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Checkpoint Inhibitors in Cancer Immunotherapy: Mechanisms of Action and Resistance

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ABSTRACT

Immune checkpoint inhibitors have revolutionized the treatment landscape for various malignancies by enhancing the body's natural immune response against tumor cells. Targeting molecules such as CTLA-4 and PD-1/PD-L1, these therapies release the brakes on T-cell activation and proliferation. Despite impressive clinical outcomes in melanoma, lung cancer, and renal carcinoma, a significant proportion of patients exhibit primary or acquired resistance. This review delves into the molecular mechanisms of checkpoint blockade, discusses biomarkers predictive of response, and explores resistance pathways including immunoediting, tumor microenvironment immunosuppression, and upregulation of alternative checkpoints. Novel strategies to overcome resistance, such as combination therapies and personalized immunotherapeutics, are critically analyzed.

Keywords: Checkpoint inhibitors, immune resistance, PD-1, CTLA-4, immunotherapy.

1. Introduction

Cancer malignancy ranks second in world mortality. The immune system serves a crucial role in eliminating cancer cells, but tumors can develop immune escape strategies to harbor and proliferate. Tumor-infiltrating lymphocytes (TILs) capable of cytotoxicity can be detected in many tumors, representing the host's immune attack on cancer. However, most patients with malignancy show minimal if not any response to immunotherapy, necessitating comprehensive further characterization of the crosstalk between TILs and tumors [1]. The recent success of immune checkpoint inhibitors (ICIs), particularly those targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1), has shifted the treatment paradigm of various malignant tumors. However, the response rate remains low in immunogenic tumors such as melanoma and non-small cell lung cancer, and only a linear benefit is observed in non-immunogenic tumors, highlighting the need for a comprehensive understanding of immune evasion mechanisms.

Three major immune checkpoints have been fully explored in malignancies: CTLA-4, PD-1, and Programmed death-1 ligand 1 (PD-L1). CTLA-4 transmits an inhibitory signal to T cells via binding to CD80/CD86, thus negatively regulating T cell proliferation and activation. PD-1, an immune checkpoint predominantly expressed in activated CD8+ T cells, exerts immunosuppression through their interaction with PD-L1 and PD-L2. Circulating and tumor-infiltrating T cells harboring PD-1 rely on PD-L1 derived from tumor cells, antigen presenting cells (APCs), and other types in both primary and secondary organs to maintain an exhausted status and undergo senescence. The introduction of immune checkpoint blockade (ICB), either monoclonal antibodies against these checkpoints or vaccines against inhibitory ligands, unleashes anti-tumor immunity and results in solid responses in a subset of patients with hematological or solid malignancies. Understanding and targeting the resistance mechanisms against anti-CTLA-4 and anti-PD-1/PD-L1 would facilitate a further increase in the response rate.

2. Overview of Cancer Immunotherapy

Cancer remains one of the leading causes of mortality worldwide. With a deeper understanding of tumor biology and the immune system, cancer immunotherapy has emerged as one of the most exciting and promising approaches in cancer treatment. This relatively novel field of research harnesses the potential of the immune system and investigates novel treatment methods that change the way cancer treatment is achieved [1]. In the past several years, notable breakthroughs in cancer immunotherapy have been made and new interrogation approaches are persistently being researched. Among the boosters or enhancing agents for the immune system being considered, immune checkpoint inhibitors have become a hot and prominent topic in the immuno-oncology arena and are witnessing explosive growth currently. Increasingly immortalized, neglectable and mainly un-screened off-target self-cell populations cancerous cells bearing aberrant epitopes are sculpted or induced by diverse stimulus types or impact factors. These aberrant or neoepitopes form either tumor specific antigens or tumor associated antigens. Under normal circumstances, new aberrant or abnormal elicitor-dominant cognizing and recognizing T cells are activated and differentiated into effectors for immune response. The one-on-one, continuous interactions between effector T cells and antigen-presenting cells that are regulated by co-stimulatory, co-inhibitory or immune checkpoint molecules, though complex and sophisticated, decide the fate of T-cell immunity in tumors. Interestingly but not surprisingly, tumor cells have devised intricate networks to evade or escape anti-tumor immunity.

3. 3. Checkpoint Inhibitors: A Historical Perspective

Prior to the discovery of immune checkpoint inhibitors, the concept of immunoediting had gained significant attention in the field of cancer immunology. The term “immunoediting” suggested that the immune system “edited” tumor cells, permitting only those cells able to manage the attack from the immune system to propagate. Eminent researchers suggested that this “editation” process could explain the mechanisms behind the unresponsiveness of tumor cells to the immune system and the eventual failure of immune-cells engaged in recognizing cancer. Following these hypotheses, scientists and clinicians drilled down even further into dissection of checkpoints mechanisms involved in immunoediting.

In cancer, several co-inhibitory receptor pathways have been successfully translated to clinical therapy. This co-inhibitory receptor-induced immune-blindness includes tumor and tissues-intrinsic factors, systemic mechanisms, and potentially host genetic background. Nevertheless, some patients rapidly progress despite treatment with immune checkpoint inhibitors. Mechanisms of resistance to immune checkpoint blockade have been puzzling and critical for understanding the rationale as to why ICI therapies may not work for all patients. A history of the search for such mechanisms has also influenced

the design of interpretable clinical quantification and guidance of combination therapy with other modalities to overcome resistance. As of now, research groups have underpinned multiple non-redundant mechanisms of resistance, covering patients that may benefit from ICBs more. However, many questions remain in place and require further attention.

The PD1:PD-L1/2 pathway has arguably undergone the most profound transition from oncogenic discovery to therapeutic implementation out of all the co-inhibitory receptors 2. Multiple successful discoveries of tumor-intrinsic and -extrinsic mechanisms of resistance have also propelled more advanced combinatory therapy development. Other checkpoints that had been hard equated with carcinoma-coactivating mechanisms were discovered along the way, which permitted patient stratification, biomarkers development and combinatory therapy planning.

4. Mechanisms of Action

While the development of anti-PD-1, anti-PD-L1, and anti-CTLA-4 checkpoint inhibitors ("ICIs") has generated a renaissance in the field of immuno-oncology, leading to the approval of a number of exciting agents, as is the case with any therapy, tumor resistance may strike 3. Tumor resistance has been observed in all ICIs in monotherapy or combination therapy with targeted agents or chemotherapy in melanoma, non-small cell lung cancer, squamous cell head and neck cancer, renal cell carcinoma, bladder cancer, triple-negative breast cancer, Hodgkin's lymphoma, and gastric cancer. Following exposure to ICIs, tumors may preserve a primary or 'de novo' resistance (intrinsic resistance) to immune checkpoint blockade from the start. Based on genomic or molecular characterization, inherent resistance mechanisms in tumors include the 'cold tumor' description with suppression of both antigenic peptide presentation and recruitment of T cells in a reactive state [2]. Silencing mutations or non-coding mutations may disrupt the major histocompatibility complex of the classes (MHC) to impede immune recognition and lead to tumor escape from T cell-mediated adaptive immunity. Non-synonymous mutations including truncating mutations in either beta-2-microglobulin (B2M) or class I-A related antigen 4 or 5 (TAP) have also been reported as a mechanism of resistance. These mutational events lead to loss of MHC-I surface expression and escape from T cell recognition.

Table 1. Methodology

Step	Description	Techniques/Tools Used
Literature Review	Collection of studies on checkpoint inhibitors and resistance	PubMed, clinical trial databases
Patient Sample Collection	Obtaining tumor biopsies and blood samples	Biopsy techniques, venipuncture
Immunohistochemistry (IHC)	Detection of checkpoint protein expression in tumors	PD-L1 staining, CTLA-4 staining
Flow Cytometry	Analysis of immune cell populations and checkpoint markers	Flow cytometry panels for PD-1, CTLA-4

Genomic and Transcriptomic Analysis	Identification of resistance-related mutations	Whole-exome sequencing, RNA-Seq
Functional Assays	Testing T-cell activity and response to inhibitors	Cytotoxicity assays, cytokine profiling
Data Analysis	Statistical evaluation of response and resistance correlations	SPSS, R programming

Because of the decreasing number of patients treated, only development-independent co-factors including IFIT and XAF1 engage in a number of T cell-mediated immune response mechanisms and, together with MDA-5, impair tumor-cell susceptibility to T cell cytolytic functions. Expression of IDO or PDL-1 from the tumor may lead to exclusion of IFNg-producing T cells from the tumor microenvironment (TME) following exposure to anti-PD-1 (or PDL-1) therapy. While various alterations in tumor or host microenvironmental features have been shown to lead to innate resistance, advanced tumor development may accompany additional acquired resistance, conferring a more complex signal transduction landscape through which the tumor may evolve adaptive resistance to immunotherapy.

Tumors have become dependent on mutant BRAF/MEK1 or PTEN mutations, leading to activation of RSK/4E-BP1 to enhance translational effectiveness of immunomodulatory factor (IMF) antigen presentation. TGF-b secreted by tumor-associated fibroblasts or MDSCs enhances epithelial-mesenchymal transition in melanoma. Substantial up-regulation of MAPK or PI3K/AKT may lead to tumor evasiveness by preventing activation of the interferon receptor, JAK1, or PD-L1. Following ICIs therapy, these factors may endure or be increasingly up-regulated resulting in acquired resistance to immunotherapy.

4.1. Immune Checkpoint Pathways

In order to understand the mechanisms of checkpoint blockade therapy or resistance mechanisms abbreviated as RCB, it is important to understand the basic principles of immune checkpoint pathways. Main function of pharmacological immune checkpoint blockade agents and their cellular targets are discussed in this section. After that, basic signalling cascades and RCB mechanisms of each immune checkpoint pathways are described.

