-tumour immunity) macrophages [208]. CAFs are one of the most abundant stromal cells in the TME and their presence has been linked to the modulation of anti-tumor immune responses on various levels [209]. These fibroblasts in TME can contribute to therapy resistance driven by the mediator TGF-β [118]. These immunosuppressant cells can hinder Teff cells' function and limit ICB efficacy [210]. Various soluble signaling molecules are secreted by cells infiltrating into TME as well as cancer cells, which could have stimulatory or suppressive effects. Cancer cells secrete IL-6 and G-CSF, blocking the differentiation of CD34 cells into dendritic cells and consequently affecting the presentation of neoantigens to naïve T cells [139]. Some chemokines secreted by cells in the TME are capable of attracting Teff cells and NK cells to enhance the anti-tumor response [211,212]. Other chemokines such as CCL2 and CCL22 can inhibit the immune response by recruiting inhibitory cells such as Tregs, MDSCs, and M2 macrophages. Based on this, the levels of different chemokines in TME can determine the cancer immune status. TGF-β is another cytokine with an important role in immunosuppression through stimulating Tregs [213,214]. High levels of TGF-β are associated with poor prognosis in multiple cancers [215]. This cytokine, TGF-β, was associated with a limited response to ICBs in murine models of cancer [216]. The improved antitumor response was observed following the inhibition of TGF-β in urothelial cancers [217]. The well-known promoter of angiogenesis, vascular endothelial growth factor (VEGF), also functions as an immunosuppressant and is associated with resistance to ICBs. The level of VEGF was found to be higher in non-responders to anti-PD1 therapy compared to responders [218]. Inhibition of VEGF was correlated with an improved response to ICB in renal cell carcinoma [219].

Hypoxia

Reduced oxygen availability in TME is one of the characteristics of tumours that leads to uncontrolled cell proliferation. Tumour-associated macrophages preferentially accumulate in TME under hypoxic conditions, and this mechanism is known to mediate resistance to multiple therapies for cancer [220].

Gut microbiome

Accumulating evidence points to the important role of the gut microbiota in the immune system's response to cancer [221]. The mechanisms involved are mainly the cross-reactivity of the microbiota (and their metabolites) and the cancer antigens, as well as the stimulation of the pattern recognition receptors. Studies on mouse models have confirmed that different gut microbiota have significantly different cancer treatment responses [222,223]. These animal studies have also been verified in human patients with different cancers [224–228]. Through altering the relative numbers of certain microbial species, antibiotics can yield either higher susceptibility or higher resistance to ICBs. Immune profiling suggested enhanced systemic and antitumor immunity in responders having a favorable gut microbiome [229].

T cell exhaustion

The $CD8^+$ T cells can become exhausted, and the intensity of PD1 expression can determine the extent of this exhaustion and thus affect the sensitivity to anti-PD1 therapy [201]. T cell exhaustion is associated with loss of function [230]. Chronic exposure to cognate antigen results in elevated PD1 levels with the subsequent impairment of T cell function and poor response to immunotherapies [201,231,232]. Epigenetic modifications were also linked to T cell exhaustion through chromatin changes [233,234]. CD28 is another co-stimulatory receptor related to CTLA4, although it performs the opposite function. It activates the immune response by interacting with either CD80 or CD82. Without CD28 exhausted T cells cannot be reactivated to perform their normal function [130]. Inhibition of CD28 resulted in the progression of colon cancer [235].

Activation of other immune checkpoints

Other immune checkpoints operate within the overall immunity process in addition to CTLA4 and PD1. These checkpoints include TIM3 (T cell immunoglobulin mucin domain 3), LAG3 (lymphocyte activation gene 3) and NKG2A (CD94/NK group 2 member A) and factors affecting their differential expression in particular cancers remain to be studied. Overexpression of alternate immune checkpoints has been linked to anti-PD1 and anti-CTLA4 therapeutic failures. Resistance was observed after upregulation of TIM3 and LAG3 [236-238]. Other immune checkpoints continue to be discovered, including B and T lymphocyte attenuators (BTLA), T cell immunoreceptor tyrosine-based inhibitor motif domain (TIGIT) and the v-domain immunoglobulin-containing suppressor of T cell activation (VISTA). Co-expression of multiple ICs has been linked to severe exhaustion of T cells [239].

