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Targeting Immune Checkpoints with Therapeutic Monoclonal Antibodies: Mechanisms and Therapeutic Advances

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ABSTRACT

Targeting immune checkpoints including PD-1, PD-L1, and CTLA-4 with monoclonal antibodies have transformed cancer immunotherapy by altering T-cell signaling pathway. This review examines the molecular mechanisms underlying immune checkpoint blockade and discusses the current landscape of FDA-approved therapies, ongoing clinical trials, and next-generation checkpoint targets. Emphasis is placed on resistance mechanisms, biomarker development for patient selection, and combinatorial strategies to overcome therapeutic limitations. Furthermore, this study explores the potential of antibody engineering in future such as bispecific antibodies and Fc optimization. Merging the mechanistic insights with advancements in clinical and future thinking, we address the issue of personalized medicinal approach, and immune related adverse reaction.

Keywords: Cancer Treatment, CTLA-4, Immune Checkpoints, Immune Evasion, Immunotherapy, Monoclonal Antibodies, PD-1

1 Introduction

ANTIGENS can be divided into two categories, which are exogenous antigens and endogenous, they could come from any source that enter into the organism from outside. The non-self-proteins such as proteins, polysaccharides, nucleic acid and lipids are recognized as exogenous antigens, while self-proteins are endogenous antigens [1]. Immunity is defined as the combined recognition of anti-pollutants and the ability to neutralize them, mainly by the activation of lymphocytes and the production of antibodies by B lymphocytes. Tumour antigens can be divided into two categories, which are tumour-specific antigen (TSA) and tumour-associated antigens (TAA). Mutations in cancer cells can lead to non-synonymous mutations in the products of some genes and resulting in production of novel polypeptides that differ in amino acid sequence from that of the corresponding protein in normal cells. Antigens recognized by T lymphocytes are 8–10-mer peptides bound to MHC classes I or II molecules that are derived from ingested proteins [2].

Tumour cells express mutated peptides that combine with high avidity to MHC class I, and they are usually referred to as neo-antigens or novel antigens. Vaccination with such neo-antigens can elicit anti-tumour immunity

and regress tumour. Mutated target sequences have been identified in various tumour types, such as lung-adenocarcinoma, melanoma, and breast carcinoma. Tumour-associated antigens received more attention for immunotherapy long before TSA was discovered. These antigens are proteins associated with tumour cells and detectable in normal tissues [3]. Some tumour-associated antigens show restricted expression to tumour cells and may be available for T-cell recognition in the absence of self-tolerance, such as alpha-feto protein, keratin 19 and carbohydrate antigen 199. Some tumour-associated antigens were also called non-mutated tumour-associated antigens (TAA). Although they are expressed at much lower levels in normal tissues, they can still induce tolerance in healthy individuals, represented by 1 TSHR, MUC1. These antigens are usually associated with and recognized by the humoral immunity [4].

2 Overview of Immune Checkpoints

The lymphocyte signaling pathways are controlled by immunoregulatory receptors, known as immune checkpoints, which affect immune homeostasis. Under normal conditions, the immune checkpoints can maintain immune self-tolerance to avoid potential damage to self-tissues. Under pathological conditions, such as tumour microenvironments or chronic viral infections, the immune checkpoints are overactivated and form negative regulatory signals toward antigen-specific T cells, resulting in immunosuppression. Immune checkpoints are composited of costimulatory molecules and inhibitory receptors on T cells. Other immune checkpoints may also be expressed on non-T cells [5].

The primary immune checkpoints already attracting interest include the costimulatory molecule pathway and the inhibitory receptor pathway. The new immune checkpoints of clinical interest include new costimulatory molecules and new inhibitory checkpoints. Monoclonal antibodies that target the immune checkpoints have been either approved for clinical use or entered clinical trials; monoclonal antibodies targeting tumour-associated antigens have been developed but discovered less affection because of the lack of broad expression [6].

2.1 Definition and Importance

Immune checkpoint inhibitors (ICIs) antibodies are a promising new class of monoclonal antibodies that modulate T-cell signaling pathways. These ICIs antibodies can reinvigorate end-stage effector T cells and stimulate robust anti-tumour immune responses. The first clinically utilized immune checkpoint was the T-cell membrane protein CTLA-4. Monoclonal antibodies that block CTLA-4 have been shown to be efficacious for metastatic melanoma. Subsequent investigation of the PD-1/PD-L1 immune checkpoint has yielded monoclonal antibodies that block PD-1 or PD-L1. ICI has been associated with unprecedented clinical outcomes that are altering the way oncologists treat a variety of malignancies [7].

The increasing array of immunotherapies is moving beyond immune checkpoints towards mechanisms that broadly target the regulation of the innate or adaptive immune systems. Numerous combination approaches are being developed, including those that combine immune checkpoint and other immunomodulatory agents [8]. Novel population strategies are also being pursued to optimize the delivery of these interventions. Each of these developments will require additional research enabling an understanding of the mechanism of action and the interactions with the immune system. Understanding the biology of immune checkpoint targets can aid the design of combination strategies and identify the patient population best suited to treatment. Combination ICI is a field of active investigation but in future, it will also entail a retreat from large combinations of agents after promising Phase I results toward combinations given with a focused rationale. Identifying new targets will involve preclinical searches for novel monoclonal antibodies supportive of the current mechanism of action or discovery of adjunctive targets such as IDO [9].

2.2 Key Immune Checkpoint Proteins

Programmed death 1 (PD-1), programmed death ligand 1 (PD-L1), and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) are piovital immune checkpoint protein that govern immune regulation and uphold self-tolerance [10]. The activated T cells proliferate and migrate to the tumour microenvironment, where they recognize neoantigens and secrete perforin and granzyme to kill the tumour cells. Immune checkpoint proteins are important physiological players that enhance the safety of the immune system by regulating the duration and limits of immune responses. The inhibitory functions of immune checkpoint receptors are finely regulated processes by modulating protein levels, receptor-ligand interactions, and signal transduction. Cancer cells often evade recognition by cytotoxic T cells through the overexpression of immune checkpoint ligands, resulting in the delivery of inhibitory signals to T cells. New immuno-oncological therapeutics designed to blockade these interactions have shown promising outcomes in the clinic, leading to durable responses in patients with advanced cancers [11, 12].

3 Monoclonal Antibodies: An Introduction

Monoclonal antibodies (mAbs) are engineered immunoglobulin G (IgG) proteins that bind to a specific epitope on an antigensubstrate. Variable domains of heavy and light chains of the antibodies are grafted onto HuMAb for displaying desired specificity; and in parallel, an IgG molecule with constant heavy and light chains is engineered by joining HuIgG1 with non-overlapping proteases enzymes cleavage sites at the hinge. Both the

constructs are expressed in mammalian cells for generating Fab&Fc-HuIgG [13]. Isolation of mAbs with desired specificity from cell supernatants is carried by sandwich ELISA and validated by functional assays. A thorough sequence, affinity, avidity, and molecular characterization of these mAbs is carried out, followed by characterization of effector functions of IgG/CD3 mAbs. The mAbs are characterized for the terminal lysine (K) and asparagine (N), oxidation of methionine, glycosylation pattern, and aggregation or fragmentation. Specificity of mAbs for targets is validated by the epitope mapping the interaction sites of mAb and antigens. After establishment of mAbs molecular and biological characterization, the therapeutic mAbs are formulated in the desired buffer and characterized by pH change and concentration variation [14].

Early trials with mAbs infrequently showed any effect on therapeutic efficacy. Such failures prompted extensive analytic characterization of mAbs and led to the 'biologics license application' for the agonist CD40 clone CP-870,893, and CTLA-4 blocking mAb. Anti-CTLA-4 mAb was observed to enhance survival in patients with melanoma [15]. Subsequent mAb based clinical successes in oncology setting transmitted promise for generalization to all malignancies. Ultimately available anti-cancer mAbs, largely against checkpoint inhibitors, and agonists of immune co-stimulatory receptors are addressed here. These increasing successes for mAbs in cancer therapy orphaned niches like malignancies resistant to immune-checkpoint blockade, and identification of appropriate re-engagement strategies became the priority. Such alternative immune evasive mechanisms beyond conventional immune-checkpoint blockade are summarized [16].

3.1 History and Development

More than 50 years ago, it was demonstrated that prey animals could reject transplanted tumours due to prior exposure to the same tumour's allogenic cells. These initial studies prompted numerous investigations into other transplantable tumours of unfortunate murine origin. Over time, it was shown that the immune system could reject transplantable tumours in syngenic hosts, and adoptively transferred lymphocytes could confer antitumor immunity. However, it soon became apparent that solid tumours more often escaped immune surveillance than did their hematopoietic counterparts. While many breast and prostate tumour cell lines could be immunogenic when administered subcutaneously with adjuvant, T-cell mediated rejection was rarely observed with established solid tumours. A great deal of effort went into developing vaccines or ex vivo manipulation of tumour antigen presenting cells to stimulate a patient's dendritic cells. Data supporting the use of these modalities gave birth to companies with unrivaled biotechnology, budgets, and best intentions but uniformly disappointing clinical results. Just as Fernando C. De La Garza, at aged 77, was investigated for the use of an "autologous, cell lysate-based" vaccine to treat a virally-induced glioblastoma multiforme, a paradigm shift occurred. Ipilimumab, an antibody that targets CTLA-4, was approved for the treatment of late-stage melanoma. How an antibody blocked T-cell co-inhibition and promoted durable antitumor immunity, was not understood prior to its approval, but has become an area of intensive investigation [17, 18].

