Immune Evasion and Resistance in Cancer Progression: Overcoming Checkpoint Inhibition Challenges with Personalized Immunotherapy Guided by PD-L1 Expression

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Abstract: Immune evasion is a hallmark of cancer development and poses an important impediment to the effectiveness of immune checkpoint inhibitors (ICIs). Cancer cells take advantage of heterogeneous intrinsic and extrinsic pathways to circumvent immune detection, such as metabolic remodeling (e.g., increased glycolysis, activation of IDO1), genomic mutation (e.g., JAK/STAT, β-catenin), and epigenetic suppression of immune-regulatory genes. Concurrently, TME promotes immune suppression through Tregs, MDSCs, TAMs, and fibroblast-mediated extracellular matrix remodeling. Hypoxia and cytokine dysregulation also undermine antigen presentation and T-cell functionality. These immunoevasion strategies form the foundation of both native (innate) and adaptive resistance to ICIs, while recent evidence places emphasis on microbiota composition being able to modify therapeutic response. The PD-1/PD-L1 pathway remains the focus of ICI therapy, but PD-L1 expression is limited by spatial, temporal, and technical heterogeneity. Beyond PD-L1, integrated biomarker approaches including tumor mutational burden (TMB), microsatellite instability (MSI), IFN-γ gene signatures, and circulating tumor DNA (ctDNA) have arisen to further inform patient stratification. Emerging therapeutic technologies—e.g., dual checkpoint blockade, engineered cytokines, personalized neoantigen vaccines, and adoptive T cell therapy (CAR-T, TCR-T)—are designed to overcome resistance and maximize clinical efficacy. Integration of multi-omics and AI-based models provides additional precision in the tailoring of immunotherapy. This review integrates existing knowledge of immune escape and resistance, highlighting dynamic biomarker development and combinatorial approaches for next-generation personalized cancer immunotherapy.

Keywords: Immune checkpoint inhibitors, Tumor microenvironment, PD-L1 expression, microbiome, combination therapy, biomarkers.

1. INTRODUCTION

The immune response has a contradictory role in tumorigenesis, both as a defense mechanism against malignancy and, conversely, as an amplifier of immune tolerance and tumor growth [1]. The binomial action is described by the model of Cancer Immunoediting, where the evolutionary relationships between tumors and the host's immune response are defined in three consecutive phases: Elimination, where immune surveillance mechanisms effectively detect and destroy immature transformed cells; Equilibrium, a prolonged phase of latency in which selective pressure from the immune system causes the survival of low immunogenic mutants; and Escape, where cancer cells acquire complex evasion mechanisms to evade immune cell-mediated killing and establish a progressive malignancy [2]. The immunological scenario of cancer involves the interaction among the primary cellular effectors, including cytotoxic $CD8^+$ T cells, antigen-presenting dendritic cells (DCs), tumor-associated immunosuppressive macrophages (TAMs), and natural killer (NK) cells, whose functional balance defines tumor development or regression [3]. However, malignant cells exploit various mechanisms of immunosuppression to reorganize the tumor microenvironment (TME) as an immune-privileged niche supporting tumor survival and dissemination.

Immune checkpoint dysregulation, in which inhibitory receptor-ligand interaction is usurped by tumors for the suppression of T cell activation and promotion of immune tolerance, is one of the most relevant immune escape strategies. Of these, the PD-1/PD-L1 pathway is a prominent mechanism of adaptive immune resistance, wherein overexpressed PD-L1 on tumor cells and antigen-presenting cells (APCs) incapacitates effector T cell function [4]. Other checkpoint regulators like cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), and T cell immunoreceptor with Ig and ITIM domains (TIGIT) also facilitate an immunosuppressive microenvironment, favoring immune evasion [5]. The advent of immune checkpoint inhibitors (ICIs), namely monoclonal antibodies against PD-1, PD-L1, and CTLA-4, has transformed the therapeutic strategy in oncology by reconstituting antitumor immunity [6]. However, the efficacy of ICIs is constrained by primary resistance

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(intrinsic tumor mechanisms excluding response) and acquired resistance (adaptive mechanisms that emerge under treatment pressure), necessitating the exploration of novel combinatorial strategies to counteract immune escape [7].