The pathophysiology of the tumor microenvironment

The construction of TME can make the delivery of immunotherapies and immune responses challenging [37]. Pancreatic cancers, for example, are characterized by having a thick stromal microenvironment constituting a physical barrier against the penetration of TME by large molecules and immune cells [240]. Moreover, the TME of pancreatic cancers has been shown to harbor bacteria that can metabolize and inactivate some chemotherapeutic drugs [241]. The presence of an adequate supply of blood and lymphatic vessels could assist immunotherapies in exerting an improved response. Organized aggregates of lymphoid cells are often found at the edge of the TME and are recognized under the name tertiary lymphoid structure (TLS). This structure can also play an important role in ICB treatment and is often associated with a good prognosis [242,243]. Other investigations, however, pointed to TLS as having the potential to increase cancer aggressiveness [244,245].

Enzymatic and metabolic signatures

Several enzymatic activities and metabolites can generate alterations within the TME, resulting in a reduced response to immunotherapies. Adenosine was shown to inhibit T cell proliferation and function via A2A receptors on T cells as well as promote metastasis via A2B on cancer cells. [246,247]. Furthermore, CD73, which is the enzyme that dephosphorylates AMP (adenosine monophosphate) to form adenosine, can also suppress immune function and promote metastasis [248]. High expression of CD73 is associated with a poor prognosis in different cancers [249–251]. CD73 also promotes T cell exhaustion and the consequent resistance to ICB. The enzyme IDO, released by MDSC and cancer cells, catalyzes tryptophan degradation to form the immune suppressor kynurenine [252]. Tryptophan metabolism is a rate-limiting step in T cell division and its depletion by IDO reduces T cell proliferation, inhibiting their function and giving rise to resistance to ICB [254-256]. Studies have demonstrated that increased levels of CTLA4 upregulate IDO in DCs [257]. Increased IDO expression has been linked to a number of cancers [253,258]. Additionally, the accumulation of Kynurenine and the depletion of Tryptophan lead to immunosuppression

through T cell anergy and apoptosis [259]. IDO-knockout mice showed improved survival after ICB compared with wild-type mice, highlighting the therapeutic value of IDO inhibition [260]. Another immune suppressor enzyme, Arginase1, was recently shown to compete with IDO to inhibit DC function [261].

Future Strategies to Improve the Effectiveness of Immunotherapies

Immunotherapies have taken a prominent role in the fight against cancer, despite remaining challenges regarding their efficacy. Further studies elucidating the mechanisms that result in resistance to immunotherapies are needed to improve clinical outcomes from these treatments [118]. The TME in immunotherapy-resistant cancers contains multiple immunosuppressive cells and molecules that require overcoming to achieve an improved response. Studies further examining the heterogeneity of tumours could be valuable and provide the fundamental basis for constructing an effective therapy. The pharmaceutical market is currently overloaded with antagonist antibodies such as those targeting PD1, PDL1 and CTLA4. However, it is evident from this review that these antagonists alone, as monotherapies, are not enough to induce a durable response. Investigating agonists such as those targeting ICOS and VISTA could yield promising results. Combined treatments against several immune and non-immune targets, such as those employing immunotherapeutic agents together with chemotherapy or with targeted therapeutics to overcome resistance and immune evasion, are the most investigated approaches [117,262-264]. Currently, a few of these have already been granted authorization by the FDA and are generally classified under three categories: a) immunotherapy combinations, b) immunotherapies and targeted therapy combinations, and c) immunotherapies and chemo (or chemoradiation) therapies, as shown in Tables 2, 3, and 4 respectively. More combination immunotherapies are in various stages of clinical trials, and this route may be a worthwhile strategy to overcome resistance. The use of small molecules to block immunosuppression is emerging as another area of intense research due to the advantages they offer in terms of reaching tumours that larger molecules are unable to.

Table 2: FDA-approved immunotherapy combinations [268]

Therapy	Tumour to be treated	Reference
Nivolumab/Ipilimumab	Metastatic melanoma	[269]
Nivolumab/Ipilimumab	Metastatic RCC	[270]
Nivolumab/Ipilimumab	Metastatic CRC	[271]
Nivolumab/Ipilimumab	HCC	[272]
Nivolumab/Ipilimumab	Metastatic NSCLC	[273]
Nivolumab/Ipilimumab	Mesothelioma	[274]

Nivolumab is a mAb against PD1, Ipilimumab is a mAb against CTLA4; RCC: renal cell carcinoma, CRC: colorectal cancer, HCC: hepatocellular carcinoma, NSCLC: non-small cell lung cancer.