Accidental observations that Gleevec Ř (Trade name of Imatinib) inhibited specific mutations that drove Bcr-Abl activated signaling cascades led to its approval a few years later. Similarly, the observation that combined blockade of PD-1 and CTLA-4 produced unprecedented tumour regressions and perpetual survivals in melanoma patients led to the use of combination immune checkpoint inhibitors. Combination therapies targeting tumour angiogenesis, mutated peroxidase, and dendritic cells have been FDA approved, as have conjugated chemotherapies, but these data have also not always translated to other tumour types [19].

3.2 Types of Monoclonal Antibodies

Monoclonal antibodies (mAbs) are widely used for therapeutic and diagnostic purposes. Originally regarded as mere passive delivery vehicles, mAbs are presently recognized as active agents capable of eliciting various immune-mediated effects. They may exert their activity via different mechanisms, including the blockade of bioactive molecule-receptor interactions, interference with receptor clustering, activation of effector immune cells following in situ candidate protein recognition, and the induction of apoptosis through Fc - receptor interactions. Broadly, monoclonal antibodies are divided into three classes based on their anatomical, biochemical, and physical properties: IgA, IgM, IgD, IgE, and IgG. However, only one format, the IgG class, has been developed for therapeutic applications [20].

The IgG subclass to which an mAb belongs influences several of its functional and pharmacological properties. In humans, the four IgG subclasses, IgG1, IgG2, IgG3, and IgG4, share a common structure characterized by a monomeric polypeptide chain composed of two identical light and two identical heavy chains. Interestingly, heavy chains comprise two constant and one variable domain and vary between subclasses. IgG1 and IgG3 contain a flexible hinge region connecting their Fab and Fc moieties, allowing greater mobility of the antigen-binding sites. This structural feature is associated with a more complete engagement of the effector immune cells, yielding a more potent antibody-dependent cell-mediated cytotoxicity (ADCC). Of note, IgG3 is the most efficient in terms of elicit antibody-dependent cellular cytotoxicity (ADCC). IgG4 possesses a

cysteine in position 241 of the heavy chain which is associated with a unique serological behavior. mAbs that have been produced in either murine or humanized formats can have the potential of inducing human anti-mouse antibodies (HAMAs) or human anti-human antibodies (HAHAs) that may reduce their efficacy or lead to life-threatening severe anaphylactic reactions. Thus, off-the-shelf mAbs of mouse origin are normally used in preclinical studies, while fully human mAbs are employed in early phase I trials in oncology [21].

4 Mechanisms of Action

The induction of an anti-tumour immune response requires an exquisitely coordinated process involving the recognition of new mutations within a cancer and the processing and presentation of the associated tumour-associated antigens by professional antigen presenting cells, such as dendritic cells, to T cells with the appropriate T-cell receptors in draining lymph nodes. In order for a productive T-cell response to occur, signals in addition to T-cell receptor signaling are needed. These signals can be provided by the engagement of co-stimulatory receptors on the T cell expressed by the professional antigen presenting cells and licensed by a relevant innate immune response to directly recognize the underlying mutations. A plethora of co-inhibitory receptors have been discovered on T cells in recent years that down-modulate T-cell activation/proliferation, including CTLA4, PD-1, and TIM-3. Their continued engagement post-activation disrupts ongoing T-cell responses. Immune checkpoint blockade can be achieved at multiple levels including the receptor–ligand interaction (by mAbs) or downstream signaling pathways (by small molecules) to re-establish an active anti-tumour immune response and restore T-cell function [22].

Just as T-cell co-stimulation is an important aspect of a productive immune response, T-cell co-inhibition is equally as important and has traditionally fallen within the domain of tolerance induction to prevent inappropriate immune activation. There are several strategies under development that attempt to overcome the developmental, 'hard-wiring' account of unresponsive lymphocyte populations. Many of these strategies involve the direct re-engagement of a co-inhibitory receptor by mAbs to block inhibitory receptor/ligand interactions. A mAb to CTLA4 was among the earliest anti-checkpoint agents to enter the clinic. This mAb requires engagement with the CD80/CD86 ligands on dendritic cells and, by preventing it from binding CD80, enhances T-cell activation in vivo and demonstrates single agent clinical activity in advanced melanoma and other malignancies [23].

In particular, this strategy has been investigated in the context of tumours which are refractory to current regimens, such as the dB/DT combination in the BRAF mutant melanoma model. The ability of the mechanism of action outcome to influence this issue has also been addressed: blocking PD-1 did not prevent dB/DT-induced reduction in tumour growth, whereas CTLA4 blockade accelerated the rate of regression. Bioengineering mAbs to prevent FcR-mediated phagocytosis is another relatively simple, but potentially powerful, methodological technique to develop depleting agents in a manner that ensures a sustained biological response [24].

4.1 Inhibition of Immune Suppression

Monoclonal antibodies that target co-inhibitory immune checkpoint molecules are either in development or are currently being used to treat a variety of cancers. T cell and dendritic cell suppression is one of the primary mechanisms of action of these antibodies, which also include CTLA-4, PD-1, and PD-L1-targeting medications. Activation of antibody-dependent cellular cytotoxicity, enhanced dendritic cell maturation and priming, and increased T cell proliferation are additional mechanisms of action. Most studies to date examining the potential mechanism(s) of action of these agents have been done using murine models, so it is still been determined whether these mechanisms operate in similar ways on the human immune system (Figure 1) [25].

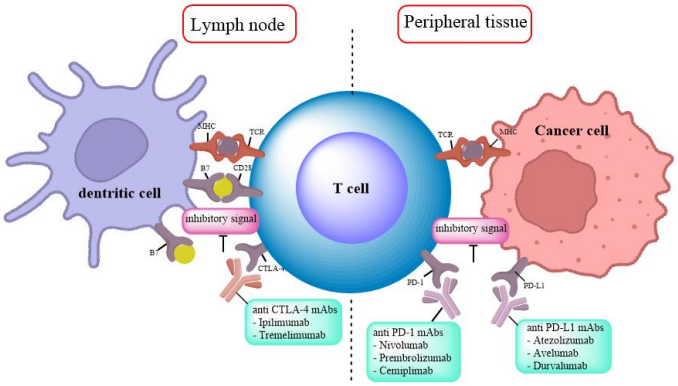

Fig. 1. Mechanisms of action of monoclonal antibodies (immune checkpoints inhibitors) in cancer.

CTLA-4 blockade using ipilimumab enhances the proliferation of CD4 and CD8 T cells in lymphoid tissue and whole blood. Unlike anti-CD28, which delivers a non-specific costimulatory signal enhancing all T-cell activity, CTLA-4 blockade enhances stimulation via the TCR and results in T-cell up-regulation of the Th1 cytokines and co-stimulatory molecules in a specific way, but fully inhibits T-cell responses to general mitogens in vitro. This would be consistent with CTLA-4 blockade acting primarily in lymphoid tissues where T-cell priming occurs [26].

Repeated doses of anti-CTLA-4 in melanoma patients have been shown to result in production of circulating effector memory T cells and a Th1-associated gene signature. In one patient with early-mixed response and

then progressive disease, the inscriptions returned to baseline before switching to chemotherapy. The T-cell response was indolent, taking more than 6 months after starting treatment to demonstrate a clinical response. Clonal populations of T cells expressing CD4 or CD8 have been identified longitudinally in the blood, suggesting that they move from the lymph nodes where priming is thought to occur and into the periphery where the anti-tumour effect is seen. Absence of the same clone in the baseline blood samples attests to the selectivity of the population [27].