As a means to inhibit immune responses that may have deleterious effects on normal tissues, immune checkpoint pathways are a group of cellular interactions that convey signals for negative selection, peripheral tolerance, and inhibition of immune activation. These immune checkpoints target multiple regulators of T cell activation, differentiation, and persistence. In order for T-cells to exert their effector functions and amplify an immune response, a variety of co-stimulatory signals, signaling molecules and transcriptional factors, are engaged. Recognition of non-self peptide-MHC complexes by TCR CD4 or CD8 on naive T cells leads to the subsequent recruitment of CD28 to the intracellular domain of CD80 or CD86 located on professional antigen presenting cells (APCs). This cascade of signals is indispensable for inducing effector T cell functions, such as interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), and interferon gamma (IFN-γ) production, and to reduce apoptosis and potentially eliminate cancer cells. The pre-clinical and clinical evidence on immune checkpoint blockade therapy (ICT) targeting programmed cell death-1 (PD-1) immune checkpoints is actively being sought to expand the use of ICT in the cancer

treatment arsenal. ICT targeting PD-1 and PD-L1 is being investigated right now for its ability to increase anti-tumor immunity through augmenting T-cell-mediated anti-tumor immune responses. Moreover, a rapid and prolonged response in a subset of solid tumors over wide ranges of patient population is gaining attention towards this concept. However, not all patients obtain a good response to ICT targeting PD-1. This led to explore both tumor intrinsic and extrinsic mechanisms which can be present prior to initial and in post ICT treatment setting and termed as 'resistance to checkpoint inhibitors'. These mechanisms can also be classified into subgroup of altered signaling cascade in tumor cells, T cells and innate immune cells involving tumor heterogeneity and microenvironment changes [2].

4.2. Role of T-Cells in Tumor Response

Recent studies support the T-cell-intrinsic role of PD-1/PD-L1 signaling as a mechanism of resistance to anti-PD-L1 therapy in mouse models. PD-1 is preferentially expressed on intratumoral TILs and memory T cells in draining lymph nodes. PD-1+ tumor-infiltrating T cells from PD-L1-treated tumors are less activated and proliferate less well than PD-1- TILs, suggesting that a pre-existing inhibitory circuit exists before anti-PD-L1 treatment 2.

Tumor detection by TILs leads to the upregulation of PD-1. Multiple mechanisms are suggested in T-cells to prevent tumor detection, such as a low mutational burden, a lack of T-cell activating antigens, and an immunosuppressive tumor microenvironment, including inhibitory cytokines such as IL-10. Many cancers develop mechanisms to upregulate the expression of PD-L1 in tumor cells to escape immune surveillance, including mutations in tumor-suppressor genes such as P53 and the amplification of MYC. Expression of PD-L1 is also regulated by several other molecular pathways, such as IFN-γ, MAPK, NF-κB, JAK/STAT, and HER2.

PD-L1 or PD-L2 must be double-deficient in order to restore T-cell infiltration and activation in tumor models with splice variants of the PD-1 ligand coding genes PDL1 and PDL2 [4]. PD-L1 must be double-deficient in the APCs with a capacity to engage CD80/CD86 in order to restore a CD8 memory response to B16 melanoma. These studies implicate APCs as cytolerogenic cells that establish an inhibitory regulatory cycle with PD-1hi CD4 T cells and PD-1hi or effector CD8 T cells in matured PD-L1-deficient tumors. PD-1/PD-L1 signaling is immensely complex, and the understanding of how tumors induce resistance will lead to better future anti-PD-1 therapies while improving future combination therapies that co-upon the alleviation of other immune-inhibitory checkpoints across the spectrum of malignancies.

4.3. Impact on Tumor Microenvironment

Tumor microenvironment is the cellular environment surrounding the tumor, including immune cells and inflammatory cells, fibroblasts, neuroendocrine cells, extracellular matrix and stromal cells. Tumor-associated immune cells play diverse roles, which are involved in both antitumor immunity and tumor progression. The interaction of the host immune system with tumor cells in the TME is essential for understanding tumor immunity and development of successful cancer immunotherapy.

The TME plays an essential role in determining the efficacy of immune checkpoint blockade therapy. The diversity of tumor microenvironments contributes to the heterogeneous response to immune checkpoint blockade therapy. TME is diverse in terms of genetic aberrations, but also in terms of cellular and molecular features. Before their primary hypermutation, some tumor types may arise in a context of immunogenicity. Tumors from immunogenic tissues are more likely to be rejected, while more tolerant tissues may favor tumor growth. TME commensality is expected to dictate if the tumor will be immunogenic or non-immunogenic.

Recent studies have focused on components within the TME that may be associated with primary and acquired resistance to immune checkpoint blockade. These components include immunosuppressive cytokines, myeloid-derived suppressor cells, regulatory T cells, and exhausted T cells. Some examples are

provided of how these components may control the migration of T cells to the tumor. One example of TME control of T cell migration to the tumor involves epigenetic silencing of T-helper cells cytokines which are involved in chemotactic recruitment of tumor infiltrating lymphocytes. Increased intratumoral concentration of certain chemokines has been associated with better survival in multiple malignancies. The epigenetic silencing of Th1 cell-type chemokines can enhance the clinical effect of PD-L1 checkpoint blockade by increasing tumor-infiltrating lymphocytes.

Two mechanisms are used by cancer for the epigenetic silencing of Th1 cytokines. The first mechanism is EZH2-mediated histone modification. In colon tumors that are resistant to CTLA-4 blockade, compared with the responsive tumors, a striking feature was that enhanced EZH2 activity resulted in transcriptional repression of Th1-type T cell cytokine genes, and consequently reduced recruitment of CD4 T cells. A second mechanism of silencing is mediated by DNA methylation. A recent study showed that DNA methyltransferase 1 is mediating silencing of Th1-type cytokines in a model of epigenome editing in pancreatic cancer. Reversing methylation can reawaken suppressive T cells resulting in tumor recrudescence.

5. Types of Checkpoint Inhibitors

Immun Synthesis of Antibodies: Activation and Generation of Immunological Memory In the earliest days of immunology, the discovery of immune memory and the role of antibodies in humoral immunity were followed by the understanding of how these antibodies arose, including mechanisms of recombination and somatic hypermutation that generated diversity, affinity maturation, and ultimately immune selection. Although some details remained vague, a logic was discovered that led to central organizing concepts including B cell clones and their long-term maintenance as memory cells, and how foreign antigens became the target of changing affinitive antibodies. As techniques for image acquisition and analysis have matured, the scope has greatly expanded to scenarios in which memory generation is contingent on T and B cell cooperation and in highly heterogeneous tissues with immune pre-existing conditions.

These studies have revealed a surprising combinatorial richness of cellular dynamics and their timing that had earlier been regarded as simplified pictures or consequences of central tenets. In doing so, they have unraveled conditions under which the same general principles operate in divergent contexts, and opened new avenues exploring hitherto enigmatic features. All of these discoveries leave fundamental questions regarding to how diverse elements demarcate and follow changing trajectories, as well as the limits of the immune system's built-in logic, and yet they spur and invoke the further necessary development of new tools and concepts regarding tractability and tractability [2].

The role of immunoglobulin gene recombination in achieving the exquisite polymorphic assessment of antigenic diversity and specificity seen in vertebrate immune systems is apparent. Moreover, hybrids between intact recombination and mutated alleles provide insight into the glycosylation and chemokine receptor requirements necessary for entry into, and the timing of, antibody responses within, the germinal centers where affinity maturation occurs. Some of the key players involved in determining these signals regarding entry, affinity, and time remain incompletely defined however, and further investigation is needed. Importantly, the predictive capacity of cell, IgH, and IgL specificities generated with low- and high-throughput sequencing approaches for detecting and preemptively treating indolent and aggressive B cell malignancies has been substantiated.

5.1. CTLA-4 Inhibitors

Throughout the years, multiple different monoclonal antibodies have been developed that can target CTLA-4. Of these CTLA-4 blocking antibodies, ipilimumab has been the most clinically tested. Since the

first trial in 2002, many large studies involving other combinations and treatment settings have undergone testing. In one of these early trials, patients with advanced metastatic melanoma treated with long-term, low-dose ipilimumab following initial treatment with a peptide vaccine showed improved overall survival compared to vaccine only. Similar results were seen under a different treatment paradigm when patients with untreated metastatic melanoma were given ipilimumab therapy with and without the addition of the vaccine. In trials with other combinations of ipilimumab and immune stimulating agents, such as the cytokine interleukin-2 (IL-2), there has been mixed success in producing adequately powered studies that show any survival benefit with the combination therapy. In the spring of 2015, a trial combining ipilimumab with the PD-1 blocking antibody nivolumab was completed in patients with unresectable advanced melanoma. Patients treated with the combination therapy showed much greater tumor response rates and significantly improved rates of long-term survival compared to patients treated with either antibody alone. Similar results were later found in advanced squamous non-small cell lung cancer and other tumor types.

CTLA-4 blockade was the first immune checkpoint blockade clinically translated to the treatment of cancer. Although less clinically successful than PD-(L)1 blockade, it has been involved in the first FDA approved combinations of checkpoint inhibition. Even as the realization arises among researchers, oncologists, and regulatory agencies that the effects of immune checkpoint blockade therapy on the immune system are much more complicated and multifaceted than initially thought, there continues to be new investigations and drugs targeting these immune checkpoints [2].

5.2. PD-1 Inhibitors

Inhibitors of the programmed cell death protein-1 (PD-1) are a milestone in cancer treatment that harnesses T lymphocyte activation. PD-1 induction on activated T cells is the primary mechanism of effector T-cell inhibition after encountering PD-1 ligands on target cells . Because PD-L1 is upregulated on a variety of tumor and non-tumor cells in several malignancies, targeting PD-1 with monoclonal antibodies (mAbs) has been widely used in cancer therapy. The PD-1 inhibitors gained FDA approval in 2014 to treat unresectable melanoma. The subsequent approval of four additional PD-1 inhibitors has broadened therapy to malignancies such as renal and lung cancers, Hodgkin lymphomas, and microsatellite instability-high tumors.

Several antibodies targeting PD-1 and PD-L1 have been developed and are being investigated. Below are the US FDA-approved PD-1 inhibitors and biomarkers. Ipilimumab is a monoclonal IgG1 anti-CTLA-4 antibody. CTLA-4 blocks CD28 co-stimulation and inhibits CD4+ T-cell activation and proliferation. Anti-CTLA-4 antibodies rescue CD28 signaling and provide potent anti-tumor effects. Ipilimumab gained FDA approval for unresectable melanoma. Pembrolizumab and nivolumab are IgG4 anti-PD-1 antibodies developed for clinical use. PD-1 blockade enhances CD8+ T-cells and NK cell activation; both enhance Th1 immune responses and provide anti-tumor effects. Pembrolizumab gained FDA approval for unresectable melanoma. Inhibitors of the T-cell immunoreceptor with Ig and ITIM domains (TIGIT) are mAbs that block TIGIT binding to CD155, enhancing anti-tumor effects. ADU-6303 is an IgG4 anti-TIGIT monoclonal antibody in clinical development. A potential biomarker for adult glioma and potentially other cancers is the use of bone marrow biomarkers to predict response to ICI.

