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Immuno Oncology Agents for Cancer Therapy

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A B S T R A C T
The immune system consists of a complex process in body fight against cancer. This process involves cells, organs, and proteins. Cancer can commonly get around many of the immune system's natural defenses, allowing cancer cells to continue to grow. Immunotherapy is a type of cancer treatment. It uses substances made by the body or in a laboratory to boost the immune system and help the body find and destroy cancer cells. Immunotherapy can treat many different types of cancer. It can be used alone or in combination with chemotherapy and/or other cancer treatments. When the immune system detects something harmful, it makes antibodies. Antibodies are proteins that fight infection by attaching to antigens. Antigens are molecules that start the immune response in body. Monoclonal antibodies can help fight cancer in different ways. For example, they can be used to block the activity of abnormal proteins in cancer cells. This is also considered a type of targeted therapy, which is a cancer treatment using medication that targets a cancer's specific genes, proteins, or the tissue environment that helps the tumor grow and survive. Other types of monoclonal antibodies boost your immune system by inhibiting or stopping immune checkpoints. Cancer cells can find ways to hide from the immune system by activating these checkpoints. Checkpoint inhibitors prevent cancer cells from blocking the immune system. Common checkpoints that these inhibitors affect are the PD-1/PD-L1 and CTLA-4 pathways. Different types of immunotherapy work in different ways. The immunotherapy treatments can help the immune system to stop or slow the growth of cancer cells.
Keywords: Immune system, Monoclonal antibodies, Cancer cells, Immunotherapy.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Cancer immunotherapy has been a game changer in cancer treatment since the approval of the immune checkpoint inhibitor (ICI) ipilimumab in 2011. Currently, 11 immune checkpoint inhibitors and 2 chimeric antigen receptor T cell (CAR-T) products have been approved in treating 16 types of malignant diseases and 1 tissue-agnostic indication. In 2018, one half of the Nobel Prize in Physiology or Medicine was awarded to James Allison, who conceptualized cancer immunotherapy by targeting the immunosuppressive signal mediated by Cytotoxic T Lymphocyte-Associated Protein 4(CTLA-4.This conceptual breakthrough led to the subsequent revolutionary development of immune checkpoint inhibitors (ICIs). In addition, co-Nobel Prize awardee Tasuko Honjo showed that a basic mechanism of activation-induced cell death in lymphocytes is mediated by Programmed Cell Death 1 (PD-1). Honjo subsequently demonstrated that the PD-1

pathway is an important negative regulator of T cell function. The field of immuno-oncology has been transformational in the care of cancer patients. William B. Coley, now widely accepted as the father of immunotherapy, first attempted to harness the power of the immune system for treating cancer in the late 19th century. As an orthopedic surgeon who operated on patients with bone sarcomas, he noticed that some patients with significant postoperative wound infections a common occurrence when aseptic technique had not yet been optimized would undergo spontaneous regression of their unresected tumours. Beginning in 1891, Coley injected more than a thousand patients with mixtures of live and inactivated bacteria such as Streptococcus pyogenes and Serratia marcescens with the hope of inducing sepsis and strong immune and antitumour responses. His cocktail of bacteria became widely known as "Coley's toxin" and represents the first documented active cancer immunotherapy intervention[1].

Coley achieved durable complete remissions in several types of malignancies, including sarcoma, lymphoma, and testicular carcinoma[1-4]. However, the lack of a known mechanism of action for Coley's toxin and the risks of deliberately infecting cancer patients with pathogenic bacteria caused oncologists to adopt surgery and radiotherapy as alternative standard treatments early in the 20th century. It would take more than half a century before a better understanding of the key mediators of sepsis would shed some light on the mechanisms of action of Coley's toxin. Those mediators constitute a cytokine family including interleukins, interferons, and chemokines[3]. Once again, the race was on to apply those novel discoveries to cancer therapy. Physicians and researchers achieved modest success with this novel approach, occasionally inducing clinical remissions with high-dose interleukin 2 (IL-2) in metastatic renal cell carcinoma[5] and debatable responses with interferon in stages III and IV melanoma. Those modest successes were often counterbalanced with significant adverse events. Although novel methods of delivery such as pegylation would abate some of the toxicities, the sporadic and unpredictable immune responses seen with those therapies meant that only a small, carefully selected subgroup of cancer patients would benefit.

The next revolutionary wave in cancer immunotherapy came with the better understanding of the process of immune surveillance, by which innate immune cells eliminate cancer cells. When the immune system detects something harmful, it makes antibodies. Antibodies are proteins that fight infection by attaching to antigens. Antigens are molecules that start the immune response in your body. Monoclonal antibodies are made in a laboratory to boost the body's natural antibodies or act as antibodies themselves. Monoclonal antibodies can help fight cancer in different ways[5-12]. Other types of monoclonal antibodies boost your immune system by inhibiting or stopping immune checkpoints. Immune checkpoints are used by the body to naturally stop an immune system response and prevent the immune system from attacking healthy cells. Cancer cells can find ways to hide from the immune system by activating these checkpoints. Checkpoint inhibitors prevent cancer cells from blocking the immune system.

2. Immune checkpoint inhibitors include

- Atezolizumab (Tecentriq)
- Avelumab (Bavencio)
- Dostarlizumab (Jemperli)
- Durvalumab (Imfinzi)
- Ipilimumab (Yervoy)
- Nivolumab (Opdivo)
- Pembrolizumab (Keytruda)

Non-specific immunotherapies:

Non-specific immunotherapies, also called non-specific immune-modulating agents, help your immune system destroy cancer cells. There are several kinds of non-specific immunotherapies that work in different ways.

Cytokines:

Cytokines are a part of the immune system. They are proteins that send messages between cells to activate the immune system. There are two types of cytokines that are used to treat cancer:

Interferons:

These proteins are produced by your immune system to alert your body that there is a pathogen, typically a virus, in your body. Interferon's can be made in a laboratory to help your immune system fight cancer. They can also slow the growth of cancer cells. The most common type of interferon used in cancer treatment is called interferon alpha (Roferon-A [2a], Intron A [2b], Alferon [2a]). Interferon can be used to several many different types of cancer. Side effects of interferon treatment may include flu-like symptoms, an increased risk of infection, skin rashes, and hair thinning[13].

Interleukins:

Interleukins are proteins that pass messages between cells. They also start an immune response. For example, the lab-made interleukin-2 (IL-2) or aldesleukin (Proleukin) can treat kidney cancer and melanoma. Common side effects of IL-2 treatment include weight gain and low blood pressure. Some people also experience flu-like symptoms.