4.2 Enhancement of T-Cell Activation

Anti-tumour T-cell activation needs dendritic cells (DCs) to capture and present tumour-derived antigens, and additional stimulation of co-stimulatory and activation pathways is important to control tumour growth. CTLA-4 inhibitors have been proven to reinvigorate immune responses that have gone cold in the tumour microenvironment via competition blockade with CD28 for binding to CD80/CD86 on DCs, and an eventual increase of T-cell activation. Modifying the Fc region of antibodies to mount IgG2a isotype to trigger Ab-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) in cross-talk with macrophages has improved anti-tumour response for CTLA-4 and PD-L1 checkpoint antibodies. Similarly, blockade of PD-L1 immune checkpoint receptor boosted CD28/B7 blockade to enhance antibody and checkpoint efficacy. However, it is still largely unclear how the sophisticated design of effector functional antibodies modulates immune responses or whether they play synergistic roles with co-stimulatory pathway agonists in the same setting. PD-1 monoclonal antibody (mAb) displaying monovalent binding to PD-1 utilizing a unique scaffold design that enables IgG-like properties, including long half-life and high serum stability, has emerged as an alternative to tumour-targeting agents. Co-administration of PD-1 mAb significantly boosted effector function of antibody treatment and completely eradicated tumour growth in many mouse models [28]. The 4-1BB pathway agonist IgG2a, which selectively depletes Tregs, has recently validated as second-line treatment combination with checkpoint blockade therapy to improve clinical efficacy in refractory patients. Nanoparticle platform boosting efficacy of checkpoint blockade therapy or anti-tumour vaccine therapy by concurrent modulation of T-cell activation and exhaustion thresholds has also emerged as a platform for combination cancer immunotherapy. Co-administration of complementary concerted ablation and co-stimulation pathways monoclonal antibodies targeting CD8+ T-cells, bloc binding of PD-L1 to reinvigorating the effector function of tumour-infiltrating T-cells, whereas release of 4-1BB on the surface of APCs at the tumour microenvironment to enhance cross-presentation of IDO1 protein which transforms tryptophan to catabolites. There is also some need for the combination platform to enhance anti-tumour efficacy by augmenting T-cell recruitment to the tumour. The use of inclusions to target CTLA-4 and B7-H3 checkpoint pathways and tumour vasculature markers has been proven to augment T-cell recruitment within tumours and infiltration of high-grade tumours [29].

4.3 Impact on Tumour Microenvironment

The tumour microenvironment (TME) is a complex milieu composed of tumour cells, endothelial cells, cancer-associated fibroblasts, inflammatory cells, and the extracellular matrix. Stromal cells such as fibroblast, Merokaryocyte, and myofibroblast and immune cells in the TME can help or inhibit the growth of HCC tumour 1. Senescent fibroblasts in the HCC microenvironment secrete TGF to induce immunotolerance. Myofibroblast and upon parasitic construction inhibit cytotoxicity of immune cells. Macrophages take action in HCC development through the promotion of tumour cell proliferation and angiogenesis. T cell recruitment, activation, proliferation, etc. are impaired by macrophage-derived PD1 to promote immune checkpoint pathways and tumour growth. It has been reported that PD-L1 may also be expressed in the tumour microenvironment including endothelial cells and cancer-associated fibroblast rather than in immune cells to inhibit T cell proliferation. Anti-CTLA-4 antibodies were among the first to be tested in the clinic. Preclinical studies showed that CTLA-4 blockade could enhance antibody-dependent cellular cytotoxicity (ADCC) by increasing retained granzyme B in T cells with multiple mechanisms. Antibody interference or blockade of these pathways is a common approach to restore anti-tumour immunity, and many such agents are now in clinical trials [30, 31]. Numerous recent studies have shown potential cooperative or synergistic immunological biology for further investigation including the inhibition of Treg, increasing PD-L1 expression on tumours cells, mediating immunogenic tumour cell death etc. Strategies have also been described for optimizing anti-PD-L1 therapy combinations through direct inhibition of immune checkpoints and recruitment and activation of immune effector cells. Clinical responses and safety data suggest that immune checkpoint inhibitors may have potential for improving outcomes for patients as therapies are moved into combination strategies [32, 33].

5 Clinical Applications

Monoclonal antibodies that antagonize immune checkpoints have produced much excitement in cancer therapy. Early monotherapy results from checkpoint inhibitors that target CTLA-4 and PD-1 followed the introduction of ipilimumab and pembrolizumab or nivolumab, respectively, and provided proof of principal that enhancing anti-tumour immunity could translate into

long-term survival for a small subset of patients. Further preclinical and clinical studies demonstrated the presence of PD-L1 expressing tumours across a range of indications whose clinical benefit from anti-PD-(L)1 therapy was greater than the unselected population. Subsequent studies demonstrated the relative advantage from anti-PD-(L)1 monotherapy for tumours with high burden of mutational neoepitopes that could elicit a more robust anti-tumour T cell response and predictive biomarker for potential therapeutic benefit. As a result, nivolumab and pembrolizumab received widespread approval in the USA and Europe in monotherapy for previously treated advanced melanoma, NSCLC, and with varying data for consideration in a broader spectrum of cancers and settings. The relative freedom from toxicity seen with the best checkpoint agents has resulted in significant interest for their combination therapy [34-36].

Combination strategies have been based on the principle that the blockade of different negative immune regulatory pathways would produce enhanced anti-tumour responses. Preclinical efficacy data have demonstrated that more than one form of checkpoint blockade is required before significant regression of poorly immunogenic tumours. This data combined with the alternative mechanism of actions and different toxicity profiles of ipilimumab and anti-PD-(L)1 agents led to the assessment of anti-CTLA-4 and anti-PD-1 therapy in patients with advanced melanoma. Results of encouraging objective response rates in this previously treated population along with moderated toxicity led to front-line evaluations of CTLA-4 and PD-1 blockade in more difficult treatment settings including advanced NSCLC and metastatic renal cell carcinoma. The combination of ipilimumab at a fixed dose with nivolumab resulted in higher response rates across a variety of metastatic solid tumours with durable deep responses and increased survival benefit compared to standard immune checkpoint monotherapy [37].

5.1 Cancer Immunotherapy

Cancer immunotherapy is a term used to describe treatment approaches that use the immune system to combat cancer. To stop tumour growth and metastasis, monoclonal antibodies have long been used to target the cell-surface tumour antigen or antigens. Disease response can occur via multiple mechanisms, with direct antigen blockade being the most well studied. However, limited efficacy with various engineered formats and approved antibodies has been reported to date [38].

Antigen targeting alone is sufficient in establishing potent humoral and cellular responses that can protect against tumour growth and recurrence. Tumour-infiltrating lymphocytes (TILs) that hold the potential to eradicate established solid tumours are present in many patients but are effectively dormant due to tumour-associated immunosuppression. For non-hematological malignancies, it has been proposed that mice with established tumours are treated with a combination of antibodies targeting CD139, PD-1, and CTLA-4, which resulted in a large number of tumours being eradicated and survival extending significantly beyond 2 months. When re-challenged, some of the latter animals remained tumour-free to-day, indicating the development of tumour-specific immunity [39, 40].

Immunotherapy aims to boost the immune system to maximize its power to fight tumours. The immune system consists of various types of cells to attack, recognize, and eliminate abnormal cells. Immune responses are mediated via a cascade of biological processes beginning at detection of foreign antigens by antigen-presenting cells (APCs) that activate T and B lymphocytes. Amplified immune activation proceeds via several mechanisms such as cytokine production, antibody secretion, T-cell proliferation, and stimulation of cytotoxic T-lymphocytes (CTLs). These immune responses are tightly regulated via signaling through numerous co-stimulatory and co-inhibitory molecules. Tumour-associated antigens (TAAs) can be broadly classified into peptide antigens produced via posttranslational modifications from mutated onco-genes/oncoproteins (neoantigens), aberrantly expressed proteins associated with cancers, and cancer-specific aberrantly glycosylated antigens and viral antigens [41].

5.2 Autoimmune Diseases

With regards to antibody susceptibility, certain immune checkpoint inhibitors are more commonly linked to specific immune-related adverse events (irAEs) in terms of antibody susceptibility. Diabetes mellitus, hypophysitis, and adrenalitis are among the autoimmune manifestations that are frequently associated with anti-CTLA-4 therapy. On the other hand, colitis, vitiligo, myocarditis, and pneumonitis are more frequently linked to anti-PD-1/PD-L1 treatments. Importantly, checkpoint inhibitors, especially anti-PD-1 drugs, can also cause rheumatism or worsen autoimmune rheumatologic diseases that already exist, such as inflammatory arthritis.[28]. Mechanistically, CTLA-4 signaling inhibits T cell motility and cytoskeletal structure, interferes with "stop" signals mediated by T cell receptors (TCR), hinders effector T cell cytotoxicity, suppresses the initiation of immune responses, and decreases the efficiency of interactions between antigen-presenting cells (APCs) and T cells. On the other hand, PD-1 signaling is essential for increasing the activity of regulatory T cells (Treg), preventing T cell priming [28, 42, 43].

5.3 Infectious Diseases

Therapeutic mAbs have been widely investigated for the prevention and treatment of infectious diseases. A few mAbs have shown promise in studies against pathogens such as HIV and various viruses. Prominent targets are the

HIV-1 envelope surface glycoprotein gp120, the West Nile Virus E protein, palivizumab against the respiratory syncytial virus F protein, and mAbs targeting neutralizing epitopes of Nipah or Hendra viruses. Furthermore, various classes of mAbs are in preclinical and clinical investigations against tuberculosis, the prototypical intracellular bacterium [44].