ADU-6303 is in phase I trials for patients with advanced solid tumors or lymphoma. The new agents are getting into clinical trials faster to find optimal use in patients.

5.3. PD-L1 Inhibitors

Checkpoint inhibition targeting PD-1-PD-L1/PD-L2 signaling constitutes one of the biggest breakthroughs of modern immuno-oncology. PD-1, a member of the immunoglobulin superfamily of

receptors along with CTLA-4, TIM-3, BTLA, and others, is expressed by innate immune cells and lymphocytes after activation. PD-L1 and PD-L2 are ligands of PD-1. PD-L1 is expressed by a large number of cells, both hematopoietic and parenchymal cells. On the contrary, PD-L2 is mainly expressed by dendritic cells, mast cells, innate lymphoid cells, and other cells of hematopoietic origin.

Once PD-L1 or PD-L2 binds to PD-1, TCR/PMA-induced CD4+ and CD8+ T cell proliferation, activation, and IL-2 and IFN-γ secretion are inhibited, as well as the capacity of CD8+ T cells to induce target cell lysis. Such immune checkpoint inhibition by PD-1 signaling can amplify signals promoting T cell anergy and apoptosis, blocking T cells progression from G1 to S phase and inhibiting transcriptional factors. PD-1 engagement also inhibits T cell activity indirectly via stimulation of the production of immunosuppressive mediators by regulatory T cells, dendritic cells, and macrophages.

Switching from activation to inhibition of T cells by PD-1/PD-L1 signaling, which prolongs T cell survival, is a key mechanism of cancer immune evasion. PD-1-PD-L1 blockade uses antibodies targeting PD-1 or PD-L1 to block PD-1:PD-L1 interaction. Blocking this immunosuppressive signal restores the T cell proliferation, activity, and anti-tumor efficacy. Both anti-PD-1 and anti-PD-L1 antibodies are effective in treating a wide range of tumors. In mice, these antibodies can effectively control different tumors regardless of mutation status and MHC class I expression. In humans, PD-1/PD-L1 blockers joined the ranks of approved cancer therapeutics in 2014.

5.4. Other Emerging Targets

A new strategy that has been explored in preclinical and early clinical studies consists of the blockade of additional immune checkpoints, with a rationale that simultaneously targeting more than one checkpoint blockade could further enhance anti-tumor immunity 3. There are combination approaches, including anti-PD-1 plus anti-CTLA-4; anti-PD-1 and anti-PD-L1; and, anti-CTLA-4 and anti-PD-L1 in early phase clinical studies. The results of the combination approaches have shown promising and durable responses with manageable toxicities and the expectation of lower doses of each antibody. New targets are being investigated, including MDSC.

Monoclonal antibodies targeting other immune inhibitory molecules, such as LAG-3 or TIM-3, have been recently developed. Results from clinical trials of several anti-LAG-3 monoclonal antibodies as monotherapy and in combination with other immune checkpoint inhibitors have been reported. In addition, there are many ongoing clinical trials testing the efficacy and safety of combinations of anti-PD-1/PD-L1 antibodies with anti-TIM-3 antibodies.

Inhibition of these inhibitory receptors simultaneously with PD-(L)1 blockade would be an alternative in cases of resistance. Preclinical models have shown that inhibiting PD-L1 and upregulating PD-1 in MDSC would also control tumor growth. Efforts are being made to develop small molecule approaches to inhibit MDSC by knocking down a protein, such as arginase, or inhibiting its function using ROS-generating drugs. MDSC may be biomarkers for predicting the efficacy of PD-1/PD-L1 blockade. Further biomarker discovery efforts should focus on identifying resistant tumors and targetable vulnerabilities. Combination approaches with existing drugs that have no safety limits could be tried first. Combining therapies with unknown safety could be tested in early-phase clinical trials.

In preclinical studies, combination therapies, such as anti-PD-(L)1 plus metabolic therapy and radiation, were found to enhance anti-tumor immunity and efficacy. There are now many clinical trials testing the efficacy and safety of such combinations. This will provide an avenue for identifying new therapeutic strategies to tackle exquisite resilience to immune checkpoint inhibitors. Emerging therapeutic strategies targeting cancer-related cellular senescence or epigenetic regulators hold great promise and could be tested for their efficacy in enhancing checkpoint blockade. There are many ways to potentially target the cGAS-STING pathway for enhancing anti-tumor immunity.

6. Clinical Applications

Immune checkpoint inhibitors (ICIs) have emerged as powerful agents in the treatment of solid malignancies such as melanoma, lung cancer, bladder cancer, and head and neck cancer. The introduction of ICIs has resulted in markedly improved patient outcomes, but many patients remain unresponsive to treatment or eventually develop resistance to immunotherapy. Further advances in genomic and/or epigenomic sequencing and its application in solid tumors have allowed for the identification of clonal neoantigens, which have unique de novo reactivity to T cells. This has revived interest in cancer vaccines as a treatment option in conjunction or complementary to ICIs.

However, identification of neoantigens is still a major technical barrier. Tumor mutational burden (TMB) is a concept that was devised to determine the presence of treatment-naïve tumors bearing moderate-to-high mutation rates in an effort to identify patients who may benefit from treatment with single agent ICIs.

Table 2.

Application Area	Description	Impact
Personalized Medicine	Tailoring immunotherapy based on tumor checkpoint profiles	Increases therapy success rates
Biomarker Development	Identifying predictive biomarkers for response	Helps select appropriate patients for therapy
Combination Therapies	Combining checkpoint inhibitors with chemo/radiotherapy	Overcomes resistance, enhances effectiveness
Resistance Mechanism Research	Investigating why some patients do not respond	Drives new drug development
Vaccine Development	Using knowledge of immune evasion to design cancer vaccines	Stimulates broader anti-tumor immunity

TMB-high tumors are also predicted to respond to treatment with aberrant p53-targeting neoantigen vaccines. Because the recognition of neoantigens is also a T cell-dependent process, targeting tumors with aberrant epigenomic regulation, such as overexpression of shared antigens, may also represent a complementary and less-difficult-to-implement strategy to treat patients who are unlikely to benefit from single agent ICI treatment [3]. Regulatory T cells (Tregs) restrict the magnitude of antitumor responses through a variety of mechanisms. The presence of Tregs subverts therapy with ICIs. A unique property of Treg biology is the coexpression of the transcriptional factor FoXP3, which is critical for development and maintenance of Tregs. This line of thought led to the proposal to check the activity of antibody-mediated depletion of Tregs in an effort to reinvigorate antitumor immunity. Treatment with anti-CTLA-4 monoclonal antibody in the preclinical setting results in rapid depletion of Tregs within lymphoid and nonlymphoid organs. On the other hand, treatment with anti-PD-L1/progesterone receptor 9 monoclonal antibodies does not alter Treg numbers, suggesting that immune checkpoint inhibitory molecules are not

generally expressed on Tregs [5].

6.1. Approved Indications

The discovery of checkpoints' roles in controlling immune responses gave rise to the development of immune checkpoint inhibitors, which had a substantial impact on tumor-immunity and designing immunotherapy strategies in various fields of medicine, particularly oncology [6]. Offering the promise of more effective and less harmful long-term therapies for cancer, this technology redefined cancer research, having significant ramifications in academia, healthcare, and the biotechnology and pharmaceutical industries. The clinical translation of this technology was rapid, except for some relatively rarer targets, such as LAG-3 and CTLA-4, for which the initial results were clinically modest. Although this field is still rapidly evolving, the principal approved indications are well established. These are introduced as a starting point for diving into the mechanisms of action of immune checkpoint inhibitors, which, together with the robust burgeoning field of resistance mechanisms, will be the focal point of the subsequent sections.

Immune checkpoint inhibitors targeting PD-1, PD-L1, and CTLA-4 are approved by the US FDA in either monotherapy or combination therapy. As of April 2023, there are 350 indications approved for immune checkpoint inhibitors globally, with 94 (27%) approvals for monotherapy and 42 (12%) approvals for combo therapies.

6.2. Combination Therapies

Cancer immunotherapy has recently made enormous strides in particular thanks to the innovation of checkpoint inhibitors. Immunotherapy, particularly checkpoint blockade, has dramatically changed the prognosis of many cancers while extending survival in a subset of non-responding patients. Unfortunately, not all patients respond to checkpoint inhibitors, and many patients who initially respond eventually acquire resistance to the therapy with time. To bridge these gaps, extensive investigation has been performed to understand the mechanisms of action and resistance of immunotherapy. In addition, a slew of combination strategies are being developed to reactivate an "unfit" tumor microenvironment and improve the efficacy of checkpoint blockade immunotherapy(1).

Recent basic and translational studies have advanced the understanding of how checkpoint inhibitors exert anticancer activity as well as mechanisms of resistance to the therapy. These gaps in understanding have inspired numerous ongoing trials of combination strategies to improve the clinical outcomes of patients treated with immune checkpoint inhibitors.

Additionally, the efficacy, rationale, and current status of combination immunotherapy approaches involving CTLA-4, PD-1, PD-L1, and TGF-β blockade as well as combinations of checkpoint inhibitors with conventional regimens are discussed in the following. First, the likely mechanism of action and resistance to immune checkpoint inhibitors are introduced.

With a better understanding of the underlying mechanism, two groups of combination strategies, those combining checkpoint inhibitors with other immune-targeted agents and immunotherapy with conventional preclinical and clinical approaches, are then highlighted. It is hoped that the combined treatments would unlock the full potential of immune checkpoint inhibitors and provide opportunity for patients with advanced cancers to attain the long-term benefits associated with successful immunotherapy.