Bacillus Calmette-Guerin (BCG):

This type of immunotherapy is similar to the bacteria that causes tuberculosis. It is used to treat bladder cancer. BCG is placed directly into the bladder through a catheter. It attaches to the inside lining of the bladder and activates the immune system to destroy tumor cells. BCG can cause flu-like symptoms. Cancer immuno-editing is the process by which various immune system components protect the host against primary tumour development or enhance tumour escape, or both, either by sculpting tumour immunogenicity or attenuating antitumour immune responses. The process is tightly regulated by immune checkpoints, which are immune-cell surface receptors controlling either the activation or the inhibition of immune responses. Activation of the immune system is, on the one hand, the desired outcome to achieve tumour control, but on the other hand, responsible for autoimmunity17-23. The discovery and development of monoclonal antibodies against the inhibitory immune checkpoints CTLA-4 and PD-1 have resulted in dramatic antitumour responses by the up-

regulation of immune activation at various stages of the immune cycle. Immune checkpoint inhibitor therapies are now widely indicated in numerous cancer types. Furthermore, numerous ongoing clinical trials are assessing the potential of other agonistic or inhibitory checkpoints to affect tumour-related outcomes. The checkpoints are not equal in their potential.

Modulating and Predicting Immune Toxicity for Better Efficacy: Immunotherapies are often limited by their immune-related adverse events (irAEs), an immune activation and inflammatory response against the host's healthy tissues. Immune activation against the host's tumour is the desired outcome, but irAEs are challenging to predict, diagnose, and treat. In the setting of metastatic melanoma, the addition of a CTLA-4 antibody to PD-1 blockade is associated with only an incremental increase in survival, but at the cost of more than double the rate of serious irAEs. A recent meta-analysis reported a fatality rate of up to 1 patient in every 77 treated using an ICI combination. For specific irAEs, such as immune-related myocarditis, the mortality rate is as high as 50% in treated patients[11].

Numerous predictors of irAEs have been proposed (baseline lymphopenia and eosinophilia, B cell changes, T cell repertoire, circulating IL-17, and gut microbiota changes[12–17]), but few have been prospectively validated. For serious irAEs, guidelines recommend broad immunosuppression consisting of corticosteroids, followed by one or more biologics (tumour necrosis factor inhibitors) or T cell suppressants (such as mycophenolate mofetil. Very little prospective knowledge has been developed about the consequences of those therapies for cancer-related outcomes. An analysis of the baseline use of corticosteroids in patients with lung cancer reported an association with worse survival outcomes. Similarly, the use of high-dose steroids in the setting of immune-related hypophysitis in patients with metastatic melanoma was also associated with worse survival[22]. On the other hand, the use of corticosteroids in other clinical settings in which patients experience irAEs was not associated with a reduced response to ICI therapy or with survival.

Targeting Tumour Metabolism in the Tumour Microenvironment: There is growing evidence that the tumour microenvironment supports inappropriate metabolic reprogramming, negatively affecting T cell function and resulting in attenuated antitumour immune responses. In that context, targeting both tumour and T cell metabolism can beneficially enhance immunity in an inhospitable microenvironment and markedly improve the success of immunotherapies. As discussed earlier, TILs in the tumour microenvironment have significant prognostic and predictive significance. Their function is limited not only by immune checkpoints, but also by increasingly recognized "metabolic checkpoints. Rapidly dividing tumour cells show complex and dynamic metabolic reprogramming and high glycolytic activity, a phenomenon called the "Warburg effect," which is recognized as one of the hallmarks of carcinogenesis. Thus, tumour cells impede the access of T cells to nutrients necessary for their activation and generate high levels of lactate. Recent evidence suggests that ICIs might directly sculpt the metabolic landscape in the tumour microenvironment, thus affecting the functioning of effector T cells. On the one hand, CTLA-4 and PD-1 binding to their respective ligands impairs the metabolic TIL phenotype by inhibiting glycolysis, thus causing reduced cytokine secretion and leading to an exhausted effector T cell phenotype[24-26].

On the other hand, ICIs also have the opposite effect on metabolic reprogramming of cancer cells. Ligation of PD-L1 directly upregulates glycolysis in cancer cells by promoting glucose uptake and production of lactate, thus promoting tumour growth and metastasis. Many therapeutic strategies have been proposed to tackle that imbalance. The PI3K/AKT/mTOR pathway is well known to play a critical role in integrating the metabolism signals of cancer and immune cells. Recent preclinical evidence suggests that rapamycin, in combination with ICIs, augments cytotoxic and memory T cell function[24,66]. Another promising therapeutic is metformin in combination with ICIs. Metformin is known to target the mitochondrial respiratory complex I and to activate AMPK pathway signal transduction, a key pathway in T cell regulatory and metabolic functioning.

A current perspective on the anti-cancer immune response: Challenges in improving the efficacy of existing immunotherapies, and the development of new ones, have led to a deeper appreciation of understanding the mechanisms underlying an effective anti-cancer immune response, as well as the "defects" that are responsible for the lack of an effective anti-cancer immune response in cancer patients.

The cancer-immunity cycle

We present a model of the anti-cancer immunity "cycle which provides a summary of our scientific knowledge on each step of an effective anti-cancer immune response. The cycle starts when tumor antigens are recognized by the immune system. Genomic instability/mutation is 1 of the 2 enabling characteristics of cancer. All cancers, regardless of their tissue origin(s), harbor genetic alterations that range from a few mutations in pediatric malignancies to dozens or hundreds in adult cancers. These non-synonymous DNA alterations can give rise to proteins that differ from the proteins expressed in normal cells, i.e., tumor antigens. As a second enabling characteristic, some cancers express non-mutation-associated tumor antigens, such as proteins normally expressed in immune-privileged sites, viral proteins, or proteins encoded by endogenous retroviral genes. When these antigens are taken up and processed by professional antigen-presenting cells (APCs), the APCs migrate to secondary lymphoid organs and activate naïve T cells in concert with a highly-coordinated hierarchy of co-stimulatory signals, such as the CD28/B7-1/2-mediated signal. To achieve homeostasis and prevent over-reaction to non-self antigens, the immune system has also developed highly coordinated negative feedback circuits. CTLA-4 is one of the major negative regulators of the T cell-mediated immune response. CTLA-4 expression is rapidly upregulated upon T cell receptor (TCR) engagement,

allowing it to outcompete CD28 for ligation by B7-1/2, and thereby negatively regulate T cell activation and effector function[27-29].

The immune microenvironment of the tumor

Study of a Tumor Immunity in the MicroEnvironment (TIME) classification system can be used as the first step in assessing anti-cancer immunity and determining underlying tumor resistance mechanisms.