The efficacy of antibodies in targeting intracellular pathogens is usually low. Receptor-blocking mAbs/recombinant antibodies, engineered to moderate antibody effector functions such as complement activation and interact with the target intracellularly, have been used to circumvent some weaknesses of antibodies against intracellular pathogens [45]. Concerns regarding their effectiveness arise since most receptors of potential antibody targets are membrane-bound glycoproteins, and mAbs would require targeting these glycoproteins inside the cell. mAbs have been engineered to carry endosomolytic peptides to escape endosomes of immune receptors such as FcRs, improving efficacy against intracellular infection. However, this approach is questionable given the cellular heterogeneity of lymphoid tissues and high doses of mAbs needed. Furthermore, most types of antibodies (IgG, IgA, IgE) that use the conventional endocytic pathway must energize the process at the intracellular level. mAbs against cancer and chronic infections require a greater understanding of mechanisms against helper cells and cross-dendritic cell immunogenicity than is presently available [46].

Combining passive mAb immunotherapy with peptide T-cell vaccines against various cancers, respiratory infections, and chronic viral infections offers very promising benefits. Combination approaches employing broadly neutralizing antibodies to prime type I IFNs with T-cell/HIV viral proteins to promote the cytolytic T cell-based immune dominance against acute West Nile infection to augment the anti-SARS-CoV-2 mAb response have also been equally or more beneficial. mAbs combined with checkpoint inhibitor antibodies targeting immune checkpoint receptors enhance the immunogenicity of mAbs and T-cell-based immunization modes. Studies show that adding mAbs against checkpoint receptors may enhance mAb immunotherapy against cancer and chronic virus infections [47].

6 Current Therapeutic Monoclonal Antibodies

Therapeutic monoclonal antibodies account for more than 40% of biological drugs currently approved for clinical use. Proteins of monoclonal origin finding therapeutic applications in diseases including cancers have made a substantial impact in modern therapeutics. However, an overwhelming majority of the monoclonal antibodies are still derived from mouse or rat and thus not optimal [45]. This makes the search for new monoclonal antibodies that are entirely of human to lessen abnormal immune response a hot research area. To this end, an alternative to the host systems for hybridoma production has been phage display in which random antibody libraries are functionally displayed on the surface of filamentous bacteriophages. Antibodies, also called immunoglobulins, consist of two heavy chains and two light chains that form a Y-shaped structure. Each chain has one variable region and one or more constant regions. The variable regions, also called Fab parts, are responsible for the selectivity of antibodies while constant regions are poorly immunogenic and are antagonistic to Fc receptors of phagocytes [48].

A general structure-based strategy to develop fully human monoclonal antibodies for therapeutic applications is essential. As an illustration, monoclonal antibodies targeting immune checkpoints to develop a new class of anti-cancer medicines have been explored in great detail. Immune checkpoints exhaust T cell responses in solid tumours and contribute to resistance in response to immune checkpoint blockade. There are two categories of immune checkpoints: stimulatory such as 4-1BB, OX40, Glucocorticoid-Induced TNF Receptor, CD137, CD27, and CTLA-4; inhibitory such as PD-1, PD-L1, and TIM. Immune checkpoint targeting monoclonal antibodies of both types have been shown to be effective in bringing on better clinical outcomes in patients with solid tumours, even making some patients completely tumour-free. To date, more than 20 immune checkpoint antibodies have entered clinical trials, more than 10 of which have gained approval [49].

In the last two decades, a new class of anti-cancer medicines: therapeutic monoclonal antibodies have yielded encouraging results. Generally speaking, therapeutic monoclonal antibodies consist of variable and constant regions and can be classified into five formats according to their source. A large number of therapeutic monoclonal antibodies have already gained approval for the treatment of cancers. Recently, therapeutic monoclonal antibodies targeting immune checkpoints have attracted wide attention in tumour immunology research. Immune checkpoints are a variety of immunomodulatory proteins that can inhibit effector T cell responses in either a constitutive or a dynamically regulated manner (Figure 1) [50].

6.1 Ipilimumab

Ipilimumab is a therapeutic monoclonal antibody that targets the protein cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), an immune checkpoint primarily expressed on regulatory T-cells. CTLA-4 dominates the CD28 pathway after a short delay following T-cell activation. This timing allows for initial T-cell activation via CD28 and for CTLA-4 to then trigger attenuation of the immune response. As T-cells migrate into secondary lymphoid nodes to encounter antigen-presenting cells, T-cell receptor signaling induces expression of ligands for

CD28 and CTLA-4, B7-1, and B7-2. Binding of B7-1 and B7-2 to CD28 generates an early positive signal that promotes T-helper 1 polarization of T-cells and drive interleukin-2 (IL-2) and T-cell proliferation. In contrast, a late negative signal occurs following interaction of CTLA-4 with B7-1 and B7-2 [51]. Together with CD28 and B7-1, CTLA-4 acts in a counter-regulatory manner to restrain excessive immune responses and restore immune homeostasis [52]. The monoclonal antibody ipilimumab locks CTLA-4 in an inactive form and prevents the downregulating signal, thus allowing a sustained T-cell immune response to persist and even amplify against the tumour 3. In the early 1990s, a rat IgG2a anti-CTLA-4 monoclonal antibody was studied in experimental mouse models of transplantable murine tumours and was found to promote T-cell responses leading to rejection of the transplanted tumours. This was followed by the generation of a number of fully human anti-CTLA-4 monoclonal antibodies, the most clinically relevant being ipilimumab. Interest in this biological agent and its use in cancer therapy mounted in the early 2000s following the first human clinical trials in advanced melanoma patients, whereby evidence of long-term survival was noted in some of the patients responding to the antibody 13. Most of the subsequent studies led to the evaluation of ipilimumab in advanced melanoma but other clinical trials were authorized after a relevance query. Almost all of the studies were first-in-man or phase I trials as extensive preclinical data were generated, exploring the limits of the cancer frontiers, but also regulatory hurdles for testing novel agents or treatment protocols in patients with goblet cell carcinoid, hepatocellular carcinoma, mesothelioma, breast cancer, glioma, kidney cancer, and uriel cancers were overcome only later by rising commercial data [53].

6.2 *Nivolumab*

Nivolumab, a monoclonal human immunoglobulin G4κ (IgG4κ) antibody, selectively binds to human PD-1 and blocks the interaction of PD-1 with its major ligands PD-L1 and PD-L2. This inhibition leads to enhanced proliferation of T and B lymphocytes and increased production of interferon-γ (IFN-γ) and other pro-inflammatory cytokines. Additionally, there is evidence for dose-dependent pharmacokinetics and a long terminal half-life of approximately 26 days. Nivolumab is administered by intravenous (IV) infusion at a dose of 3 mg/kg every 2 weeks [54].

Nivolumab monotherapy was evaluated in patients with advanced squamous non-small-cell lung cancer (NSCLC) who had received > 1 previous chemotherapy regimen plus a platinum-containing chemotherapy and had progressive disease on or after the last regimen. Nivolumab therapy resulted in an ORR of 15% and a DCR of 55%. The median PFS and OS were 2.1 and 9.1 months, respectively. The 1-year and 2-year OS estimates were 41% and 24%, respectively. PD-1 ligand status (PD-L1 ≥ 5% vs. PD-L1 < 5%) was not predictive for response, but clear clinical benefit was observed in both PD-L1 specks. Nivolumab was well tolerated, and the most frequent treatment-related AEs were fatigue and rash. Nivolumab was evaluated in patients with melanoma versus the investigator's choice of chemotherapy after progression on ipilimumab and a BRAF inhibitor. Nivolumab led to significantly improved ORR, DCR, and OS compared with chemotherapy [55].

6.3 *Pembrolizumab*

Targeting the PD-1 receptor, pembrolizumab is a fully humanized IgG4 monoclonal antibody. Since its initial approval by the U.S. Food and Drug Administration (FDA) in 2014 for the treatment of melanoma, the drug's clinical indications have grown to include a wide range of cancers. Pembrolizumab is currently used to treat a number of cancers, including locally advanced or metastatic triple-negative breast cancer (TNBC) with a Tumour Proportion Score (TPS) ≥1%, PD-L1-positive (≥1%) non-small cell lung cancer (NSCLC), recurrent or metastatic head and neck squamous cell carcinoma (HNSCC), as well as traditional Hodgkin lymphoma, unresectable melanoma, gastric cancer, urothelial carcinoma, and cervical cancer. Pembrolizumab is currently one of four PD-1-targeting antibodies that have FDA approval [56].

Monotherapy or drug combinations have expanded the use of the antibodies for various tumours beyond those originally approved. The approval of pembrolizumab, along with its competitive partners, is based on the understanding of the structural basis of antibody action. Further detailed studies on the epitope mapping and the structural interaction of antibodies with PD-1 will pave the way for the next-generation antibodies against PD-1. Additionally, it enables screening out false positive PD-1 antibodies in early discovery [57].