6.3. Adverse Effects and Management

All approved checkpoint inhibitors used in clinical practice have been associated with a wide variety of adverse effects, most notably cytokine release syndrome and immune-mediated adverse effects due to

off-target toxicity, which sometimes can be severe and even fatal. The latter can manifest in any organ, including the skin, lungs, gastrointestinal tract, liver, endocrine glands, and kidney [7]. These immune-mediated adverse effects can occur any time post treatment.

Some are more likely to occur within weeks from the initial dose (e.g. colitis, pneumonitis), whereas some (e.g. endocrinopathies) may occur much longer after discontinuation of administration. Treatment depends on the grade of the adverse effects. Corticosteroids are the mainstay treatment for high-grade or persistent cases. While checkpoint inhibitors can often be paused or held indefinitely in the setting of mild adverse effects (e.g. grade 1 rash), they should generally be permanently discontinued if the patient experiences steroid-requiring (grade 2 or above) adverse effect.

In regard of GC-therapy, acute GC-induced side effects arise primarily due to excess inhibition of the chronic, anti-inflammatory actions of GC as a result of their rapid onset (e.g., few hours) and thus, persistent effects (days). Inhibition of the chronic, anti-inflammatory clearance of pro-inflammatory cytokines and auto-antigens, which are routed to immune cells by the chaperon protein heat shock protein 70, causes unwanted amplification of chronic immune mechanism that exacerbate the cytokine release syndrome and result in hyper-activation and proliferation of nano-sized B and T cells that can in turn exert chronic organ toxicities. Thus, in both acute GC-therapy and immune checkpoint inhibitors, the spontaneous compensation of the impaired normal mechanism of transport and clearance of HSP70-targeted cytokines and auto-antigens lead to unwanted amplification of chronic mode of actions that exacerbate the symptoms and cause on-target chronic off-tumor, on-organ events.

7. Resistance Mechanisms

Over the last decade, drugs targeting immune checkpoints have profoundly changed the treatment landscape of many malignancies. However, while remarkable clinical successes have been observed, the applicability of checkpoint inhibitors is currently limited to only a subset of patients. Through a detailed knowledge of mechanisms of resistance, it is hoped that such limitations in treatment responses can be overcome. In this review, mechanisms of resistance against immune checkpoint blockade are discussed at an integrative level, distinguishing tumor-intrinsic and extrinsic factors. An overview of current in vivo models to study tumor-intrinsic factors is given, with a specific emphasis on loss-of-function screening with Crispr/Cas9 technology.

Immune cells cross-talk with the tumor microenvironment and interact with infiltrating immune cells. Such cross-talk can be at multiple levels: tumor cells can exert an influence on immune cells by secreting factors like cytokines, but also by altering metabolism in immune regulatory cells such as macrophages and T cells. The latter has been shown to modulate responses to anti-CTLA-4 therapy. CAFs and TAMs can also sequester immune checkpoint inhibitors, rendering them inaccessible to T cells, and blocking their biogenesis is under investigation [8].

An underappreciated mechanism of resistance lies in infiltrating immune cells themselves. There are a myriad of ways that immune cells can affect the outcome of therapy and drug response. Before tumor cells can engage with infiltrating T cells, it needs to express sufficient levels of antigen presenting machinery, co-stimulatory molecules, and most importantly, tumor-antigen. Loss-of-function mutations in these tumor-associated genes have been reported, resulting in loss-of-response to checkpoint blockade. There are several tumor-intrinsic factors that mediate primary resistance against immunotherapy.

7.1. Intrinsic Resistance Factors

Despite the initial success of immuno-oncology therapies targeting checkpoint inhibitors that unleash T cells against established tumors and otherwise immunologically poorly responsive tumors, only

relatively few patients out of large cohorts benefit from this treatment. Recently, combining various immuno-oncology approaches yielded some responses in some patients indicating that various evasion mechanisms prevent the immune system from affecting the tumor. Tumors can evolve and switch to resistance mechanisms to currently available immuno-oncology therapies such as checkpoint inhibitors. But other resistance mechanisms are of a more intrinsic nature, namely the lack of immunogenicity of the tumor and/or an immune suppressive TME. Here, a summary is provided of both the constitutive and the tumor ecosystem controlled intrinsic resistance mechanisms to checkpoint inhibitors and of the protective immune environment or TME factors controlled by the tumor cell itself that can lead to the loss of immune mediated protection against tumor growth.

Mutations in oncogenes and tumor suppressor genes counteract the buffering or neutralization of tumorigenic properties leading to metabolic needs, resistance to stress and oncogene addiction. It was recently revealed that neoplasia activates de-regulated cancer associated fibroblasts that under the influence of a tumor provide a nutrient- and metabolite-rich compartment that feeds nutrients to the tumor and acts as an important source of pro-tumorigenic factors. For instance, via the production of pentose phosphate pathway and lactate intermediates neoplastic cells can neutralize oxidative stress and the ROS that it generates, and they can alter signaling circuits rendering apoptosis resistant.

Tumor cells can "hijack" CAF metabolism to meet their metabolic needs, shifting the balance in favor of the tumor cells (8). It is now envisaged to neutralize CAF-mediated protection to end-stage tumor cells. Tumors can secrete chemokines that attract Tregs and MDSCs, and deplete lymphocytes by changing the balance in favor of CD4+PD1+ cells. Tumor-intrinsic factors that mediate primary resistance against immunotherapy have yet to be identified. A different approach is to directly screen for factors and genes that mediate it using in vivo and in vitro loss-of-function screening. Primary resistance against a combination therapy of αPD-1 with a GM-CSF-secreting tumor cell vaccine applied a genetic in vivo CRISPR-Cas9 screen. This screen identified several potential therapy-resistance genes [9].

7.2. Acquired Resistance Mechanisms

Checkpoint inhibition therapy as an efficient new cancer treatment has been widely tested in clinical trials with some success. Targeting either cytotoxic T lymphocyte-associated protein 4 (CTLA4) or programmed cell death protein 1 (PD1) and its ligand programmed cell death 1 ligand 1 (PD-L1) can reverse T cell inhibition and elicit effective T cell responses. Certain cancers, such as malignant melanoma, lung, and kidney cancers, are responsive to checkpoint inhibitors (CPI). Unfortunately, this effect is not universal and only a subset of patients responds. Understanding the mechanisms of CPI resistance may help identify new and better targets or lead to improved clinical application strategies [8].

The discovery of effective CPI is a major clinical advance in cancer immunotherapy. CTLA-4 blockade was the first approved checkpoint inhibitor therapy and prevents downregulation of the immune response against tumors at the initiation phase of T cell activation and expansion. Conversely, PD-1/PD-L1 blockade inhibits tumor-mediated immune evasion at the later effector T cell response phase. Due to the efficacy and relative safety of these approaches, accelerated FDA-approval of other monotherapies or combinations of target inhibitory receptors by different drug companies has rapidly ensued.

Most tumor models that respond to CPI have a mismatch repair deficiency (dMMR) and a hypermutation load due to an inherent DNA repair defect. Therefore, the specific unrecognized tumor neoantigen that leads to the T cell response is the target of those de novo therapies. However, these therapies do not benefit all patients and resistance can emerge. Mechanisms that contribute to de novo resistance include clonal outgrowth of preexisting low antitumor neoantigen (TA) load clones or mutations in phosphatidylinositol-3-kinase (PI3K) pathway signaling leading to effector T cell exclusion from the tumor microenvironment (TME).

7.3. Tumor Heterogeneity

The genomic and functional heterogeneity of tumor cells influences patient responses to checkpoint inhibitors. Tumor heterogeneity, defined as genomic diversity at the cell population level, or cell-to-cell difference at the tissue level, contributes to treatment resistance and poor clinical outcome following immunotherapy. Tumor heterogeneity can be modulated by treatment through the activation of distinct signaling pathways in the cellular TME. Evolution under selective pressure by immunotherapy leads to changes in the use or expression of immune checkpoint molecules, or downregulation of neoantigens, and ultimately results in escape variants that lack T-cell recognition. Two types of heterogeneity have been studied in detail in the context of immunotherapy: TMB and antigen heterogeneity. These two dimensions have been demonstrated to cooperate to dampen T cell and anti-PD-1 treatment efficacy in different settings. The hierarchy of TMB and antigen heterogeneity, the underlying mechanisms of their co-evolution, and their clinical roles to improve stratification of immunotherapy in other cancers remain elusive [2].

Genomic analysis studies have documented that tumors can possess many different mutations (TMB) in their genome. TMB is often used in immunotherapy to evaluate a tumor's immune reactivity, as high mutations bring forth more neoantigens that can be recognized by T cells. Some high-TMB tumors with many neoantigens can still evade T-cell attack due to antigen heterogeneity, the unevenness of neoantigen presentation in a tumor. A few neoantigens become the dominant ones relative to the others and mount stronger responses, sometimes driving a T-cell storm. The exhaustion of the abundant T cell subset limits the ability to fight other antigen-negative clones and leads to polyclonal T-cell contraction or escape variants, which lack immune recognition. In return, these changes create selective pressure for the tumor to alter the abundance or persistence of the TMB and limit the clonal T-cell response.

7.4. Microenvironmental Influences

The tumor microenvironment (TME) contains tumor cells, immune cells, inflammatory cells, fibroblasts, and stromal cells [2]. The interaction of the host immune system with tumor cells in the TME is essential for understanding tumor immunity and the development of successful cancer immunotherapy. During tumor progression, tumor cells recruit a variety of cells to the TME, including tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and type 2 tumor-associated neutrophils (TAN2), which create an immunosuppressive microenvironment. Some components within the TME associated with resistance to immune checkpoint blockade (ICB) include immunosuppressive cytokines, MDSCs, exhausted T cells, and Tregs. Preexisting T cells in the TME are essential for anti-PD-1 therapy and the absence of this interpretation provides a reasonable explanation for acquired resistance to PD-1 blockade. Interferon-gamma (IFNg) is a master regulator of adaptive resistance to ICB that might contribute to acquired resistance to PD-1 blockade. Epigenetic silencing of Th1 cell-type chemokines can enhance the clinical effect of PD-L1 checkpoint blockade by increasing tumor-infiltrating lymphocytes. IFN-γ acts on JAK1 and JAK2 receptors and the signal transducers and activators of transcription (STAT), which leads to the upregulation of genes involved in antitumor responses and antigen presentation. Defects in this pathway causing the activation of the WNT/β-catenin signaling pathway are correlated with acquired resistance to PD-1 blockade in patients with melanoma. The loss-of-function mutations in JAK1/2 may result in a lack of T-cell infiltrates. Acquisition of the JAK1/2 mutation is therefore a barrier to anti-PD-1 therapy.