TIME classification is based on two major factors:

(1) Tumor expression of PD-L1, and the presence of immune cell infiltration, mainly tumor-infiltrating lymphocytes (TIL). Correspondingly, 4 distinct TIME subtypes can be described: T1 (PD-L1$^-$, TIL$^-$), T2 (PD-L1$^+$, TIL$^+$), T3 (PD-L1$^-$, TIL$^+$), and T4 (PD-L1$^+$, TIL$^-$). In cancers with no immune cell infiltration (T1 or T4 TIME), no anti-cancer immunity exists at the cancer site(s), suggesting defects in cancer antigen release (Cancer-Immunity Cycle Step 1), presentation (Cancer-Immunity Cycle Step 2), immune cell priming and activation (Cancer-Immunity Cycle Step 3), or trafficking of immune cells into cancer sites (Cancer-Immunity Cycle Step 4). In these cases, normalization of cancer immunity using anti-PD1/PD-L1 therapy may not work, since no cancer immunity exists to be de-repressed. On the other hand, the majority of solid tumors (approximately 70%) have a T4 TIME, which underscores the importance of developing rational IO combinations to address both a lack of effector cell infiltration and the presence of non-PD-L1/PD-1 immunosuppressive components. Furthermore, T1 or T4 TIME tumors often exhibit low levels of tumor mutation burden and tumor antigens. For example, androgen-dependent prostate cancer usually presents with a T1 or T4 TIME, with little lymphocyte infiltration. In other cases, physical barriers can inhibit TIL infiltration such as in pancreatic cancer, even though an anti-cancer immune response emerges in some tumors, an immune-excluded phenotype is commonly observed because the desmoplastic stroma precludes the immune cells from penetrating into the tumor.

From enhancing immunity to normalizing TIME

Historically, cancer immunotherapy has focused on amplifying tumor immunity above physiological levels, which is associated with clinical response in a minority of patients, in highly selected cancers (e.g., kidney and melanoma), and with off-tumor toxicities. It is becoming increasingly appreciated that many cancer patients have anti-cancer T cells, but the TIME can effectively suppress their immune response by harnessing immune homeostasis mechanisms to negatively regulate anti-cancer immunity or cell survival. As a result, cancer cells that can evade immune attack are naturally selected for survival. Hence, Lieping Chen and his colleagues have emphasized that, instead of enhancing the immune system, it is important to restore the function of the TIME. The lessons we have learned from the failure of boosting immunity and the success of ICI development substantiate this notion of TIME normalization. It is now crucial that we determine how to normalize the defects in TIME. In particular, targets for normalizing T1 (PD-L1$^-$, TIL$^-$) TIME remain to be discovered and validated. Searching for and defining such targets from T1 tumors are anticipated by Chen to be the next game changer in cancer immunotherapy[30-34].

Fast and furious development of cell therapy

Although a paradigm shift from immunity enhancement to normalization of TIME may be advisable in IO development for solid tumors, immunity enhancement remains a mainstay therapeutic strategy for hematologic malignancies. CD19-targeted CAR-T cells for B cell neoplasms have opened up a new era in synthetic cancer immunotherapy. There are two approved CD19-CAR-T cell platforms: tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta). Besides CD3ζ chain, Tisagenlecleucel uses CD137 (4-1BB) as additional co-stimulating signal (COS), while axicabtagene ciloleucel uses CD28 a COS. Both agents utilize a single chain anti-CD19 fragment to target malignant B cells. Tisagenlecleucel is approved for the treatment of patients up to 25 years of age with B cell precursor acute lymphoblastic leukemia (ALL) that is refractory to standard therapy or in at least second relapse.

History of immuno-oncology

It has long been known, but is now increasingly appreciated, that tumour cells can be recognised and disabled by the immune system. Some tumours show evidence of spontaneous regression early in their development, suggesting that the immune system may be capable of recognising and eliminating early-stage tumour cells. Observation of spontaneous remissions in patients led to the foundation of the area of IO. A spontaneous remission is defined as a reduction in severity of, or disappearance of, the signs and symptoms of a disease, without any apparent cause and in the absence of treatment. This is most often noted in patients who have recently had acute infections, especially when this results in fever which appears to stimulate the immune system. It is now recognised that, in some cases, the immune system is capable of completely eliminating a tumour. Spontaneous remissions have been observed in most cancer types, but most frequently in advanced melanoma, renal cell carcinoma (RCC) and urothelial carcinomas, although the phenomenon has also been reported in breast cancer, neuroblastomas, some sarcomas and embryonal cancers.

William Coley was the first to investigate the potential for IO, and successfully treated malignancies based on immune stimulation in the 1890. After discovering that cancer patients who contracted post-surgical infections seemed to improve faster than those who did not, he investigated the use of bacteria to stimulate and enhance the body's natural immune response to fight cancer. Through these studies, he later developed Coley's toxin, which was based on attenuated bacteria and is thought to be the first known IO therapy.

A later development involved the Bacillus Calmette-Guerin (BCG) vaccine, originally produced in the early 1900s for use against tuberculosis (TB), and first used therapeutically for TB in the 1920s. However, its role in cancer therapy dates back to 1929 when a reduced incidence of cancer among patients with TB was observed at autopsy. Experiments revealed that BCG produced a profound

stimulation of the mononuclear phagocyte system (also known as the reticuloendothelial system), which was recognised as an important defense against cancer. Furthermore, it was observed that neonates who had been immunised with BCG had a significantly lower incidence of leukaemia later in their lives[35-39].

This background and basic understanding of IO sparked an interest in the use of BCG for other types of malignancies, in particular bladder cancer. Early investigations demonstrated responses in patients with melanoma metastatic to the bladder when treated with intralesional BCG. In light of this success, work in animal models led to publication of the results of the first successful clinical trial of intravesical BCG in patients with recurrent bladder cancer. It is now understood that intravesicularly adminstered BCG attaches to bladder tumours and urothelial cells via specific fibronectin and integrin receptors. Following internalisation by macropinocytosis, the mononuclear phagocyte system is stimulated by the BCG, inducing a local inflammatory response characterised by the infiltration of granulocytes, macrophages and lymphocytes. Important elements of the humoral immune response to BCG include the interleukins (ILs) IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, tumour necrosis factor alpha (TNF-a) and interferon gamma (INF-g). More recently, studies have shown that BCG contains high levels of CpG oligodeoxynucleotide motifs that are known to induce the TNF-related apoptosis-inducing ligand (TRAIL) through IFN production. Intravesical BCG is still indicated for the treatment and prevention of recurrence of some types of non-invasive bladder cancers.

Current research

As of September 2017, 58% of all clinical trials evaluating IO therapies were combination trials, 82% of which involved either another IO agent, a targeted therapy and/or a cytotoxic agent, while around 16% of combination trials involved PD-L1 antagonists and 20% CTLA-4 inhibitors. However, as of September 2019, there were 1,469 more active clinical trials evaluating PD-1/PD-L1 mAbs alone or in combination with other agents, with 76% of these active trials investigating combination therapies[40-42].

NSCLC, melanoma and non-Hodgkin's lymphoma have been at the forefront of IO research since its infancy, although, in recent years, interest in other malignancies such as renal, pancreatic and advanced (metastatic) cancer have significantly increased. However, since 2014 the average number of planned enrolments has declined from an average of 429 to 129 patients per trial, reflecting the shift in focus from major tumour types (e.g. melanoma and breast cancer) to rarer cancers with a significantly smaller eligible population.