Antibody-mediated blockade of immune checkpoint (IC) proteins has become the new standard treatment for multiple tumours. PD-1 is mainly expressed in immune cells such as T cells, B cells, dendritic cells, and NK cells. PD-1 is a crucial IC for maintaining immune tolerance in peripheral tissues and T cell exhaustion in tumours. Tumour derived PD-L1 and PD-L2 are mainly responsible for PD-1 activation, enabling the tumour cells to escape from T cell immune responses. Inhibition of the interaction of PD-1 with its ligands using monoclonal antibodies can thus restore T cell immune responses against cancer cells. As a result, not only PD-1, PD-L1, but also CTLA-4 have become the focus of oncolytic monoclonal antibodies [58].

6.4 *Atezolizumab*

Atezolizumab (MPDL3280A), a humanized immunoglobulin G1 (IgG1) monoclonal antibody, has a higher affinity for PDL1 than PDL2. It was the first anti-

PD1/PDL1 agent approved for use 16. The PD1 checkpoints, in contrast to CTLA4, were recognized as downregulators of T cells relatively recently. The PD1 is immunoglobulin superfamily molecule with its ligands being PDL1/PDL2. PDL1 is regulated at both transcriptional and translational levels by numerous factors including interferon-gamma, telomerase, NF-kB, and various oncogenes. It is expressed on various hematogenous tumours, breast cancers and a wide variety of solid tumours. PD1, an immunoglobulin superfamily member containing a single extracellular IgV domain, a cytoplasmic immunoreceptor tyrosine-based switch motif (ITSM) and an immunoreceptor tyrosine-based inhibitory motif (ITIM), is expressed by activated immunity cells 1. PD1 ligation to its ligands results in downregulation of T cells via recruitment of phosphatases to specifically dephosphorylate CD3ζ. Such naïve CD8 positive T cells, upon contact with tumour cells, can divide and acquire TE markers like granzyme B, perforin, and IFN-γ. In preliminary studies, MPDL3280A was shown to be safe and efficacious in managing a variety of malignancies [59].

Monoclonal antibodies against PD1/PDL1 including Nivolumab, Pembrulizumab and Atezolizumab are now approved in the USA and worldwide for treating unresectable metastatic melanoma. Ongoing and future studies will determine the potential of melanoma vaccination as a sensitizer or adjuvant for anti-PD1/PDL1 antibodies and as a means to identify patients at risk for decreased response. Head and neck squamous cell carcinoma patients have a distinct immune related toxicologic profile compared to melanoma and lung cancer. Secondly, potent immune checkpoint regulators other than PD1/PDL1 are actively undergoing evaluation. Anti-PD1 monoclonal Ab plus anti-CTLA-4 monoclonal Ab, or triple DDG + anti-PD1 mAb + anti-CTLA4, or TLA/DKD + anti-PD1 mAb + anti-CD40, are being investigated for safety and efficacy [59].

6.5 *Durvalumab*

Durvalumab is a human immunoglobulin G1 lambda (IgG1λ) monoclonal antibody targeting PD-L1 (programmed cell death ligand 1) and, in turn, preventing PD-1 and CD80 binding. It has shown activity across many solid tumours and hematological malignancies. In hematological malignancies, durvalumab is approved in combination with ibrutinib in chronic lymphocytic leukemia (CLL) and is expected to receive approval as a combination therapy with daratumumab in multiple myeloma (MM) [60]. In solid tumours, durvalumab has been evaluated with and without chemotherapy in most tumour types. In lung cancer, it is approved both as first-line and second-line therapy in combination with chemotherapy and monotherapy in second-line locally advanced disease. Several randomized trials have explored durvalumab in the curative treatment of unresected stage III non-small cell lung cancer (NSCLC) after platinum-based chemotherapy and radiotherapy and ongoing trials of pre- and postoperative treatment are addressing its role in respectable disease [61].

Among the PD-1, PD-L1, and CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), antibodies durvalumab (anti-PD-L1 antibody) has shown clinical activity in different cancer types, including hematological malignancies. MM is a hematological malignancy characterized by a monoclonal expansion of immunoglobulin-secreting plasma cells in the bone marrow (BM). In various subtypes, the disease is incurable and is often treated with a translational approach based on an ICIs strategy [60]. As a result, the PD-1, PD-L1, and CTLA-4 monoclonal antibodies are currently under evaluation in MM. Several ICIs have been combined with CRM in upfront and relapsed settings and have produced promising efficacy and activity in patients with an early response. More data are needed in order to understand selection criteria and optimal combinations and timing. MAbs inhibiting numerous immune checkpoints are being investigated for improving anti-tumour immunity in different hematological malignancies depending on sensitivity to T-cell mediated immune destruction. However, the challenge remains to combine ICIs without excessive toxicity considering the narrow therapeutic index of some of these agents. So far there have been no major safety issues in adult or pediatric populations. Chronic immune mediated adverse events (imAEs) involving skin, lung, colon, and liver after chronic PD-1 blockade are now being recognized in children. Patients having transaminitis following first dose of nab-paclitaxel will still tolerate PD-1 inhibitor. Destiny-CRC03 study evaluated efficacy and safety of trastuzumab deruxtecan (T-DXd) and durvalumab with or without chemotherapy in patients with HER2 mutant colorectal cancer or HER2 expressing colorectal cancer. PD-L1 antibody was generally well tolerated when combined with antibody-drug conjugate trastuzumab emtansine in HER2 positive breast cancer and no new safety signals were observed [62].

7 Clinical Trials and Efficacy

The efficacy of immune checkpoint blockade has been demonstrated in over 30 clinical trials targeting PD-1, PD-L1, or CTLA-4. Several immune-related adverse events are unique to immune checkpoint blockade treatments with monoclonal antibodies targeting CTLA-4 or the PD-1 pathway likely because of their unique anatomical exposures and pathways of movement in the body [63]. The therapeutic monoclonal antibodies currently approved for use in the United States and European Union for relapsed and/or refractory cancer are ipilimumab, pembrolizumab, nivolumab, atezolizumab, and durvalumab [60]. Antibodies against immune checkpoint receptors are reasonably well tolerated. Generally speaking, antibody

therapies tend to have a lower incidence of adverse events compared to other forms of anti-cancer therapy such as chemotherapy. The immune-related adverse events that occur with immune checkpoint blockade treatments with monoclonal antibodies targeting PD-1 or PD-L1 tend to be mild and reversible (often resolving without intervention or with topical corticosteroids or systemic steroids). However, some immune-related adverse events can be severe and irreversible especially those related to the endocrine organs (such as the pituitary, thyroid, and adrenal glands), the lungs, and the liver. Therefore, close follow-up and monitoring are warranted. Nevertheless, most side effects are generally manageable, and gradual tapering of steroid doses is possible with clinical improvement. The effect on overall odds ratio favors treatment with antibodies against immune checkpoint receptors [64].

Immune checkpoint blockade has emerged in recent years as a new and effective class of cancer immunotherapy that can achieve responses in many patients with relapsed and/or refractory cancer that can last for many years. Understanding the biology and mechanism of action of immune checkpoints has led to the identification and targeting of new immune inhibitors with the goal of developing new cancer therapies. The limited clinical development of anti-PD-1 versus anti-PD-L1 antibodies is also noteworthy [60]. Monoclonal antibodies against PD-1 such as nivolumab and pembrolizumab have been successfully developed for the treatment of a wide variety of malignancies with multiple FDA approvals, and combination therapy of dual immune checkpoint blockade with nivolumab and ipilimumab has the potential to expand the treatment to even more malignancies. The efficacy in a wide variety of solid tumours has also stimulated the further study of monoclonal antibodies targeting LAG-3, TIM-3, VISTA, and other inhibitory immune checkpoint receptors [65].

7.1 Overview of Key Clinical Trials

Inhibiting the PD-1 pathway in advanced cancers has shown promising activity with two IgG4 monoclonal antibodies. One was given to patients with PD-L1 positive advanced melanoma as monotherapy on a three-weekly basis at a dose of 10 mg/kg and was associated with modest grade 3 adverse events in 16% of patients. Objective responses were noted in 23% of patients with at least one dose where market authorization was granted. The rate of sustained responses lasted longer than sixteen months in patients with high PD-L1 expression and showed high dynamic range of response from 0% in tumours with no PD-L1 expression. Based on these results, we now have diagnostics that allow for prospectively assessing PD-L1 expression by next generation immunohistochemistry [66]. A trial comparing one of the antibodies with another highlighted the value of PD-1 pathway inhibition in melanoma independently of PD-L1 status. Approval was expanded to include both metastatic non-squamous and squamous NSCLC on the basis of its activity as third-line therapy in early phase studies, with more substantial activity seen in tumours expressing PD-L1 [67].

A trial of PD-1 blockades in previously treated patients with NSCLC with activity independent of PD-L1 expression was the impetus for two studies of the blockade versus another treatment in squamous and non-squamous NSCLC respectively. Subsequently, a greater than 50% improvement in overall survival was observed in the squamous cell lung cancer trial and 73% survival rates at 18 months were observed. These results saw a rapid expansion of clinical trials investigating novel combinations within expanded access protocols based on high-level activity seen in almost every tumour type tried [68].