Table 3: FDA- approved immunotherapies combined with targeted therapies [268]

Therapy	Tumour to be treated	Reference
Pembrolizumab/Axitinib	RCC	[264]
Avelumab/Axitinib	RCC	[275]
Pembrolizumab/Lenvatinib	Endometrial carcinoma	[276]
Atezolizumab/Bevacizumab	HCC	[277]
Atezolizumab/Cobimetinib/ Vemurafenib	Melanoma	[278]
Nivolumab/Cabozatinib	RCC	[279]

Pembrolizumab is a mAb against PD1, Axitinib is a tyrosine kinase inhibitor against VEGF receptors, Avelumab is a mAb against PD1, Lenvatinib is a tyrosine kinase inhibitors against multiple targets including VEGF receptors and FGF receptors, Atezolizumab is a mAb against PDL1, Bevacizumab is a mAb against circulating VEGF, Cobimetinib is an MAPK signalling pathway blocker, Vemurafenib is a selective inhibitor of mutated BRAF protein leading to reduced signalling via MAPK pathway, Nivolumab is a mAb against PD1and Cabozatinib is a tyrosine kinase inhibitor against a number of targets including RET, MET and VEGFR2. RCC: renal cell carcinoma, HCC: hepatocellular carcinoma.

The application of CRISPR-cas9, or similar technology, to identify genes involved in resistance could also be a promising strategy to develop immunotherapies against new targets [265-267]. Identifying new biomarkers

associated with response and resistance to immunotherapies is also a good strategy and could potentially be exploited for developing new medicines [45]. Enhancing CD28 receptor expression to rescue exhausted T cells and promote the durability of anti-tumour immune responses could also constitute a potential means of overcoming resistance to ICBs and other immunotherapies that rely on the function of T cells.

Table 4: FDA- approved immunotherapies combined with chemotherapies or chemoradiation therapies [268]

Therapy	Tumour to be treated	Reference
Pembrolizumab/Pemetrexed/Platinum	NSCLC	[280,281]
Chemoradiation followed by Durvalumab	NSCLC	[282]
Pembrolizumab/Chemoradiation	NSCLC	[283]
Atezolizumab/Bevacizumab/Paclitaxel/Carboplatin	NSCLC	[284]
Atezolizumab/Etoposide/Carboplatin	ES-SCLC	[285]
Atezolizumab/Nabpaclitaxel	Triple negative breast cancer	[286]
Pembrolizumab/Chemotherapy	HNSCC	[287]
Atezolizumab/Nabpaclitaxel/Carboplatin	NSCLC	[288]
Durvalumab/Chemotherapy	SCLC	[289]
Ipilimumab/Nivolumab/Platinum chemotherapy	NSCLC	[290]
Chemotherapy followed by Avelumab	Urothelial carcinoma	[291]
Pembrolizumab/Chemotherapy	Triple negative breast cancer	[292]
Pembrolizumab/Chemotherapy	Gastro and gastro-oesophageal adenocarcinoma	[293]

Pembrolizumab is a mAb against PD1, Pemetrexed is a type of chemotherapy, Platinum refers to a group of chemotherapy, Durvalumab is a mAB against PDL, Atezolizumab is a mAb against PDL1, Bevacizumab is a mAb against circulating VEGF, Paclitaxel is a chemotherapy targeting the mitotic spindle assembly, Carboplatin is a chemotherapy that causes inter- and intra-DNA strand cross linkage, Etoposide is a chemotherapy that inhibits DNA synthesis through forming a complex with topoisomerase II, Nabpaclitaxel is a chemotherapy where Paclitaxel is bound to Albumin, Ipilimumab is a mAb against CTLA4, Avelumab is a mAb against PD1. NSCLC: non-small cell lung cancer; ES-SCLC: extensive-stage small cell lung cancer; HNSCC: head and neck squamous cell carcinoma.

Conflict of interests

The author declares no conflict of interests.

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N/A

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