T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibition motif domains (TIGIT) is an immune receptor present on the surface of some T-cells and natural killer (NK) cells. Similar to PD-1, it is an inhibitory checkpoint that is upregulated in multiple cancer types (e.g. melanoma, colon and renal cancer) and also plays a role in the activation and maturation of T-cells and NK cells. The associated ligand, poliovirus receptor (PVR), is highly expressed on the surface of dendritic, endothelial and some tumour cells. TIGIT plays a vital role in suppressing the antitumour immune response within the tumour microenvironment. Therefore, blockade of binding to the ligand PVR may suppress its immunosuppressive signalling and allow the co-receptor CD-226 pathway to resume its T-cell activating functions. Recent evidence suggests that activation of the STING pathway, a major innate immune pathway, is involved in the generation of spontaneous antitumour T-cell responses. STING activation within antigen-presenting cells in the tumour microenvironment leads to production of IFNb and spontaneous generation of antitumour CD8 T-cell responses. In addition, it has been observed that a deficiency in this pathway increases susceptibility to tumour progression.

Indoleamine-pyrrole 2,3-dioxygenase (IDO) is a heme-containing enzyme encoded by the IDO1 gene. With other related enzymes it catalyzes the first and rate-limiting step in the kynurenine pathway (i.e. the oxygen-dependent oxidation of L-tryptophan to N-formylkynurenine). It has been implicated in immune modulation by limiting T-cell function and engaging mechanisms of immune tolerance. Previous studies have suggested that IDO becomes activated during tumour development, helping malignant cells escape eradication by the immune system. Furthermore, IDO expression is closely linked to both CTLA-4 and PD-1/PD-L1 expression via several complex pathways.

For example, the IDO enzyme, which is an intracellular target, can be induced by the interaction of PD-1 with PD-L1 on the surface of mast cells. It is expressed by various tumour types and, in many, high IDO expression correlates with poor survival and prognosis[81]. Pre-clinical and early phase clinical trials have shown that a combination of CTLA-4 blockade with IDO inhibition can provide more effective antitumour immunity, making IDO a potential novel target for IO therapy. Current IDO inhibitors are small-molecule rather than antibody-based, and examples include indoximod, epacadostat and navoximod, which have been studied both alone and in combination.

Another experimental IO target is the glucocorticoid-induced tumour necrosis factor receptor (GITR), a surface receptor molecule involved in inhibiting the suppressive activity of T-regulatory cells and extending the survival of T-effector cells. Thus, GITR has the capacity to promote effector T-cell functions and impede T-regulator suppression. The anti-GITR antibody TRX518 was developed to target GITR and bind in an agonistic fashion. This agent reached phase I clinical trials in 2010, with safety reports published in 2019, and led to combination studies with anti-PD-1 agents in patients with advanced refractory tumours. Another study investigated the use of the anti-GITR antibody MK-4166, both as a monotherapy

and in combination with pembrolizumab. Overall the results showed that mild immune-related adverse effects(irAEs) were common, occurring in more than 20% of patients after treatment with MK-4166 in combination with pembrolizumab, with only one dose-limiting toxicity of bladder perforation in a urothelial patient-reported[43-25].

Lymphocyte activation gene 3 (LAG3), also known as CD223, is a cell surface protein expressed on activated CD8+ T-cells and other immune cells, which enhances the regulatory T-cell activity and negatively regulates cellular proliferation and activation and T-cell homeostasis. It specifically inhibits CD8+ effector T-cell functions and can enhance the suppressive activity of T-regulators. Multiple models have demonstrated that blockade of LAG3 with mAbs can augment T-cell function, although the mechanism(s) by which this occurs are poorly understood. LAG3 is often co-expressed with other inhibitory proteins, especially PD-1. Several pre-clinical studies have suggested the potentially greater therapeutic benefit of dual blockade of these receptors (LAG3 and PD-1) compared with a single agent blockade. The dual blockade approach has demonstrated promising survival benefits and durable response rates in early phase I clinical trials in small subgroups of patients with specific cancer types (e.g. RCC), although detailed knowledge of the biology of LAG3 is presently lacking.

Cost of immuno-oncology therapies

There are significant cost implications associated with IO-based therapies. For example, the one-year global cost of treating NSCLC with selected ICPis has been estimated at over US$80 billion[112]. The estimated cost per patient per year for a variety of IO agents is over £100,000, which places significant pressure on healthcare systems[115]. Costs for implementing these newer targeted therapies have escalated dramatically, and the duration of treatment has also lengthened because many cancer types are increasingly being treated as chronic rather than acute diseases. In the UK, the National Institute for Health and Care Excellence (NICE) is the organisation responsible for determining whether new treatments are cost-effective for the NHS. The cost of a new therapy is evaluated for its clinical effectiveness using a standardized measurement known as a quality-adjusted life year (QALY). In order to be deemed cost-effective for the NHS, a therapy should cost no more than £20,000–30,000 per QALY gained, or £50,000 for end-of-life therapies. New IO agents are increasingly exceeding these thresholds, resulting in rejection by NICE and reduced access for patients.

The Institute for Clinical and Economic Review, a US-based non-profit organization providing comprehensive clinical and cost-effectiveness analyses of treatments, tests, and procedures, has studied the cost-effectiveness of the three leading immunotherapies (i.e. atezolizumab, nivolumab and pembrolizumab) and concluded that each therapy would need to be discounted by 31%–68% to reach the QALY threshold. Taking this into account, NICE has stated that nivolumab cannot be recommended for routine use in the NHS with estimated QALYs of £58,791 and £78,869 versus paclitaxel and docetaxel, respectively, for treatment for urothelial cancer after cisplatin chemotherapy. NICE has also recommended that use of these agents should not be supported by the Cancer Drugs Fund (a 'back-up' government-sponsored fund allowing patients to obtain expensive cancer treatments through the NHS) because they do not have the potential to be cost effective[46-49]. Many pharmaceutical industry analysts have suggested that, moving forward, there should be a greater emphasis on the value and affordability of novel IO agents, rather than on generating larger numbers of potential candidates of similar therapeutic activity. There is no easy solution to this problem as it is difficult to curtail the enthusiasm of the biotechnology sector; however, it is evident that a longer-term more-sustainable research and development strategy for novel IO therapies is required.

Future of immunotherapy

This area appears to be moving away from the development of agents selective for a given cancer type. IO agents are now rarely approved for one particular type of cancer; instead, there is a focus on the pathways involved and the expression of specific biomarkers in tumours, regardless of their origin or location (i.e. 'tissue agnostic' therapies)[131]. This pan-cancer approach is evident with the first tumour-agnostic approval of Keytruda by the FDA, in 2017, for patients with unresectable or metastatic solid tumours based on their MSI-high and dMMR status, as opposed to the location or origin of the tumour. Merck, the company which developed Keytruda, is now seeking a second pan-cancer indication against the TMB biomarker, aiming to widen patient access still further. There has been a similar trend towards a tumour-agnostic approach in the small-molecule oncology area; for example, in the past two years, the kinase inhibitors larotrectinib and entrectinib have been granted accelerated approval by the FDA for use in patients with any solid tumour-type that has the NTRK fusion mutation.