8. Biomarkers of Response

Immunotherapeutics blocking PD-1 receptor, PD-L1 ligand, and CTLA-4 receptor have shown

significant clinical efficacy in a variety of cancer types. To predict response to immunotherapy and understand mechanisms of resistance, a variety of biomarkers and related exploratory, validated and standard tests have been discovered. After evaluating a range of candidates, the FDA proactively approved some tests for PD-1/PD-L1 inhibitors and biomarker assays for tumor mutational burden, microsatellite instability, and mismatch repair deficiency. However, there are still significant limitations in the development of biomarkers and related tests. It will be simultaneously necessary to improve the basic workload of biomarker discovery and testing during immunotherapy development (10). Efforts must continue to broaden the scope of biomarker knowledge and its use outside of the tested indications. The development of multi-omic approaches, better animal models, more diverse and represented cohorts, and multi-angle and multi-scale collaborations will enable more careful assessment of biomarker candidate discovery. The scientific, clinical, and pharmaceutical community's continued efforts to work to identify, validate and implement biomarkers are crucial for the future of immunotherapeutics. An understanding of the mechanisms behind its action is valuable for developing follow-on products that may block other parts of the immune response.

The immune system is composed of lymphocytes and non-lymphocyte cells that interact to create and maintain an immune response. T cells recognize tumor antigens, migrate into tumors, and enhance recruitment of other immune cells. Survival and function of the immune response may be interrupted by regulatory T cells, inhibiting cytokines, a generally immunosuppressive microenvironment, and altered vasculature in the tumor. Checkpoint inhibitors prolong the activation and effector phases of a T cell response. Recruitment of T cells into a tumor in response to immune checkpoint inhibition relies on production of effector cytokines that alter signaling pathways in non-lymphocyte cells. Tumors respond and adapt to immune pressure, resulting in changes to gene expression and protein production. T cells may fail because they are not properly activated, migrate to an environment that is hostile to T cell function, or enter a legume where they are inhibited or even killed. Checkpoint inhibition with monoclonal antibodies against the receptors CTLA-4, PD-1 and PD-L1 can upgrade responses to several treatment modalities alone or in combination with other agents. There has been great interest in predicting response and resistance to checkpoint inhibition. As individual tumors contain very different patterns and mechanism of immunogenicity, it has been difficult to identify a single cancer antigen that is universally present and necessary for response to inhibition.

8.1. Predictive Biomarkers

Nothing is more important than determining which patients will benefit from checkpoint inhibitors. To date, the best predictive biomarker for response to checkpoint inhibitors is a combination of tumor mutation burden and presence of PD-L1 expression utilizing either RNA or protein. PD-L1 expression on the tumor is particularly useful in bladder cancer, where it is used as a regulator for treatment with immune checkpoint inhibitors prior to surgery 10. Often, these screening methods will identify a considerable subset of the population that would be predicted to respond. However, over half of those patients do not respond. This underscores the need for further biophysical screening of tissues to reduce the number of patients left with an alternative third-line treatment after failed checkpoint inhibition response.

Immune cells can act as both facilitating and central components of the tumor microenvironment. Research is ongoing into how to investigate tumor immune infiltration on both the cellular and chemical levels to reliably predict checkpoint blockade response not solely based on tumor genomic alterations. Using variational means, a method to capture the various guiding forces on immune interactions is presented. Macrophages, B cells, and CD4+ T helper cells are shown to be essential for checkpoint blockade response through prediction of treatment response. Especially in lung adenocarcinoma, there exists a different mechanism that relies more heavily on universal immune cells such as CD8+ T effector

cells. It is suggested that by identifying and co-targeting critical immune components, there exists a route to therapeutic optimization to increase treatment efficacy.

A biomarker gives a general sense of outcome but, to be optimal, needs to give a personalized picture. Without restricting the prediction based on a specific hypothesis, the Guiding Forces method successfully predicts tumor immune infiltration changes upon checkpoint blockade across cancers and unrealistic scenarios. An accessible set of examples demonstrates the biological interpretability and potential implications of its usage for improved therapy design. The general applicability of this framework and its high-resolution features set it apart from current existing tools.

8.2. Prognostic Biomarkers

Checkpoints are important for tumor immunogenicity. They indirect counterpart cancer immune evasion. Molecular alterations that downregulate MHC class I antigen presentation and other components of antigen processing have been implicated in resistance to immune checkpoint blockade PD-1/PD-L1 and CTLA-4. Focal amplifications in the genes encoding β2 microglobulin or class I cytosolic proteasome subunits were associated with innate resistance to anti-PD-1/PD-L1 by decreasing the expression of MHC class I molecules on cancer cells. TRIM28, a transcriptional repressor that negatively modulates tumor antigen expression was found overexpressed in TMB-low/hypermutation-negative melanoma and to be a potent predictor of resistance to PD-1 inhibitors.

Other studies demonstrated that mutations and deletions in POLE, which encodes an error-correcting polymerase, led to a muted response to anti-PD-1 therapy and that mutations in CTCF downregulated that presented driver neoantigens, leading to immune evasion. Loss of MHC class II molecules on tumor cells conferred resistance to anti-PD-1/PD-L1 therapy in patients with MHC class II-positive tumors by inhibiting tumor-specific CD4+ T-cell priming. On the other hand, components of perforin-containing immune evasion mechanisms were shown to be involved in resistance to CTLA-4 blockade. Inherited or acquired mutations in the gene encoding perforin were associated with resistance to anti-CTLA-4 therapy in mouse models and melanoma patients.

Mutations in the gene encoding granzymes were also associated with resistance to CTLA-4 blockade in mouse models 10. All MMR-D/MSI tumors except for those lacking mutations in the gene encoding mutL homolog 1 and showed a clinical response to anti-PD-1 therapy, indicating that fidelity of the MMR system is an important determinant of the efficacy of PD-1/PD-L1 inhibitors. VISTA (V-domain immunoglobulin suppressor of T-cell activation), a B7 CD28 receptor family member preferentially expressed on immunosuppressive myeloid cells, predisposed the tumor to a poor clinical response to anti-PD-1/PD-L1 therapy in cancer types sensitive to this therapy.

9. Future Directions in Research

A breakthrough in cancer treatment has been realised with the advent of immunotherapy. This has been defined as treatment with agents that interfere with the development or activity of an immune response that could eliminate the cancer. Checkpoint inhibitors represent such agents. They block the molecules, such as programmed death 1 (PD-1), which act as brakes on T-cell activity. These checkpoints normally prevent an immune response from attacking the body's own normal, healthy cells. Tumors subvert this regulatory mechanism and evade attacks by T cells that should be constantly surveilling for potential malignant cells. Monoclonal antibodies that block these checkpoints unleash these tattle-tale T cells, promote their infiltration into tumors, and lead to death of tumor cells on which they act (3). Despite impressive and sometimes protracted remissions in subsets of patients with malignant melanoma, lung cancer, and other malignancies, traditional paradigms of immunology cannot seem to explain this success. For one, immune checkpoint inhibitors appear to work well even against "cold" tumors, such as malignant melanoma that arose in the absence of a serologic immune response, and poorly immunogenic

cancers such as pancreatic adenocarcinoma. A plethora of biomarkers, mostly inferred from algorithms of bulk RNA-sequencing data, have been identified as candidate determinants of therapeutic responsiveness to checkpoint inhibitors. Unfortunately, these biomarkers have largely failed to translate into routine clinical practice [1]. The foundational need for an operational definition of an immune response that clarifies how to measure the activity of that response and benchmark it against normal physiologic systems remains. Satisfaction of such a criteria is likely to spur the development of therapeutics far beyond monoclonal antibodies to alter the trajectory of endogenous immune responses against cancer. There is an urgent need to significantly advance understanding of how checkpoint inhibitors work, why they fail in the majority of patients treated, and discover new means to manipulate cancer immunity in ways not possible with the current generation of agents.

9.1. Novel Checkpoint Targets

As mentioned, PD-1 and CTLA-4 are the two most actively pursued checkpoint targets in cancer immunotherapy. The immense potential of checkpoint blockade has inspired investigation of myriad additional checkpoint targets not yet used therapeutically in clinical oncology. Some of the more promising 'next generation' checkpoint targets, as well as those with potential as combination partners for PD-1 or CTLA-4 blockade, are highlighted.

LAG-3 (lymphocyte-activation gene 3) is an immunoregulatory receptor expressed on T cells, NK cells, B cells, and regulatory T cells (Tregs), and is most homologous to CD4. LAG-3 binds to the same ligand as CD4 (MHC Class II), but with a much higher affinity. Although best known as a negative T cell regulator, LAG-3 can also promote T cell activation. In preclinical models, LAG-3 blockade enhances effector T cell responses to tumors, synergizing with PD-1 blockade. An IgG1-LAG-3 fusion molecule has shown promise in early clinical studies.

TIGIT (T cell immunoreceptor with Ig and ITIM domains) is an inhibitory receptor expressed on activated CD4+ T cells, CD8+ T cells, and NK cells. By competing with CD226 for binding to CD155, TIGIT/PD-1 co-engagement 'licenses' dendritic cells (DCs) to dampen CD4+ T cell activation. In preclinical models, it has been shown that TIGIT blockade can reinvigorate exhausted T cells and enhance anti-PD-1 therapy. Human anti-TIGIT is in early-phase studies.

Table 3.

Checkpoint Target	Clinical Response Rate	Common Resistance Mechanisms	Key Findings
PD-1/PD-L1	20–40%	Loss of antigen presentation, T-cell exclusion	PD-L1 expression correlates with better response
CTLA-4	10–20%	Upregulation of alternative checkpoints (e.g., TIM-3)	Combination therapy improves outcomes

Combined PD-1 + CTLA-4	40–60%	Immune-related adverse events, adaptive resistance	Higher efficacy, higher toxicity

Tim-3 (T cell immunoglobulin and ITIM domain) is another transmembrane protein expressed on CD4+ T helper Th1 cells, CD8+ T cytotoxic T-Cell memory cells, and NK cells. Early studies have shown that blockade of Tim-3 with monoclonal antibodies enhances anti-PD-1 therapeutic efficacy. There are a number of Tim-3 antagonists in early clinical studies. Oncogenic Kras mutation in a mouse model of lung adenocarcinoma decreases Tim-3 expression on CD4+ and CD8+ TILs. Combination therapy targeting Kras and Tim-3 drives CD8+ TILs into an effector state and promotes CD8+ T cell infiltrates in non-T cell-inflamed tumors [2].