While the ICIs have demonstrated magnificent efficacy across several types of malignancies, the clinical trials are limited by selective patient populations, reliance on PD-L1 as an imperfect biomarker, and relatively short follow-up in some important studies. Furthermore, the challenge of generalizing trial results to real-world cohorts, with which the comorbidities and treatment tolerability may vary substantially.

7.2 Efficacy in Different Cancer Types

The FDA approval of CTLA-4 blockade (ipilimumab) for malignant melanoma was a landmark event in immunotherapy with enormous ramifications across all areas of oncology. A host of anti-PD-1/-L1 agents followed close behind in 2014, transforming both the landscape of non-small-cell lung cancer (NSCLC) therapy with durable long-lived responses observed across all pathologies, and treatment strategy in renal and bladder cancers, malignancies previously deemed non-immunogenic [69, 70]. Combo immunotherapy (anti-CTLA-4 and anti-PD-1) then established unequivocally the potency of dual checkpoint blockade in treatment-naïve melanoma 1. In 2020, the first generation of immune checkpoint inhibitors (ICIs) targeting CTLA-4 and PD-1/-PD-L1 secured clinical approval across an impressive range of malignancies. Monoclonal antibodies targeting co-inhibitory checkpoint proteins CTLA-4 and PD-1/-PD-L1 first brought hope to patients with immunologically cold tumours. Monoclonal antibodies can activate co-stimulatory pathways and the immune system to eradicate tumours, evidenced by remarkable durable complete metabolic and clinical responses [58]. However, development of ICIs has been complicated by the emergence of immune-related adverse events. Notably, ICI responses appear slower, taking 3-6 months longer to manifest than targeted therapies or chemotherapy. Consequently, early switching of therapy may be warranted for cases of (pseudo)progressive disease, especially for ICIs alone when given a thorough chance

(i.e., ≥6 weeks of full dosing). There is a pressing need to create a comprehensive real-world cohort to examine the evolving adoption of ICIs, as well as the incidence, timing, and clinical significance of immune-related adverse events and (pseudo)progressive disease [66]. In an effort to delineate de novo responses from pre-existing immunity, biomarkers of response to anti-PD-1 were explored, with PD-L1 expression on tumour cells or immune environment conferring the greatest predictive value at the time of treatment initiation across multiple malignancies. As our understanding of the tumour microenvironment has evolved, emerging immunotherapy biomarkers have allowed for a broader view of the tumour's broader 'immunoscape' beyond mere PD-L1 expression. Although responses to CTLA-4 monoclonal antibodies are generally heterogeneous, substantial literature has suggested associations between tumour mutational burden, clonal neoantigen burden, timely tumour biopsies to delineate pre-/post-treatment changes, tertiary lymphoid structure, hypomethylation, and abundance of effector T cells [71].

7.3 Determinants of Response and Resistance

While the ICIs have transformed the therapeutic landscape, only a subgroup of patients achieve progression-free survival. Current evidence indicates that both tumor-intrinsic and host-related factors govern this diversity in treatment reponse. Responders are usually with a high tumor mutational burden (TMB) and neo-antigen load, and this in turn enhances immunogenicity and promotes infiltration of effector T cells. High PD-L1 expression, presence of tertiary lymphoid structures, and interferon-γ–driven signaling pathways further correlate with improved outcomes. To enhanced responsiveness, host immune competence, including favorable gut microbiome composition, and HLA diversity has been linked [72].

On other hand, resistance to ICIs may occur at two levels. The primary resistance is usually associated with "cold" tumours with defective antigen presentation machinery, lacking sufficient T-cell infiltration, or a suppressive tumour microenvironment rich in myeloid-derived suppressor cells and regulatory T-cells. The second resistance mechanism is the acquired resistance which observed after initial responses and commonly arises via adaptive tumour progression such as epigenetic reprogramming, loss of interferon-γ pathway signaling, up-regulation of alternate checkpoints (TIM-3, LAG-3, TIGIT), and metabolic constraints like hypoxia and IDO activity. These mechanisms emphasize the dynamic interplay between host and tumor that shapes therapeutic outcomes. Therefore, biomarker-driven patient selection, rational combination strategies, using next-generation checkpoint inhibitors, represent the key avenues to overcome resistance and broaden the clinical benefit of ICIs [73].

7.4 Long-term Outcomes

Immunotherapy targeting immune checkpoints has succeeded in altering a host-anti-tumour immune response and producing durable tumour regressions with prolonged survival in some patients with metastatic disease. PD-1 and CTLA-4 blockade have transformational benefits for the treatment of metastatic melanoma, non-small cell lung cancer, and other cancers. The duration of response with PD-1 blockade can exceed 3 years or longer, at rates greater than 25%. Historically, the median survival of metastatic melanoma patients treated with standard therapy was less than 1 year. The newly approved mAb immunotherapeutics are now integrated into the routine care of patients with these common malignancies [74].

However, a critical knowledge gap is the long-term outcomes for patients treated with these agents. Pending impartial meta-analyses of individual patient data from the now-concluded phase III program in metastatic melanoma should provide a clearer picture on durability, postsurgical impact, and late responses. Alongside the manic implementation of these agents in practice, there has also been a striking increase in the number of patients experiencing unintended adverse consequences from treatment. By successful targeting of an adaptive immune system, an oncogenic host response has been activated that rarely occurs as widely or as devastatingly with traditional agents. Adverse events and complications from Janus kinase immunotherapy are well documented but no single medication has so markedly altered the tumour-host relationship as mAb checkpoint blockade. Adverse effects include a wide spectrum of new airways disease, colitis, steroid-responsive, and difficult to control neurologic syndromes, and other more rare manifestations. Furthermore, this chronic toxicity is likely to continue and even worsen in a number of patients after treatment is withdrawn. Administration of these agents produces a dramatic worsening in outcomes in the context of already existing unrecognized immune disturbances and may increase the risk for new autoimmune diseases to emerge. In a manner similar to thalidomide and other compounds, it is possible that checkpoint blockade avoidance may foster a new population of treated patients with similarly debilitating and terminal diseases [75]. Although the anti–PD-1/PD-L1 and anti–CTLA-4 antibodies have transformed certain types of cancer such as melanoma and NSCLC with durable responses exceeding three years in subsets of patients, the overall survival outcome remains uncertain and heterogeneous for many other type of cancers[58].

8 Adverse Effects and Management

Pharmacological therapies targeting immune checkpoints have demonstrated the possibility of curbing malignancies; however, such therapies have also introduced a new class of prescription-drug-induced immunotoxicities. Potential

organ systems affected are numerous, including but not limited to the skin, gastrointestinal tract, liver, pituitary gland, lungs, heart, kidney, and neuromuscular junction [76]. Most commonly, dermatomyositis, vitiligo, autoimmune colitis, oculomotor palsy, hepatitis, type I diabetes insipidus, thyroiditis, pneumonitis, myocarditis, psoriasis, encephalitis, and sarcoidosis have been documented and reported in long-term follow-ups 20. Such events have been confirmed to occur by mechanisms of autoantibody production or proliferation of T effector cells, which initiate inflammatory cascades culminating in cell death. Severe events can be lethal, especially if involving the lungs, heart, or muscles, and can result in permanent disability. Prolonged treatment with immunosuppressants, often the first-line therapy for irAE, can in turn shield unwarranted immune attack allowing for tumour reinvigorating. The offsetting nature of these toxicities presents a conundrum of immune homeostasis, which continues to be an obstacle in using costoimmunotherapies in tunable and efficacious ways [77].

The mechanisms underlying such variables from benign cutaneous itchiness to life-threatening pneumonia have remained largely unclear. Vital to their elucidation are longitudinal studies of dose escalations, adjuvant therapies, or patient populations, with a special emphasis on unique immunogenetic predispositions. However, such studies have remained few and far in between. Pharmacogenetic associations, especially of HLA alleles, have been shown to exist and more are being explored in this area [76]. As tools for precision medicine, they may allow a reduction in the incidence of immunotoxicities while still maintaining drug efficacy. Investigating better-tolerated agents that modulate immune checkpoint pathways are also avenues of exploration. Identification of biomarkers predictive of toxicity would also represent therapeutic opportunity. A lineage-tracking-based system reported the involvement of a distinct CD4+ T-cell population that differentiated from bulk T cells based on restricted TCR use and stillness toward the tumour correlated with a slightly increased incidence of irAE. Further confirmation of such previously undiscovered populations in larger cohorts may assist in the real-time prediction of toxicity [78].

8.1 Common Adverse Effects

Immune checkpoint inhibitors are a promising new class of anticancer drugs. However, one of their important limitations is that they can cause severe adverse effects. Immune-related adverse events associated with checkpoint inhibitors can involve virtually every organ system, including the skin, endocrine system, lungs, liver, gastrointestinal tract and musculoskeletal system. Although many of these immune-related adverse events are potentially severe, they may be managed and treated effectively. The incidence of immune-related adverse events can vary considerably depending on the specific checkpoint inhibitors used, their dosing schedule, tumour type, and perhaps more importantly, the presence of other immune-related co-therapies [79].