Current Immunotherapy Modalities: an Overview

Immune checkpoint inhibitors currently represent the most promising cancer therapeutics where even monotherapies can produce durable responses in 40-50% of patients, persisting long after treatment has ceased. The main strategies are those stimulating effector mechanisms and those neutralizing immunosuppressive mechanisms. Vaccine-based oncotherapy using tumor antigen infusion enhances the innate anti-tumor ability of a patient's immune system. Additional stimulatory approaches administer genetically engineered OVs to initiate systemic immune responses, use ACT to directly deliver immune cells into patients, or apply co-stimulatory mAb's specific to members of the tumor necrosis factor receptor (TNFR) superfamily to bolster T-cell function. Immunosuppressive tumor mechanisms include checkpoint inhibitor mAb's targeting inhibitory T-cell checkpoints of PD-1 and CTLA-4, and other targeted antibodies (e.g., against CD25) that deplete inhibitory regulatory Treg cells.

Although single-agent immunotherapies, especially checkpoint inhibitors, have demonstrated promising efficacies in some patients with late-stage cancers, however, benefit in most cases was limited. In addition,

even effective treatments suffered from significant toxicity (3, 25). Checkpoint inhibitors can induce pressing "immune-related adverse effects" (irAEs) due to supra-stimulation of immunity.

This could impact upon normal adaptability of vital organs such as liver, heart, kidneys, and pancreas and give rise to type 1 diabetes, pancreatitis, arthritis, and lymphocytic myocarditis. Also, autoimmune diseases such as hypophisitis, autoimmune hepatitis, pneumonitis, and inflammatory colitis have been reported frequently with use of nivolumab and ipilimumab. Thus, risk of immune reactions of healthy organs to checkpoint inhibitors remains an understudied area, and immuno-oncologists must tread a "very fine line" between maximizing anti-tumor efficacy and triggering autoimmunity. More seriously, in a study on a mixed cohort of cancer patients, CTLA-4 or PD-1 blockade was found to induce a 2-fold *increase* in tumor development and 50% increase in tumor burden. Patients with rare, extra copies of MDM2/4 ("murine double minute 2 homolog") proto-oncogenes had the greatest risk of such "hyper-progression". In another recent study on a murine model of non-Hodgkin's lymphoma, PD-1 signaling prevented cancerous T-cell proliferation, i.e., PD-1 blockade would actually reactivate cancerous T-cells to promote their replication and hence accelerate malignant growth. All these highlight the need for profiling individual cancers and patient genomes for best treatment outcome. Overall, therefore, there are significant limitations in immunomonotherapies given also the intricate heterogeneity and stemness of human tumors. Although corticosteroids and supplementary immunosuppressive therapy can help alleviate undesirable side effects, it is synergistic "combination immunotherapy" that holds the greatest promise[50].

Combinations simultaneously targeting different components of tumor development/progression can significantly enhance efficacy, response rates, and durability relative to single-agent first- and second-generation immunotherapies. These "third-generation" novel combinations are increasingly based upon the PD-1/PD-L1 blockade "backbone," given its relatively favorable safety profile and efficacy compared to other checkpoint inhibitors. Improved immune targeting and combination therapies owe their enhanced efficacy over monotherapies to the strengthening of multiple components of T-cell anti-tumor responses. This improvement results from (i) functioning of effector T-cells inside TME, including the capacity to evade immunosuppressive checkpoints and soluble factors; (ii) effective extravasation of T-lymphocytes from lymphoid organs into TME; and (iii) production of adequate quantities of effector T-cells inside lymphoid organs.

Emerging Targets and Combination Therapies

In the following, we outline emerging targets and possible combinations with checkpoint blockers.

Second Generation Immunotherapy Targets

Many recent reviews have highlighted emerging alternative checkpoint inhibitors as targets for future monotherapies and/or inclusion in combination therapies. Whilst CTLA-4 and PD-1 checkpoint inhibitors are the crux of current clinical focus in immunotherapy, other checkpoints with potentially greater potency are emerging and promise to broaden the therapeutic "toolkit" and improve patient benefit. However, it remains essential to maintain the delicate balance between suppressive and stimulatory checkpoint modulation, using techniques such as multiplex immunoassays. VISTA, LAG-3, TIGIT, and TIM-3 immunomodulatory pathways are now well established as novel "next-generation" therapeutic targets.

Most recently, P-selectin glycoprotein ligand-1 (PSGL-1), a glycoprotein with a critical role in cell adhesion and inflammation and regulator of T-cell responses in TME, was also found to be a potential "checkpoint". Notably, ligating PSGL-1 to exhausted CD8+ T-cells inhibited T-cell receptor (TCR) signaling, decreased pro-inflammatory IL-2 and elevated PD-1 levels. Thus, PSGL-1 deficiency would reduce PD-1 expression and significantly enhance antitumor T-cell responses to melanoma. Anderson et al. postulated (i) that CTLA-4 and PD-1 could serve as "first-tier" co-target receptors responsible primarily for maintaining overall immune self-tolerance and (ii) that "second tier" receptors (TIGIT, LAG-3, and TIM-3), which have overlapping effects on NK and CD8+ T-cell effector functions, would exert more specific roles. LAG-3, TIM-3, and TIGIT are all highly expressed in dysfunctional T-cells in tumors. Synergizing their corresponding blockades would abrogate Treg cell-mediated immunosuppressive effects and enhance CD8+ and NK cell function within tumor tissues, demonstrating improved safety profiles over CTLA-4 and PD-1 inhibitors. Thus, emerging synergies of first- and second-tier blockades promise to produce stronger responses against a range of malignancies.

Costimulatory mAb's

This approach aims to generate synergies between checkpoint inhibitors and costimulatory receptor mAb's. The first signal necessary for T-cell activation is triggered when APCs present antigens to TCRs via MHCs. The second/final signaling occurs when co-stimulatory receptors on T-cells (e.g., CD28) interact with compatible APC surface proteins. Progress in this approach was initially slow, owing to the clinical failure of the CD28 super-agonist mAb TGN1412 that induced "cytokine storms" and life-threatening organ failure in 17% of patients.

This antigen is appealing given its expression on both T-cells and APCs, coupled with its ability to boost T-cell effector functions, expansion, and survival. In a murine colon adenocarcinoma model, significant synergy was reported for 4-1BB agonists plus PD-1 blockade combination resulting in total rejection of tumors. This effect involved increased levels of intra-tumor IFNγ-producing CD8+ and CD4+ T-cells, compared to monotherapies. Furthermore, the extent of irAEs was much improved and there was no overt toxicity. A further study on mice showed, however, that while 4-1BB mAb agonists alone halted progression of c-Myc-driven B-cell lymphoma in 70% of cases, combination of 4-1BB agonist with PD-1 blockade unexpectedly reduced this antitumor effect[51].