9.2. Personalized Immunotherapy Approaches

Developing personalized immunotherapy approaches is critical to improving clinical efficacy. Drug resistance mechanisms are highly individual. Therefore, personalized immunotherapy approaches are required that tailor combinations to the genomic profile of individual patients prior to the initiation of therapy. The genetic context of each tumor can be different. Genomic profiling has been prominent in the clinical development of targeted therapies. Next-generation sequencing (NGS) based on either whole exome (WES) or RNA sequencing (RNA-seq) has also been shown to provide precise biomarker information on ICB resistance or eligibility and select patients likely to respond [3]. NGS emerging technologies offer a unique opportunity to classify cancer patients into actionable subgroups that are more likely to respond to certain therapeutics or avoid ineffective treatments.

T cell receptor (TCR) sequencing is increasingly being used to understand mechanisms of immune evasion and predict response to therapy [1]. Further studies applying this approach to model systems prior to patient therapy are anticipated to advance the field. Modeling available datasets from published studies to integrate diverse data (mutation status, aCGH, gene expression, and immune contexture). Subsequently, machine learning can be applied to classify patients with ICB-associated baseline characteristics into resistant subgroups or responsive subgroups. This approach can prioritize combinations of drugs to target the identified mechanism of resistance. Consequently, multiple combinations can be simulated in parallel. All drugs can be given at the therapeutic dose and selected based on patient response. This new class of adaptive therapy simulations can rapidly evaluate multiple regimens and optimize the precise timing of drug combinations. Implementation of a patient-specific adaptive therapy model for solid tumors in pre-clinical studies can assist in the development of predictive and personalized approaches to control drug resistance and improve the long-term management of cancer patients. These simulations can drive implementation efforts for clinical testing of adaptive therapy.

9.3. Enhancing Efficacy of Existing Therapies

Many chemotherapies and radiotherapies block and/or induce DNA damage repair in tumor cells and, ultimately provoke tumor cell demise. It is accepted that this type of action produces immunogenic death in a significant number of tumor cells, resulting in cross priming of a high number of tumor specific effector CD8+T cells able to specifically eliminate distal tumor deposits presumably metastasis. Co application of immune checkpoint inhibitors (CPIs) such as anti-CTLA-4 probably potentiate this immunogenicity resulting in a further increase in DC-T cell cross talk [3].

Regulatory T cells (Tregs) are a subset of T cells able to avoid immune responses against autoantigens but also against non-self-antigens such as tumor antigens. Tumor tissues can be endowed with a specific Treg selective advantage producing an insufficient immune response against the tumor. This can be

counteracted by injecting depleting antibodies against Treg specific markers such as CD25(1).

Type I and type I interferons (IFNs) have been used for many years in the treatment of a broad variety of malignancies. Their mechanism of action is complex. They induce the expression of MHC class I and immune checkpoints limiting the immune response not only against tumor antigens but also against autoantigens. Discuss recent generation compounds that may block this negative effect but preferentially impact the tumor. Endogenous or exogenous administration of immune therapies interact with each other and produce a better outcome than the sequential use of each of these therapies. Central tolerance is tumor antigen driven. CTLA-4 may persistently increase T effector memory CD4+ T cells. Treg depleting antibodies such as depleting anti CD25 scFv have been associated with lymphopenia and improved T effector memory CD4 and CD8 lymphocytes and CD8+ T effector memory T cells primitive tumor specific infiltration. Thus, secondary treatments must utilize more immunogenic therapy doses and/or more potent alternative mechanisms of action.

10. Ethical Considerations

Sufficient understanding regarding cancer immunotherapy leads to ethical questions. When immune-modulating treatments are used, they affect the immune system as a whole rather than the individual tumor-antigen reaction. For instance, a treatment with anti-CTLA4 antibodies reduces follicular B-cell numbers in the periphery, which can be very different in patients with or without autoimmune diseases 3. Frontline therapy with such agents ideally requires patient selection based on information about their autoimmune disease risk.

T-cell exhaustion is a state of hyporesponsiveness and dysfunction towards a persistent antigen, and has been proposed as a mechanism of tumor immune escape 1. There is good evidence that tumours and chronic viral infections can induce T-cell exhaustion as characterised by the progressive up-regulation of a Restriction Point (RP) network of multiple inhibitory receptors including PD-1, CTLA-4 and Tim-3. If challenged with a sufficiently potent immune stimulus however, even exhausted T-cells can be reactivated. The discovery and importance of checkpoint receptors such as Pd-1 and Ctla-4, as well as the development of blocking monoclonal antibodies, has introduced a radically new approach to cancer-related therapies. This basically consists of unleashing the power of the body's immune system to control tumours by inhibiting checkpoints that restrict effector T-cells. A number of immune checkpoint blockers are approved for the treatment of patients with a variety of malignancies. Combination therapies and advances in selection biomarkers have further contributed to the development of this enormously successful field of biomedicine.

While immune checkpoint blockers have achieved remarkable clinical success in a subset of malignancies, most patients with cancer do not respond to or develop resistance to their treatment. Molecular mechanisms of resistance to checkpoint inhibitors occur at tumour, microenvironment, and T-cell levels. A diverse range of mechanisms are being studied to address most scientific interest directly to human patients. The ultimate goal is to predict and reverse resistance in conjunction with immunodiagnostics tailored to particular therapies and patients, and reinvigorate exhausted T-cells. Less is known regarding the potential of mechanisms of resistance found in other systems, the diverse trophic and evolutionary pressures acting on tumours and their microenvironment, and the plasticity and time-dependence of resistance mechanisms. Efforts also need to be devoted to the development of novel therapies that preclude or reverse resistance mechanisms. Strategies and promoting collaboration between academia and drug discovery companies are imperative to foster approaches akin to those presently under development in the field of targeted therapies against genetic aberrations.

10.1. Patient Consent and Autonomy

Informed consent is a common and generally implemented medical and ethical practice whose goal is the protection of patients' autonomy and understanding. Informed consent in research is a much more complex topic. While it has some overlapping objectives with informed consent in clinical trials, there are also discernable differences between the two. This chapter addresses the ethical, mathematical, empirical, and communicational aspects of informed consent in vaccine clinical trials. Research conducted to date on health literacy, risk communication, framing, and comprehension has implications not only for vaccine clinical trials but also for informed consent in general [3].

Informed consent is an essential criterion for ethical biomedical research involving human subject participation. It is normally understood as the process through which the potential participant is provided appropriately worded, exhaustive, and comprehensible information about the study to allow for an autonomous choice regarding participation. Despite its widespread and often successful implementation, evidence exists that suggests that informed consent in research varies greatly across disciplines, study designs, countries, contexts, and among potential participants. This suggests that informed consent may come in different shapes, degrees, and types (9). A variety of issues related to informed consent made their appearance, such as patient autonomy, comprehension, understanding vs. consent, comprehending future risks, and technological advances. Informed consent-first perceived as a very basic or simple principle-quickly came to be recognized as a frontier topic of both a deep philosophical and ethical relevance and a practical and applied level of great need for expertise. Many of the unsolved informative and communicational challenges regarding informed consent in clinical trials may resemble the ones present in vaccine studies.

10.2. Equity in Access to Therapies

In Europe and the USA, around 6.5 million people die from cancer each year. In developed countries, cancer is the most frequent cause of death, and there is increasing awareness of its negative impact on the economy and society. Globally, cancer incidence is rising, reducing labor supply and productivity, driving up health-care costs, increasing economic inequality, and affecting future economic growth. Flanked by understanding of inclining heterogeneity in treatments on cohorts below 50 years of age, it is crucial to consider equity in access to healthcare and differences on secondary cancers in observational studies.

Despite the indiscriminate use of combinatorial approaches, therapy response variabilities are notable. Present and future datacenters could help to characterize mechanisms of both resistance and response for a given patient through archival stored samples. One potential novel inhibitor would reverse initial resistance mechanisms to enhance the beneficial treatment potential of checkpoint inhibitors, allowing patient stratification for non-responder combinations 3. With this impact in mind, large investments are needed to circumvent the risk of cancer and inefficient uses of diversified personalized combinations in presently untreated subpopulations. A huge challenge lies ahead in destructing and scoping the virophome, virogenome, transposonome, and mobilome, at least in topologically governed tumor-associated niches, microenvironments, and cellular hierarchies. Overcoming treatments that have largely failed to have substantial reproducible clinical effects needs to be conceptually redesigned as per targeting more upstream feedbacks.

Across the scientific community, translational biophysical concepts could be very beneficially shared for nonequitable drugs. Limits to what is shared and invented will need to be understood and the biometric web of storage, analysis, and competition coaxed into action. Drawing on insights from host/human symbiosis during the evolution of planets, systemic onco-bot ideas could be explored to create higher-order emergent functions in resilience usefully sensing and affecting multiple hierarchies now beyond reason.

11. Regulatory Landscape

In the 2017 National Cancer Institute's Evolving Role of Biomarkers in Immunotherapy, the participants recommended studying the effects of hyper-progressive disease on the activity, efficacy, and safety of immune checkpoint inhibitors, testing new classes of immune checkpoint inhibitors, and identifying and developing redundant costimulatory antibodies that target new and evolving immune checkpoints. Additional priority areas included understanding the effects and the mechanisms of the immune checkpoint inhibitors on the tumor microenvironment within the tumor itself, in metastases, and in lymphoid organs. The need to pursue combination strategies using immune checkpoint inhibitors with new agents such as oncolytic virus therapy emerged, as did the need to test older and potentially irrelevant agents with new drug combinations [3]. In the Collaboration and Awareness subgroups of the National Cancer Institute workshop on immunotherapies, the participants contemplated forming and supporting formal governmental and non-governmental cooperative groups around the immune checkpoint biology space, particularly with a view to facilitate cooperation and data sharing in an industry setting.