Common adverse effects of immune checkpoint inhibitors (ICIs) include immune-related adverse events (irAEs). The majority of these adverse events are manageable [79]. ICI toxicity mechanisms are multifactorial and can involve various organ systems. Toxicity severity can range from mild to life-threatening. The reported incidence of ICI interactions between cancer immunotherapies and supportive care treatments or traditional immunosuppressive drugs is low. Patients treated with a combination of immune modulators show a higher risk of immune-related adverse events. Management of toxicities due to antibody-mediated ICI therapy is variable because they may result in severe irAEs or inadequate antitumour immune responses following systematic corticosteroid therapy, or other immunosuppressants, or temporary discontinuation of therapy [79].

8.2 Management Strategies

Strategies to enhance the efficacy of immune checkpoint agents are currently much pursued in the oncology field. An alternative approach is to study the mechanism of actions of these agents. For example, mediation of the role of the T-cell effector functions via inhibitor engagement on T cells provides a rationale for combining CTLA-4 blockade with co-stimulatory agents that directly enhance T-cell activation [80]. This suggests that a CTLA-4 blocking agent may be likely to enhance the efficacy of T-cell co-stimulation or to induce durable responses. Combination of CTLA-4 blockade with a CD137 agonist, which is still under investigation, has generated complete durable responses in preclinical models of melanoma as well as prostate, breast, and colon carcinoma. Similar results in the immunocompetent setting have supported ongoing early clinical trials examining a CD137 agonist in addition to anti-CTLA-4 in advanced cancers, including NSCLC and melanoma. Blockade of PD-1-L1 or PD-L1 engagement additionally may further enhance this combination and is being actively investigated in several clinical trials. Study of other CTLA-4 or PD-1 pathway blocking agents or other immuno-drugs that target different immune checkpoints is an active area of development [81].

Integrating immune checkpoint inhibitors with traditional or novel anticancer therapies like targeted therapies, cytotoxic chemotherapy, radiotherapy, or immunomodulatory tactics like cytokine-based therapy or vaccines is an alternate strategy. Radiotherapy, in particular, is known to have immune effects, especially enhancing T-cell priming and activation, and may induce expression of B7-H1, which is a PD-L1, on the irradiated

tumour [82]. In preclinical model melanoma and lung cancer, anti-CTLA-4 or anti-PD-1 acted synergistically with fractionated irradiation, enhancing local responses and helping to control distant untreated lesions. The combination of ipilimumab (anti-CTLA-4) ± radiation was explored in the clinic. Local immune activation, as measured by the appearance of skin autoimmune/inflammatory changes, was observed in patients treated with ipilimumab plus SBRT, supporting the radio-immunotherapy concept. Combination of ipilimumab with chemotherapeutic agent to improve response rates was another strategy. Combination of ipilimumab and the BRAF inhibitor vemurafenib in a phase I trial of melanoma was highly promising, but this combination produced significant hepatotoxicity, requiring the termination of the trial. Other investigational strategies include studying targeted agents in combination with checkpoint inhibitors [83].

9 Future Directions in Research

Currently, immune checkpoint blockade therapies have been approved for the treatment of several cancers; other monoclonal antibodies targeting immune checkpoints are undergoing preclinical and clinical evaluations. Five immune checkpoints are currently targeted by FDA-approved therapeutic monoclonal antibodies: PD-1, PD-L1, CTLA-4, LAG-3, and TIM-3 4. All of these therapeutic monoclonal antibodies are IgG1 isotypes that bind to immune checkpoints in the same way: As these immune checkpoints are all cell-surface membrane molecules, these therapeutic antibodies prevent receptor-ligand interactions between immune checkpoints and their cognate ligands, thereby exerting their antitumour effects. While these investigations provided invaluable insights into the biology of immune checkpoints, there are several important issues that remained unaddressed. For instance, how are immune checkpoints regulated at both the cell-surface and intracellular levels? Receptor-ligand interactions are only one aspect of signal transduction, and how checkpoints are activated once they are engaged by cognate ligands is poorly studied [84]. There is now an increasing appreciation of how immune checkpoints exert their inhibitory functions on T and B cells. However, whether checkpoints exert immune enhancing effects on other immune effector cells, such as innate immune cells, is largely unexplored. There are several exciting avenues deserving further exploration that might further enhance the efficacy of current immune checkpoint blockade-based therapies as well as lead to the development of new therapeutic monoclonal antibodies targeting immune checkpoints. These are:

- To investigate how to take advantage of the inhibitory functions of immune checkpoints for the treatment of autoimmune diseases;
- To explore endocannabinoids receptors and their ligands as new immune checkpoint targets for cancer immunotherapy;
- Studies of immune checkpoints in combination treatments;
- To further investigate epigenetic and microenvironmental regulation of immune checkpoints;
- To explore biomarkers of immune checkpoint blockade therapies and strategies to overcome resistance mechanisms;
- Masking immune checkpoints with antibodies for enhanced bioavailability;
- Targeting immune checkpoints in cancer biology other than monoclonal antibodies; and
- To develop better in vitro preclinical assays [63].

9.1 Novel Immune Checkpoint Targets

Despite the promise of the current immune checkpoint inhibitors in the clinic, not every cancer patient is a candidate for treatment. It is imperative to develop the next generation of immune checkpoint inhibitors targeting additional molecules involved in the co-stimulatory and co-inhibitory pathways. In this regard, several promising immune checkpoint molecules that have been proposed to be potential targets for the next generation of immune checkpoint inhibitors are profiled [85].

Due to an increase in tumour neoantigens recognized by T cells in the immunogenic mutant landscape of melanoma, tumours inevitably develop checkpoint mechanisms to protect themselves. Understood with the immunological checkpoint landscape in melanoma, novel therapeutic antibodies targeting additional immune checkpoints have been developed to improve on the efficacy of anti-PD1 treatment. The checkpoint inhibitors that show clinical efficacy in melanoma and other cancers with the most advanced research fields are anti-CTLA-4, anti-PD-1, and anti-PDL-1 antibodies. Extreme efficacy has been reported in a small fraction of patients following single-agent anti-PD-1 or anti-PD-L1 treatment and even greater efficacy when these agents are used in combination with anti-CTLA-4 therapy 1. However, the majority of patients do not respond to immunotherapy despite the presence of mutation-derived neoantigens that provoke a robust T cell anti-tumour response. Although anti-PD-1 and anti-PD-L1 therapies are beneficial, not all cancers respond to them. Furthermore, a large percentage of patients develop eventual resistance to therapy following initial response. New and novel checkpoint inhibitors targeting additional immune checkpoint pathways are thus required for improved patient outcomes [86]. In spite of the initial robust responses, some patients eventually relapse due to resistance mechanisms such as adaptive upregulation of alternative checkpoints (e.g., TIM-3, LAG-3, VISTA), loss of antigen presentation, immunosuppressive tumor microenvironments, and T-cell exhaustion.

Chemokines and their receptors are among the most important components in the immune system, mediating the migratory movement of immune cells and also directly involved in the modulation of innate and adaptive immune responses. Chemokine-based immunotherapy holds great promise for cancer treatment in the future. CX3CR1 is a chemokine receptor selectively expressed in a subset of immune cells. Blockade of CX3CR1–fractalkine signaling led to potent anti-tumour effects in mouse tumour hosts at different animal ages and tumour stages. CX3CR1 was identified as a potential receptor for immune checkpoint blockade therapy that might be used alone or more likely in combination with other cancer immunotherapy. Genomic analysis of lots of tumour samples is needed to screen the potentially beneficial patients for clinical trial designs [87].

9.2 *Combination Therapies*

Combinations of immune checkpoint inhibitors (ICIs) drugs with other types of therapy are already being pursued in clinical practice, and numerous preclinical combinations are being studied in hopes of discovering combinations that will be effective in humans. Some ideas about drugs and strategies that might augment checkpoint blockers are described here. Inhibitors of the immune checkpoints CTLA-4, PD-1, and PD-L1 induce objective tumour regressions in a subset of patients with metastatic cancer [82]. Best results have been observed in melanoma, lung, kidney, head and neck and bladder cancer; however, various other tumour types are being tested 21. Monoclonal antibodies to CTLA-4 have produced objective regressions in melanoma, both in the treatment naive setting and following anti-PD-1 therapy. Direct comparisons of CTLA-4 and PD-1/PD-L1 therapy have not yet been made, but pharmacodynamically relevant differences between these approaches suggest that they may be complementary. Rational combinations utilizing both have been shown to produce durable responses in murine tumour models and are undergoing clinical study. The targeting of PD-1 and PD-L1 has led to the approval of five monoclonal antibodies to date: nivolumab, pembrolizumab, cemiplimab, atezolizumab, and durvalumab. Response rates vary by tumour type, and the best responses have been observed in tumours with a high burden of genetic alterations, with more modest rates of response in most squamous cell carcinomas. Effective doses of PD-1 antibodies appear to be markedly lower than those of other therapeutic antibodies [88]. PD-L1 antibodies can potentially provide improved pharmacokinetics, less toxicity, and superior activity in some tumour types, but these drugs are not yet available in the treatment of solid tumours. The latter two classes deliver PD-1 blockade via a different mechanism than PD-1 antibodies, but they also have distinct pharmacokinetic and toxicity profiles. PD-L1 antibodies deactivate PD-1 signaling, while PD-1 antibodies attach to PD-1 and, in the case of immune co-culture, prevent PD-L1 from binding to PD-1, fulfilling a similar functional purpose [89].