Checkpoint Blockers with Conventional Therapies

Radiotherapy results in stimulation of DNA-damage repair mechanisms and release of proinflammatory cytokines and tumor antigens. Localized radiotherapy (even sub-therapeutic dosages) can also cause significant immunostimulatory regression of distant, non-irradiated tumors, known as an "abscopal effect." The latter was exploited in a combination with checkpoint blockers (ipilimumab or pembrolizumab) against metastatic melanoma. Such coupling of checkpoint inhibitors with radiation significantly enhanced tumor CTL infiltration and elevated ORR in prostate cancer, NSCLC and glioblastoma. Furthermore, only low-moderate toxicity (~10% irAEs) was reported for combination of PD-1 or CTLA-4 blockade with radiotherapy against metastatic lung cancer. Interestingly, a triple combination of anti-CTLA-4 + anti-PD-1 + radiotherapy induced complete responses in mouse pancreatic cancer and melanoma models, not seen with dual-checkpoint blockade alone. In certain cases, however, radiotherapy + anti-CTLA-4 of patients with high tumor PD-L1 levels (type I TME) did not respond, contrary to anti-PD-1 treatment alone. Hence, future trials combining anti-PD-1 and radiotherapy could enhance ORR especially in patients possessing TMEs rich in PD-L1 expression and CD8+ lymphocyte infiltration.

Chemotherapy can also promote anti-tumor immune response by stimulating proinflammatory cytokines, reducing cytotoxic T-cell loss, and specific immunomodulatory effects (98). Examples of the latter include myeloid-derived suppresor cells (MDSCs) and Treg cell depletion by taxanes and cyclophosphamide, respectively. A phase Ib trial on advanced or metastatic NSCLC patients found that atezolizumab followed by carboplatin/nab-paclitaxel induced a response rate of 75% (*cf.* ~30% obtained with single-agent platinum doublet treatment) (NCT00527735). A primary clinical objective is to convert "cold" non-immunogenic tumors into "hot" immunogenic tumors more receptive to immunotherapy by priming T-cells already present. In this regard, chemotherapy-based immunomodulation before checkpoint blockade shows promise.

Bifunctional Agents

These include bispecific antibodies (bsAb's) and double-headed fusion proteins. bsAb's have dual specificity, binding simultaneously to two antigens, and high affinity. Bispecific T-cell engagers (BiTE's) represent an innovative format comprising two single-chain variable fragments (scFv's) joined in tandem via a flexible linker, where one antibody is specific for CD3 (a surface co-receptor on T-cells) and the other for a selected antigen on malignant target cells.

Blinatumomab, the first FDA-approved bsAb/BiTE, binds T-cell CD3 and CD19-expressing B-cell acute lymphoblastic leukemia (B-ALL), thus eliminating tumors by redirecting T-cells onto them. Blinatumomab and other BiTE antibodies aim to overcome tumor immune evasion mechanisms by directly engaging endogenous T-cells. This could prevent the need (i) to expand and reintroduce T-cells, including specific clones, *ex vivo*, and (ii) to use costimulatory molecules. Significant advantages over standard mAb's include enhanced cytotoxic potential, ability to bind weakly-expressed tumor antigens, superior protein stability and high potency in redirecting T-cells to target tumors even at low dosages (10–100 pg/ml).

Cancer stem cells (CSCs) play a significant role in tumor initiation and progression, and their eradication is critical for preventing chemoresistance and eventual disease recurrence. The single-chain BiTE Solitomab (MT110) simultaneously targets the epithelial cell adhesion molecule (EpCAM) CD326, a transmembrane glycoprotein and promising CSC biomarker, and CD3 on T-cells. In a mouse model of human pancreatic CSCs, MT110 stabilized tumor growth and small remaining tumors contained no CSCs. BiTEs are being developed for a range of hematological and solid tumors, including ALL, non-Hodgkin lymphoma (NHL), glioblastoma, melanoma, and cancers of breast and prostate. However, some side effects of cytokine release syndrome (CRS) have been reported, and benefits appeared short-lasting possibly due to the small size of BiTEs (~55 kDa)/short half-lives, requiring repeated administering every 48 h. BiTE performance is also being evaluated in combination with anti-PD-1 + anti-CTLA-4 immune blockades to enable even greater T-cell activation.

Bispecific fusion proteins (created by joining parts of two different genes) are being used to simultaneously to block PD-1/PD-L1 and growth factor/cytokine signaling. A first such protein (M7824) has recently been investigated in phase I trials against several types of advanced solid tumors and has produced promising complete or partial response rates of up to 21% (NCT02517398, ongoing). M7824 simultaneously blocks PD-L1 and TGF-β immune-inhibitory pathways to both restore and enhance host immune responses. The rationale is based upon averting the immunosuppression of effector T-cell function by PD-L1 and sequestering TGF-β (secreted by malignant cells, MDSCs, and Treg cells), hence preventing TGF-β-mediated tumor development and metastasis.

Epigenetic Modulators

Here, a checkpoint inhibitor is combined with an epigenetic modulator, such as an inhibitor of histone deacetylases (HDAC) or DNA methyltransferase (DNMT). This is viable since HDAC is commonly overexpressed in tumors and its inhibition downregulates the expansion of MDSCs that normally accompanies and promotes the cancer process. Additionally, most epigenetic drugs demonstrate only minor toxicity at clinical dosages. A major study focused on complementing the high-efficacy/short-term effects of targeted inhibitors with the low response rate/durable efficacies of single-agent immunotherapies. Mouse carcinoma models were used to examine the efficacy of ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) synergized with 5-azacytidine (DNMT inhibitor) and entinostat (HDAC inhibitor).

Checkpoint Blockers with Targeted Therapies

In this combination, checkpoint inhibitors are coupled with a modulator of growth factor signaling, mainly an inhibitor

of protein kinase or phosphatase. In particular, receptor and non-receptor tyrosine kinases play a significant role in tumorigenesis as well as in immunogenicity and cytotoxicity. Consequently, their inhibitors (TKIs) would offer natural synergy with checkpoint blockers. The angiogenesis-inducing growth factor, VEGF, restricts T-cell infiltration across the tumor endothelium and amplifies MDSCs and Treg cells within tumors. Against metastatic melanoma, combination of bevacizumab (a VEGF inhibitor) with ipilimumab induced a disease-control rate (DCR) of 67% and promoted T-lymphocyte infiltration of tumors with favorable tolerance. Combinations are now being sought that might synergize anti-PD-1/PD-L1 mAb's with VEGF blockade for even greater efficacy.