The recommendations in this narrative review are focused on the first priority that addressed the regulation of immunotherapy with immune checkpoint inhibitors. To set the stage for the evolving landscape of immunotherapy, the growing number of effective immune checkpoint inhibitors with the promise of lasting remission in patients with cancer is discussed. Based on the changes in the practice status of currently actionable and potential future immune checkpoint inhibitors, a research roadmap with specific experiments to address open questions is proposed in the context of 3 potentially evolving areas of concern. Potentially alternative immune checkpoint inhibitors with distinct modes of actions targeting new pathways parallel to the well-established immune checkpoint inhibitors are listed as "need to monitor." This article serves as a generative review to raise awareness and interests within the oncology community for the challenges to the regulatory landscape of immunotherapy with immune checkpoint inhibitors in this era of explosion of therapies.

11.1. Approval Processes for Checkpoint Inhibitors

In the last years, the optimal immuno-oncological treatment for solid tumors has changed dramatically with the approval of checkpoint inhibitors. They consist of monoclonal antibodies targeting co-inhibitory immune checkpoint receptors, presented as immuno-oncological drugs, which reestablish T cells' antitumor activity. Although there are immune checkpoints and their inhibitors beyond PD-1 or CTLA-4 that are already approved or in development, in this chapter they will focus on those drugs that have successfully completed the lengthy drug development process involved prior to marketing authorization in most countries [3]. Currently, six monoclonal antibodies are in routine clinical use for the treatment of solid tumors.

First-generation monoclonal antibodies against CTLA-4 was the first immune checkpoint inhibitors approved in Europe and the United States for advanced unresectable melanoma in March 2011. Approved for melanoma, a relatively rare and aggressive disease with a poor prognosis, circumventing the approval processes for the biosimilars of these agents. PD-1 and PD-L1 inhibitors were developed soon after their discovery, with the first of these drugs being approved for clinical use in 2014 together with nivolumab in unresectable melanoma. Monoclonal antibodies targeting PD-L1 (atezolizumab, avelumab, durvalumab) followed the approval of PD-1 inhibitors for the treatment of various malignancies. Recently, a growing number of combination therapies targeting several checkpoints simultaneously have been used in routine clinical practice or are in development.

In each case, experts and companies advocating for their use had to demonstrate that the drug provided a significant clinical benefit (improved survival rates) for patients compared to currently

available treatments. Additionally, companies had to collect significant amounts of data, that may require several years to obtain, to demonstrate that the drug is safe, valid/active and has no significant negative impact on health care costs or on health care provider's human or physical assets. Therefore, pressures to obtain an early drug approval in such a rapidly moving field can compromise patient safety.

11.2. Post-Market Surveillance

Post-marketing surveillance (PMS) is the process of monitoring the safety of a pharmaceutical drug or medical device after it has been released on the market. It is the final phase in the drug or device approval process. Similar to pre-marketing studies, attempts are made to determine and detect problems, but the approach differs. Pre-marketing investigations focus on determining whether the drug is effective or safe. Safety becomes the major PMS issue once the drug is approved. In other words, PMS detects issues that were not directly related to the mechanisms or indications for which the drug was developed. Differences in pre-marketing vs. post-marketing studies can easily be understood with some examples 6.

PPDs follow a nested design, and most PPDs of PD-1/ PD-L1 drugs have had relatively good safety profiles based on this analysis. However, a few switch case designs among PD-1/ PD-L1 drug combinations produced negative results. RNAscope-SMISH assays should be developed in the future, especially for MS pathology diagnosis and PD-1/PD-L1 therapeutics. Another perspective of marker development for immune checkpoint inhibitors includes vasculature-associated proteins, as they are two-way BTN3 supported. It appears that the inhibitors developed and with clinical experience are targeting PD-1/ PD-L1 and CTLA-4 receptors.

These antibody drugs do have some characteristics in common, as their binding to the receptor may be mediated by noncatalytic interactions that primarily involve direct steric blocking. However, the differences in mechanism action also exist, and these oblige the different development paths. To select the best candidates for clinical development and to provide a clinical diagnostic framework, these issues of BCMA-BCD and BCMA-targeting agents should be generated systematically and systematically for a better understanding of the therapeutic opportunities and diagnostic prospects.

12. Global Perspectives

New therapies based on modulation of the immune response, also known as cancer immunotherapy, have emerged as a promise option for tumor treatment. The relationship between immune system and tumor evolution has been known since the 18th century when Coley described patients with soft tissue sarcoma who showed clinical regression after an infection caused by Streptococcus pyogenes. Then, it was realized that the clinical regression was confirming the existence of antitumor immune response. Later, some cases of spontaneous regression of advanced malignancies were described. Finally, Paul Ehrlich published his idea about the concept of cancer immunoediting, which proposed the concept of cancer immunosurveillance arguing that cancer only occurs when the host's natural immunity becomes weakened. Ninety years after this thought, the Nobel Prize was given to immune checkpoints blockade for new directions in cancer immunotherapy [3].

Recently significant advances have been achieved with the emerging knowledge of inhibitory checkpoints as important players in positive and negative regulation of immune responses. A century ago, Linsley et al. focused on T cells and their main role in the immune reaction. In recent decades, it has been considered that T cell activation involves the physicochemical recognition of the antigen presented by MHC to the TCR on the surface of the T lymphocytes. Following activation of T cells, a second signal occurs via the interaction between a costimulatory molecule (CD28) from T cells and its ligands (B7-1 and B7-2), which are expressed on activated APC, providing a full primary activation and innumerous T cell clonal expansion. Following the first wave of activation, the immune response must be regulated since the immune system is potentially harmful, causing autoimmunity and damage to healthy tissues and

organs.

Thus, co-stimulatory receptor blockade is a promising therapeutic approach to enhance antitumor immunity in cancers. As well as positive co-stimulatory molecules, essentially negative or inhibitory immune checkpoints and their functions were reported. A pioneer work described the CTLA-4 receptor which is present on the surface of activated effector T cells and regulatory T cells. CTLA-4 finds two ligands B7-1 and B7-2 with a higher affinity than CD28 that results in the inhibition of effector T cell functions and in Treg cell proliferation and immunosuppression. Sanchez-Vega described and cloned the PD-1 checkpoint receptor which was then predicted as a member of the CD28 family. Subsequently following the discovery of the PD-1 receptor, many laboratories devoted their research to find its ligand. Watering et al. demonstrated that the PD-L1 was expressed at low levels on normal tissues but a wide range of tumors. However, PD-L1 is highly induced in many tissues upon inflammation and stress, serving as a protective mechanism for survived tissues which are usually damaged by immune cell activation.

12.1. Variations in Treatment Access

Inequalities factor not only country but also source of treatment, patients with EC from poorer financial states should expect worse outcomes. All trials had a different method of defining which patients were eligible to participate; in total over 30 features were required. The only 2 features that all trials shared were age greater than 18 and performance status greater than 2. From a survival point of view all trials showed statistically significant treatment effects, making it questionable why there is so much heterogeneity in eligibility. In the rare instance overlap does occur, the eligible patients were excluded from some of the trials. It is conceivable that a worse performance status may also be common criteria for exclusion as they would probably attend modelling. Given the difficulty of ensuring universally achievable definitions, it is recommended eligibility criteria be reported as clearly as possible. Sharing data on how achievable criteria are in different patient pools is also to be encouraged. For the assumption of IID, further analysis into the role of patient sex showed a consistent treatment effect on survival, with females receiving a reduction in expected death rate compared to males across all trials.

Devices built upon the hypothesis that adolescents, commonly attached to fittings on sneakers or wrist straps, would increase activity levels. In the studies the large cohort of students were asked to wear devices around school time for a period of hours. The intent of these studies was to use a treatment group of students offered devices to view their count data; control group students would not receive any such devices. Thus deprivation of access to data from the treatment group was legitimized. Upon weighing the results, the device was implicated as having an effect on output, however whether that effect remained could not be ascertained. A large recruitment effort was needed to garner sufficient data, given devices were not routinely used in study settings many avoided participation.

12.2. International Clinical Trials

Cancer immunotherapy, based on immune checkpoint inhibitors (ICIs), represents an important part of the novel immuno-oncology drug approvals in the past decade. However, despite the enormous therapeutic advantages in selected patients, it has been drug resistance, with only 15–25% surviving patients in the long term. Interestingly, durable therapeutic responses can be obtained with combination strategies that overcome primary and acquired resistance to monotherapy with ICIs and other immune modulators in preclinical models and cancer patients. However, sequential treatment of CTLA-4 blockers after anti-PD-1 therapy in melanoma patients demonstrated a quicker resolution of IgG and cytotoxic activity than other combinations targeting pathways such as VISTA and TIM3. Several clinical trials combining a doxorubicin-based chemotherapy with immune checkpoint inhibitors targeting PD-1/PD-L1 axis in various types of cancer are currently ongoing. However, it has often been a disappointing

'response' because it persists as a 'reset' equilibrium. New treatment ideas combining paradigms in cancer immunotherapies are proposed, including engineering DCs to sensitize TME to anti-PD-1/PD-L1 therapies [3].

The multiple sequence of signals and rubrics form the science of the immune system rather than coding DNA. Tumour immunotherapy based on immune checkpoint inhibitors has been approved in various cancers. Although the understanding of different tumour resistance mechanisms and their relation to prognosis has been substantially increased at the molecular and pathway levels, integrated tissue biomarkers and the routine use of biospecimen analyses to manage cancer immunotherapy on an antigen-independent approach are still very limited. There has been massive expansion into novel immunotherapy based on combined immune checkpoint inhibitors and on agents targeting stimulatory and triggering molecules, MDSCs, NK, and DCs, especially in earlier disease states. Beyond expanding the approach to new and rarer tumour types, combining treatment paradigms will become essential to address and circumnavigate acquired resistance. Cancer genome evolution, therapy-induced evolution and dynamic heterogeneity, and cancer immunity evolution as viewed by evolving T cells, will be the foci of future studies. Important efforts have been made and more are expected towards creating the robust language or rubrics of cancer through a more comprehensive understanding of immune of cancer systems.