9.3 *Personalized Medicine Approaches*

The idea of personalized cancer medicine, postulated in 1970s, has stayed at the concept stage until recently, mainly due to the limitation of investigational tools. Genome-wide DNA copy number changes in tumours were one of the earliest observations in personalized medicine. The development of inductive nano-alloy chips enables further investigation of the abnormality of human genome-wide DNA, and therefore may provide more information with clinical utility on targeted therapy. Personalized medicine receives more and more attention [90]. Progress has been reported on designing next-generation targeted therapy drugs for either oncogene-addicted or -common malignancies. There was an emphasis on using next-generation sequencing to identify actionable mutations and developing third-generation EGFR inhibitors that selectively target EGFR T790M mutations in nonsmall cell lung cancer. Personal cancer immunology, concerned with how to exploit a person's unique tumour antigens to stimulate antitumour immunity, was another hot topic in the symposium. Designing antibody drugs to inhibit tumour growth and restore immunity was another hot topic. Targeting PD-1, identified as a second immune checkpoint after CTLA-4, which may interfere with T cell activation signaling, has very recently been demonstrated to be a major mechanism of losing T cell immunity. Many pharmaceutics have developed PD-1 and PD-L1 antibodies, which have shown responses against a variety of cancers. Recent progress includes the FDA approval of pembrolizumab in September 2014 for treatment in refractory melanoma [91].

10 Regulatory and Ethical Considerations

All the clinical studies assessing immune checkpoint blockade have undergone a thorough review by regulatory bodies to determine their safety and risk-benefit ratio 3. Consequently, the regulatory dossier for the first drug in the area, ipilimumab, was one of the largest regional filings ever for a new drug application. Regarding clinical results, the first studies indicated that an arguably low percentage of patients achieved an objective response of the type generally considered clinically significant, i.e., a partial or complete response (CR) lasting more than 6 months 24 [6]. The distribution of responses was, however, unusual as the majority of patients had stable disease for several months, and there was an unusual percentage of early mortality. Response rates fell short of those seen with combination chemotherapy for other tumour types, and there was an overall disproportionate level of adverse dysimmune effects. Therefore, even considering the massive effort invested by the researcher teams, potential beneficiaries,

and the many hurdles faced, the final decision was close to a delicate balance of uncertain benefits versus assessed risks. Once on the market, immune modulators and especially immune checkpoint blocking agents have expanded dramatically in clinical application. They are now currently used by several hundred treating hospitals and in thousands of patients, with the number of treated patients rapidly exponentially growing. The growing patient population exposed has also led to a considerable interest in toxicity profiling across patient populations, treatment settings, and tumour types. This has revealed that, at least outside of their mild nature, mechanisms of toxicity overlap considerably within single drug families. As safety profiles are especially nuanced with respect to ingested drug characteristics, knowing parameters that partly but not wholly account for clinical outcomes is vital. In addition to full knowledge concerning the pharmacokinetics and mechanisms of action of the individual agents, at least a minimal characterization of a patient's basal immune system and tumour immune parameters would help achieve maximal benefit from the drugs employed, while minimizing toxicity [6].

10.1 Approval Processes

Monoclonal antibodies are proteins that can bind to specific targets – antigens. Antigens are usually present on the cell's surface or secreted by cells. The specificity of the monoclonal antibody is based on the epitope recognition, and several methods have been developed for testing the specificity of the monoclonal antibodies [21]. Generally, approval processes for all antibodies (both therapeutic and diagnostic) depend on several factors including type of antibody preparations (e.g., murine vs. chimeric vs. human etc.), the nature of indication (e.g., oncology vs. non-oncology etc.), and clinical development schemes (e.g., phase I vs. phase II/III trials), etc. Otherwise, the process for mAb approvals is similar to that of small molecular drugs, which is a multi-step program, even though the review and approval times are generally longer than that of small drugs [92].

Monoclonal antibodies have been the most successful biopharmaceuticals among the biologics with more than 100 mAb approvals worldwide over the past decades. The commercialized therapeutic monoclonal antibodies are important in the treatment of disorders, such as cancers, inflammatory diseases, viral infections, and hormonal imbalances. Numerous technical platforms and modalities to generate mAbs have been developed and extensively utilized for both research and therapeutic purposes [93]. Monoclonal antibodies face unique regulatory challenges, due to the complex biological nature of the products and the difficulties in determining their quality attributes (and methods for characterizations), mechanisms of action and efficacy, safety, effective forensic tracking of products across patient's treatment, etc. The proteomic nature of mAb and the degenerate nature of the epitope recognition make nearly all immunogenicity of positive safety issue with the unknowns on how to better assess it. Moreover, rapid market approval after laboratory validation and before full scale clinico-pharmaco-tox/side to an mAb based biopharmaceutical, creates many public health uncertainties, especially after single input portions for new-to-country nodes [94].

10.2 Ethical Implications of Immunotherapy

At a time when oncology has become more focused on treating tumours through immune modulatory mechanisms, it will be helpful to understand better the entry of immune checkpoint inhibitors into common practice in the treatment of malignancy. Cancer immunotherapy gained proponents over a century ago, but a pro-tumour part of adaptive immunity was discovered in the 1970s. This information led to novelty in immunotherapy targeting a vicious part of adaptive immunity, namely increasing immune checkpoints. Immune checkpoint inhibitors delivered a breakthrough with remarkable clinical success but with many unresolved issues and open, candy doors for active research. In addition, the scientific and clinical application of immune checkpoint inhibitors in cancer are discussed here along with related questions. Pro-tumour mechanisms were not detectable until the mid-1990s when CD28/B7-mediated immunity was explained. The understanding of innate immune mechanisms of action began in those years, but a comatose part of adaptive immunity was not uncovered. Immunotherapy has an extensive history, but remarkably, until today there is no acceptable treatment strategy to restore a missing piece of adaptive immunity, enabling tumour growth [95].

Restoration of broken innate anti-tumour immunity has been possible, resulting in a good, but inadequate, response. Then, the understanding of adaptive immune mechanisms was advanced in the early 2000s. A nasty part of adaptive immunity which initiated and promoted tumour growth was identified. Coining of the term immune checkpoint came later, opening a new field of research. Originating from discoveries made in normal conditions without considering cancer, the search for specific therapeutics intensified. First in the lab and clinic, and then in many other settings, the results have been compared to a dawn, with profound scientific and therapeutic changes [6]. Immune checkpoint inhibitors pose new, different questions rather than answers. An increasing number of published works are attempting to summarize what we already know, and then consider what is still unknown and should perhaps be pursued to enable translation of knowledge into practice and benefit patients. How and why are immune checkpoints pro-tumour? What should be targeted and how are still pressing questions. Another camp proposes ways to address acquired drug

resistance, limit side effects, and treat indications with no clinical success as the first step before considering the uncharted territory of immune checkpoints operating in normal conditions [96].

11 Conclusion

Anticancer immunity depends on T cells that recognize changes in self on tumour cells and robust activation of pre-existing T cell responses. Tumours exploit co-inhibitory pathways, termed immune checkpoints, to escape tumour-specific immune responses. New therapies are being developed to block these pathways with monoclonal antibodies to enhance anti-tumour immunity. CTLA-4 and PD-1 blockade have gained the most clinical momentum. Clinical responses with anti-CTLA4 mAbs indicate that T cell responses are necessary, but not sufficient, for anti-tumour immunity in some patients. Long-term survival has been observed in patients with no evidence of disease for decades with CTLA-4 blockade. Blockade of PD-1 and PD-L1 mAbs have demonstrated the ability to achieve durable complete responses in a host of malignancies from melanoma, lung cancer, head and neck cancer, kidney cancer, and beyond. Safety of immune checkpoint blockade is markedly different than that of cytotoxic therapies.

Understanding the mechanisms of action of immune checkpoint mAbs, their clinical activity, and potential safety issues will be critical for further development and expansion of the most active agents. Inhibitors of the immune checkpoints CTLA-4, PD-1, and PD-L1 are working their way rapidly into routine clinical practice. To ensure the optimal and least toxic application of these agents, an understanding of their mechanisms of action and preclinical models that have depicted in vivo efficacy as monotherapy and in combination with novel agents are explored.

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