In a mouse model of gastrointestinal stromal tumor (GIST), imatinib (a broad-spectrum TKI) was combined with an anti-CTLA-4 mAb to block T-cell immunosuppression mediated by indoleamine 2,3-dioxygenase (IDO). Synergistic activity was reported that reduced Treg cell population and enhanced tumor infiltration by CD8+ T-cells. Thus, CTLA-4 and IDO blockade combination significantly decreased tumor volume by 50% after 80 days, while during CTLA-4 blockade and imatinib administration alone tumors expanded by 40–60% over a similar period. Colony-stimulating factor 1 (CSF1/M-CSF) also contributes to resistance of melanoma to PD-1 blockade. Activated CD8+ T-cells, upon releasing IFN-γ and TNF-α into the TME, experience a "vicious cycle" whereby these immunosuppressive cytokines trigger melanoma to adaptively secrete CSF1.

IKKβ (IkB-Kinase β) represents a major component of the NF-kB signaling pathway, responsible for mediating T-cell development and activation. Mature Treg cells avert autoimmunity yet limit antitumor immune responses via CTL inhibition, and are heavily reliant upon NF-kB signaling for their development. Consequently, in melanoma murine models, IKKβ inhibition with KINK-1 (Kinase Inhibitor of NF-kB-1) reduced circulating Treg cells by ~50% with no change in CTL levels (125). The latter is due to CTLs being less reliant upon IKKβ for proliferation and survival than Treg cells. Thus, combining Treg-nullifying IKKβ inhibitors with other immunoactive pharmacological agents could bolster therapeutic efficacy.

Cyclin-dependent kinases 4 and 6 (CDK4/6) are core cell-cycle components, essential to initiation and development of breast cancer and T-ALL. CDK4/6 inhibitors (CDK4/6i) showed effectiveness against glioblastoma, breast cancer and melanoma by arresting tumor cell cycle at G1, via inhibition of retinoblastoma tumor suppressor phosphorylation. CDK4/6i can also induce antitumor immunity by overcoming two tumor immunoevasion mechanisms via (i) presenting tumor surface antigens with enhanced efficiency and (ii) inhibiting immunosuppressive Treg cell proliferation. Indeed, in murine breast cancer models, abemaciclib (CDK4/6i) + anti-PDL1 reduced tumor volume by 70% after ~2 weeks (stable up to 35 days) while abemaciclib or anti-PDL1 monotherapy was effective only temporarily. In colorectal CT-26 mice models also, this combination produced prolonged 100% regression, accompanied by resistance to further disease inducation[52].

Potential, Novel Immunotherapy Co-targets

In this section, we highlight a number of less widely recognized, emerging mechanisms that could potentially serve as co-targets in combination immunotherapy.

3. Metabolic Components

A number of metabolic mechanisms have been shown to be essential for immune evasion of tumors and could serve as co-targets in immunotherapy. Tumors demand an expansive, adaptable metabolic framework to thrive in specific niches, and all contemporary cancer hallmarks require metabolic engagement to some degree. Recent evidence suggests that tumors may perpetuate their survival by reprogramming host metabolism. In patients with both anorexia and tumors, the increased metabolic stress causes elevation in systemic glucocorticoid hormones that alone can significantly decrease antitumor T-cell immune response, cause tumor growth and self-perpetuate the cycle. Novel combination approaches should therefore aim to normalize metabolic stress in parallel with checkpoint blockade to optimize clinical outcome. Notable metabolic targets of therapeutic interest include the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), Notch homolog 1 (NOTCH1), and cyclooxygenase-2 (COX2).

Migration of immunosuppressive Treg cells to inflamed malignant tissues relies upon glucokinase-mediated glycolysis. Glycolysis is initiated by glucokinase (GCK), itself induced via the P13K-mTORC2 signaling pathway. Treg cells lacking components of this pathway remain immunosuppressive. Patients possessing a polymorphism causing elevated GCK activity saw enhanced Treg cell motility, given that GCK promotes cytoskeletal restructuring via actin association. Consequently, there exists potential for inhibition of glycolytic enzymes to manipulate the migration capacity of T-cell subsets, and thus to "soften" the immunosuppressive role of the TME.

Aerobic glycolysis, characteristic of growing tumors, fuels optimal T-cell effector function. In highly antigenic regressive tumors, competition for glucose in TME was found to be sufficient alone to drive cancer progression (159). This would occur as tumors surpass T-cells for glucose, directly sub-optimizing T-cell function by impeding their IFNγ production, critical for anti-tumor activity. Combination strategies that couple the depletion of tumorigenic immune cells with glycolysis enhancement in infiltrating T-cells, therefore, may prove effective at metabolic remodeling of the TME. This could also explain why combined anti-CTLA-4 + anti-PD-1 checkpoint blockade is particularly effective since anti-CTLA-4 would deplete Tregs whilst anti-PD-1 would directly dampen tumor glycolysis by inhibiting the mTOR pathway.

Drugs targeting tumor metabolism are in early trials. COX2 is essential for the production of the tumor-sustaining

mediator prostaglandin E2 (PGE_2), a prostanoid lipid that enhances cancer survival, metastasis, and immunosuppression.

Exosomes

Exosomes are specialized, nano-sized lipid bilayer vesicles that enable a novel means for intercellular communication, shuttling bioactive DNA, mRNA, miRNA, and oncogenic proteins between cells, thereby enabling genetic reprogramming of cellular networks. Various stages of the cancer process involve exosomal interactions. Thus, exosomes transmit messages from tumor cells to both stromal and immune cells, facilitating immune evasion, and establishment of the tumor niche. Exosomes may be therapeutically exploited via three approaches, as follows:

i. *Direct exosome-based immunotherapy*. This is exemplified by "dexosomes" (dendritic cell-derived exosomes) loaded with whole antigen or peptide fragments, and have proven ability to induce systemic T-cell responses. Immunostimulatory dexosomes are especially promising, stimulating antitumor responses with greater accuracy than possible using non-cellular approaches, and possessing higher biostability and bioavailability as well as cost-effectiveness compared with other cellular therapies. Treatment of human breast cancer with dexosomes resulted in incorporation into tumors and subsequent expression of dexosome immunostimulatory molecules (e.g., CD86, CD81, MHCI/II + tumor antigen) on tumor cell surfaces, thus boosting tumor immunogenicity and T-cell engagement. Dexosome-treated tumors indeed contained a much higher proportion of T-cells secreting IFN-γ immunostimulatory cytokines. However, early clinical trials on colorectal and NSCLC have yielded only moderate efficacies.

ii. *Exosome elimination in patients with advanced cancer*. This represents a new treatment concept, demonstrated for the blood-pressure dampening drug, amiloride. This decreased MDSC immunosuppressor functions in colorectal cancer patients by inhibiting exosome formation.

iii. *Exosomes as "natural nanoparticle" drug delivery vehicles*. As such, exosomes exhibit favorable biocompatibility and biodistribution. Indeed, use of macrophage-derived exosomes to transport paclitaxel into multidrug resistant (MDR) tumors enhanced treatment efficacy by 50-fold relative to paclitaxel administration without exosomes.