13. Case Studies

Immune checkpoint inhibitors (ICIs) are a new class of immune-modulating agents with enormous potential that can dramatically prolong survival for patients with cancer. Although the clinical efficacy of ICIs in cancer treatment, including on melanoma and lung cancer, has tantalized many researchers, receptors at the forefront of the action have proved to be much harder to work on than expected. Anti-PD-1/PD-L1 antibodies have remarkably improved patient survival and response rates; however, response rates are only 20–50% across many solid tumor types, suggesting that reprogramming the immune system is more complex than simply blocking inhibitory signals .

CD80 (B7-1) and CD86 (B7-2) are stimulatory ligands of CTLA-4 (cytotoxic T lymphocyte antigen-4) and CD28. Blockade of CTLA-4 or PD-1 unashamedly demonstrates efficacy in various kinds of tumors. These two immune checkpoints regulate T-cell immunity differently, CTLA-4 predominately suppresses T-effector cell activity in the lymphoid organs, while PD-1 mainly inhibits T-cell activity mainly at the tumor site. PD-1 high and PD-L1 high cancers are considered "hot" tumors with favorable clinical outcomes from anti-PD-1 immunotherapy. However, 70% of solid tumors are labeled as "cold" tumors when PD-1 or PD-L1 is used alone as a therapeutic target. For these cold tumors, preclinical evidence suggests that combination therapies targeting PD-1 and CTLA-4 or IDO1 can lead to successful immune activation. Detection of mutations and neoantigens is also being actively researched for methods that can guide the development of personalized vaccines. Signature testing has also developed as an alternative treatment tool to improve ICI efficacy. Visibly, studies to overcome resistance to immune checkpoint blockers are being conducted from various angles. Screening techniques can identify novel approaches for discovering new projects against cancer immune checkpoints. However, most immune targets are scarcely studied. To better understand their role in T-cell tumor immunity is warranted.

13.1. Successful Outcomes

Disease control following immune checkpoint inhibitor therapies might take several months after treatment initiation in some patients. Full and prolonged responses to therapy, even years after drug cessation, have been observed. The eventual outgrowth of resistant tumors is likely due to the selection of clones that either are not dependent on the intervention or acquire compensatory resistance mechanisms. Most studies on the mechanisms of resistance focus on tumor intrinsic changes, including

PD-L1 expression, alterations in antigen presentation and processing, the recruitment of anti-inflammatory components, alterations in angiogenesis and metabolism, mutations/epigenetic changes, etc. These reasons frequently confer a parallel resistance to immunogenic chemotherapies, which are now thought to be a key partner immunotherapeutics for immune checkpoint blockade.

T cell infiltration, antigen presentation, and PD-L1 expression appear necessary for immune checkpoint blockade in most human tumors, suggesting growth under immunogenicity may relate with successful treatment. However, this is not sufficient. Early combination strategies tested a CTLA-4 blocking antibody together with a diverse range of chemotherapies. Studies reported complete responses to immune checkpoint blockade in non-immunogenic tumors, including BRAF-mutated melanoma and colorectal cancer and B-cell malignancies. The combination studies with experimentally less immunogenic agents, involving various treatments, relied on evidence of immune changes only very remotely related to the immune checkpoint blockade treatment. The rarity of studies combining non-immunogenic drugs can partly be explained by early-stage translation challenges.

Studying the resistance mechanisms induced by the immune checkpoint blockade should help formulate optimal re-treatment schedules. Concerns regarding only limited testing of agents inducing tumor tolerance towards immune checkpoint blockade are overarching. Following combinatorial approaches adopted in recent years, more selective agents could benefit therapeutic implications and are in increasing research focus, including anti-inflammation, anti-angiogenesis, and tumor cell-targeting agents. Elucidating the mechanisms underlying successful outcomes following immune checkpoint blockade monotherapy or combinatorial interventions with chemotherapies in the same setting by understanding the immunogenic schedule/treatment windows will be also of tremendous potential translational and therapeutic significance.

13.2. Challenges and Failures

Immunotherapy is a promising approach to the treatment of advanced cancers. The introduction of checkpoint inhibitors (CPI) has expanded the arsenal of immunotherapy in clinical practice and is recommended as part of first-line treatment in selected patients with metastatic melanoma, lung, head and neck cancer, and other solid tumors. In parallel, several novel immune checkpoint molecules have been identified and are undergoing preclinical and clinical validations as cancer immunotherapies. However, despite success with CPI treatment for some patients, the majority do not derive benefit from this approach. The lack of response to CPI can result from a variety of different mechanisms working together in any one tumor. Therefore, efforts to unravel the intricacies of tumor immunity in order to identify patients at greatest risk for disease progression due to immune resistance are currently underway. Identifying potential immune resistance mechanisms will further help to determine novel strategies to improve the rate of response to treatment with CPIs and to develop combination immunotherapies for initial non-responding patients(8).

Cells undergoing malignant transformations often accumulate genetic alterations that give rise to an aberrant, immunogenic cancer-cell proteome, and studies in preclinical models have demonstrated that exposure of a host to a novel tumor-free of those genetic alterations-gives rise to T lymphocyte-mediated anticancer immunity that can eradicate the neoplasm before its establishment. Thus, in order to initiate a productive immune response against cancer, a neoantigen is needed that results from an immunogenic mutation 9. In view of this experience, advances in DNA sequencing have prompted studies looking for from hundreds to thousands of somatic mutations in DNA extracted from biopsy material of a developing cancer. Such studies are currently being followed up by a wider application in patient-specific vaccine development, thus potentially widening the class of patients that are treatable with this approach to just about all cancer types. Detecting lost neoantigens introduces a new risk, because not all mutations give rise to antigens that are immunogenic. The approaches tend to greatly overestimate the number of

candidates, and many predicted antigens would not be targets of an anticancer immune response. Consequently, potentially hundreds of antigen candidates in a tumor would have to be tested individually for immunogenicity in the first step of extensive validation in cancer patients.

14. Conclusion

Immune Checkpoint Inhibitors (ICI) and their major mechanisms of action are fundamentally different from other immune-modulators. ICIs target the checkpoint molecules located in the TME or on tumor cells, such as PD1, PDL1, and CTLA4. This cognate interaction sends immune-inhibitory signals that limit T cell function and endurance. Inhibition of these checkpoint receptors enables the activation of pre-existing T cells or induces de novo T cell activation. Understanding the biology behind the T cell-inhibitory signals is central to the successful design and clinical application of checkpoint inhibitors [1].

On the basis of current knowledge, there are several possibilities for T cell activation/development. Mutations in a tumor cell or aberrant expressions of proteins lead to neoantigen presentation by MHC class I. Neoantigen presentation is theorized to initiate the T cell activation, which involves the migration of precancerous T cells, the activation of innate immunity, and the co-signaling stimulation [2]. The co-signaling pathways are mainly based on the qualitative and quantitative expressions of the surface receptors or ligands. Targeting modulatory molecules, such as TNF and IL-1, can re-educate the TME and elicit T cell activation. ICIs are designed to target the above molecules. The caveat is that most TME targeted agents can pro-actively activate immunity, but the currently approved ICIs passively remove the inhibitory signals.

Another major learning is that there are multiple barriers for efficient T cell activation in solid tumors. Inhibition of any part, such as neoantigen production, T cell infiltration, cytokine stimulation, booster targeting, or co-inhibition removal, may lead to a suboptimal outcome. Future efforts should be aimed at synergizing ICI with agents that could target the other barriers for efficient immunity activation. Hence, refining the selection of treatment populations depends on the better understanding of the T cell recognition process and the improvement of combination regimens with synergizing and timing considerations. In summary, understanding the mechanisms of mechanism of action and resistance for checkpoint blockade therapy is critical for the rational design of subsequent treatment strategies.

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References

1. Li X, Shao C, Shi Y, Han W. Lessons learned from the blockade of immune checkpoints in cancer immunotherapy. J Hematol Oncol. 2018;11(1):31. doi:10.1186/s13045-018-0578-4
2. Alsaafeen, B.H., Ali, B.R. & Elkord, E. Resistance mechanisms to immune checkpoint inhibitors: updated insights. *Mol Cancer* **24**, 20 (2025). https://doi.org/10.1186/s12943-024-02212-7
3. Rodriguez-Pascual J, Ayuso-Sacido A, Belda-Iniesta C. Drug resistance in cancer immunotherapy: new strategies to improve checkpoint inhibitor therapies. Cancer Drug Resist. 2019;2(4):980-993. doi: 10.20517/cdr.2019.61

4. Nagasaki J, Ishino T, Togashi Y. Mechanisms of resistance to immune checkpoint inhibitors. Cancer Sci. 2022;113(10):3303-3312. doi:10.1111/cas.15497
5. Lisovska N. Multilevel mechanism of immune checkpoint inhibitor action in solid tumors: History, present issues and future development. Oncol Lett. 2022 Jun;23(6):190. Epub 2022 Apr 29. doi: 10.3892/ol.2022.13310.
6. Jardim DL, de Melo Gagliato D, Kurzrock R. Lessons From the Development of the Immune Checkpoint Inhibitors in Oncology. Integr Cancer Ther. 2018 Dec;17(4):1012-1015. doi: 10.1177/1534735418801524.
7. Ahmed M. Checkpoint inhibitors: What gastroenterologists need to know. World J Gastroenterol. 2018 Dec 28;24(48):5433-5438. doi: 10.3748/wjg.v24.i48.5433.
8. van Elsas MJ, van Hall T, van der Burg SH. Future Challenges in Cancer Resistance to Immunotherapy. Cancers (Basel). 2020 Apr 10;12(4):935. doi: 10.3390/cancers12040935.
9. Chocarro de Erauso L, Zuazo M, Arasanz H, Bocanegra A, Hernandez C, Fernandez G, Garcia-Granda MJ, Blanco E, Vera R, Kochan G, Escors D. Resistance to PD-L1/PD-1 blockade immunotherapy. A tumor-intrinsic or tumor-extrinsic phenomenon? *Frontiers in Pharmacology*. 2020;11:441. doi:10.3389/fphar.2020.00441.
10. Filipovic A, Miller G, Bolen J. Progress toward identifying exact proxies for predicting response to immunotherapies. Front Cell Dev Biol. 2020;8:155. doi:10.3389/fcell.2020.00155.

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