Dividing T-Lymphocytes

The asymmetrical division of T-cells observed in murine models represents a novel opportunity as an unconventional immunotherapy target. When a "mother" T-lymphocyte naive to immune stimulation undergoes mitosis, mTORC1 (an enzyme responsible for protein synthesis) is divided unevenly between the two daughter cells. The progeny with the higher mTORC1 becomes strongly activated as a potent killer T-cell whilst the "sister" cell displays behavioral traits more associated with memory T-cells.

This raises the possibility of exploiting mTORC1-expressing T-cells as a target for long-term potentiation of immunotherapy, by skewing development toward memory T-cells. Proteasome-activators such as Cyclosporine were found to tip the balance of dividing $CD8^+$ T-cell progeny toward memory T-cells (exploiting the fact that effector and memory T-cells have differing proteasome activity levels).Thus, there exists potential for synergizing immunotherapy with proteasome modulators.

Ion Channels

A variety of ion channels, including voltage- and ligand-gated ion channels, are expressed in cells of the immune system and make significant, dynamic contributions to immune functioning. Here, we highlight voltage-gated sodium channels (VGSCs/Nav's), voltage-gated potassium channels (VGPCs/Kv's), and calcium-activated potassium channels (K_{Ca}'s).

VGSCs may manifest themselves in immuno-oncology and serve immunotherapy in several different ways. First, Nav1.5 was shown to control the positive selection of CD4+ T-cells from CD4+/CD8+ thymocytes in response to stimulation by APCs. The selected cells would play a central role in immune functioning via production of cytokines and chemokines, facilitating antibody production by B-cells, maintaining immunological memory and priming CD8+ CTLs. Consequently, VGSC blockers could reduce the CD4+:CD8+ ratio, thus boosting CD8+ CTL populations that drive early immunosurveillance antitumor responses. Furthermore, high CD8+ TIL content of tumors is predictive of pathological complete response to primary systemic therapy regardless of cancer subtype. However, as a monotherapy, VGSC blockers may yield only short-term success since depleted CD4+ T-cells would ultimately reduce immunological memory and compromise CTL tumor re-challenge. Accordingly, tumor vaccine delivery after VGSC inhibition as a sequential "one-two punch" could activate new thymic CD4+ helper T-cells to restore lost immunological memory and sustain efficacious CTL antitumor responses. Additionally, VGSC blockers would increase tumor "hotness" by enhancing CTL presence, and thus synergize with PD-1 blockade. Second, functional VGSC expression occurs in macrophages, another cell type in the innate immune system. When recruited to tumors, macrophages can accelerate cancer progression.

A recent study by Roh-Johnson et al. on zebrafish and mouse models of melanoma showed that recruited macrophages transferred their cytoplasm into melanoma cells and this promoted metastasis. How such intracellular communication is regulated and the nature of the transferred molecules were not known. Interestingly, however, VGSC activity drives macrophage motility. Accordingly, VGSC blockade could eliminate this component of immune response and could form the basis of mono- and/or combination immunotherapy with tumor vaccines or with PD-1 blockade to dampen TME immunosuppression, overcome PD-1 resistance and enhance patient responses (149). Third, the predominant

VGSC in cancers of breast and colon is the neonatal splice variant of Nav1.5 (nNav1.5).

The role of Kv1.3 channels in the immune responses to tumors may be more complex, dependant dynamically on disease stage. On the one hand, tumor infiltration may involve downregulation of the channel. On the other hand, Kv1.3 (and $K_{Ca}3.1$) channels are expressed predominantly in CD8+ T-cells and contribute to membrane electrogenesis and calcium influx, crucial to their antitumor granzyme B and cytokine production. Kv1.3 activity also promotes T-cell proliferation and high-level expression of Kv1.3 correlated with elevated levels of Ki-67. Finally, a novel novel role for Kv1.3 has been proposed in TME where cell death within a necrotic region can release cellular K^+ into the extracellular spaces. Exposure of T-cells to such high K^+ can suppress their activation and functioning by increasing intracellular K^+ and inhibiting PP2A-dependent/TCR-activated Akt-mTOR signaling. Accordingly overexpression of Kv1.3 restored antitumor T-cell functionality by facilitating efflux of the high intracellular K^+, leading to enhanced survival of tumor-bearing mice. Overall, therefore, Kv1.3 expression in T-cells can promote the immune reaction to tumors once the cells enter TME. Calcium-activated potassium $K_{Ca}3.1$ channels are upregulated in activated T-cells and also play a significant role in regulating cellular migration and proliferation. Upon activation by tumor cells, adherent NK (A-NK) cells preferentially up-regulated $K_{Ca}3.1$ channels. Blocking $K_{Ca}3.1$ activity with TRAM-34 increased the degranulation and cytotoxicity of A-NK cells, and induced increased ability of A-NK cells to reduce tumor growth *in vivo*. Taken together, these results rationalize the co-targeting of $K_{Ca}3.1$ and PD-1 on NK cells in future cancer immunotherapy. NK cells suppress metastasis by inducing degranulation-mediated tumor cell lysis via release of perforins and cytotoxic granzymes. $K_{Ca}3.1$ blockers TRAM34 and NS6180 increased NK cell proliferation and enhanced degranulation rate of the non-adherent K562 erythroleukemia cells *in vitro*. On the other hand, Kv1.3 blockers Stichodactyla toxin (ShK) and 5-(4-phenooxybutoxy) psoralen (PAP1) decreased proliferation and degranulation, consistent with Kv1.3 being essential to NK-induced cytotoxicity.

4. Conclusion

Cancer immunotherapy has dramatically changed the survival and quality of life for patients. Despite the rapid advances made in the field, immuno-oncology is still in its relative infancy, with numerous challenges and hurdles yet to be overcome. Over time, a realization grew that the standard tools used to assess choice of treatments in the era of chemotherapy and targeted therapies might not be valid for the new immunotherapies. The T cells are currently widely recognized as the key mediators of antitumour efficacy with immune therapy treatment suggests that use of the Immunoscore is an attractive option to help guide treatment selection in other cancer types as well. Still, that option does not exclude the possible use of additional parameters that might provide further insights into the specifics of each case[53]. At present, combination therapy is still in the exploratory stage, with the efficacy and superposition of toxicity of immunotherapy studies still requiring confirmation in subsequent experimental studies; however, overall, combination therapy may benefit numerous patients, and merits extensive further investigation. With the continuous in-depth exploration of the mechanisms of resistance, immunotherapy may be applied to the treatment of a wider range of cancers. The development of a variety of high-tech technologies, effective biomarkers should be explored to screen patients based on different tumor characteristics and different microenvironment phenotypes. The future research directions of cancer treatment modes are multidisciplinary (such as surgery, internal medicine, radiotherapy) and the combination of multiple drugs, which can develop the best individualized treatment plan according to the condition of each patient. Coping with these challenges requires the joint efforts of clinicians and scientists performing basic research, and the focusing of resources to accelerate the understanding of the complex interactions between cancer and immunity with the aim of developing improved treatment options for cancer patients and promoting the advancement of novel cancer immunotherapies.

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