The objective of this review is to provide a comprehensive evaluation of the mechanisms of immune evasion and therapeutic resistance, with special emphasis on PD-L1 as a dynamic and predictive biomarker for the management of immunotherapeutic approaches. Since PD-L1 expression is heterogenous, influenced by inflammatory cues, hypoxia, and oncogenic processes, its utility transcends the crude static determination of ICI response [8]. Instead, PD-L1 is an active immunodynamic marker signifying persistent tumor-immune interaction and guiding patient-specific therapies [9]. The main focus will be put on personalized immunotherapy, which involves integrating multi-modal strategies such as combination checkpoint blockade, cytokine modulation, tumor vaccines, adoptive T cell therapy, and small-molecule immune modulators to enhance clinical benefit. Focusing on the molecular mechanisms of resistance and employing precision oncology, this review outlines the way forward for immunotherapy to overcome checkpoint inhibition challenges and increase lasting antitumor activity.

2. MECHANISMS OF IMMUNE EVASION IN CANCER

Tumor-Intrinsic and -Extrinsic Immune Evasion Mechanisms Synthesized

Cancers bypass immunity through a tightly interlinked program of cell-intrinsic metabolic programs and cell-extrinsic microenvironmental signals that collectively reprogram the tumor microenvironment (TME) towards immunosuppression. Metabolic rewiring in cancer cells—defined by increased glycolysis (Warburg effect), glutaminolysis, and altered lipid metabolism—instigates lactate build-up and extracellular acidosis that suppress cytotoxic T lymphocyte (CTL) and natural killer (NK) cell function. Lactate preferentially inhibits interferon-gamma (IFN-γ) expression by CTLs and facilitates polarization of tumor-associated macrophages (TAMs) to an M2 phenotype that produces transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10), enhancing immune tolerance (Chen J *et al*., 2025; Chattopadhyay A *et al*., 2023; Noe JT *et al*., 2021) [10,11,12]. Another prominent tumor-intrinsic adaptation is induction of indoleamine 2,3-dioxygenase 1 (IDO1), which depletes tryptophan in the TME, blocks effector T-cell proliferation, induces apoptosis, and skews differentiation toward regulatory T cells (Tregs); kynurenine also signals through the aryl hydrocarbon receptor (AhR) to enhance suppression (Nguyen NT *et al*., 2014) [13]. Targeting such metabolic weaknesses presents opportunities to reinstate immune surveillance [13].

These inherent programs interact with—and assist in the formation of—an immunosuppressive TME consisting of heterogeneous immune, stromal, endothelial, and fibroblast populations that cooperate to suppress anti-tumor activity [14]. TAMs often present in an M2 phenotype, secrete IL-10, vascular endothelial growth factor (VEGF), and TGF-β, and may express PD-L1, driving T-cell dysfunction and exhaustion directly (Chen Y *et al*., 2019) [15]. Myeloid-derived suppressor cells (MDSCs) suppress through arginase-1-mediated depletion of arginine, reactive oxygen/nitrogen species, and prostaglandin E2 (PGE2), further limiting T-cell and NK-cell activity (Groth C *et al*., 2019) [16]. Cancer-associated fibroblasts (CAFs) lay down dense extracellular matrix that impedes immune infiltration and secrete chemokines (e.g., CXCL12) that attract suppressive cells, reinforcing exclusionary niches (Cheng B *et al*., 2023) [17].

Hypoxia, which is characteristic of solid tumors, amplifies these consequences through stabilization of HIF-1α and induction of VEGF, adenosine-generating enzymes (CD73/CD39), and TGF-β. Aside from angiogenesis, VEGF disrupts dendritic cell maturation and antigen presentation; adenosine signaling through A2AR on T cells suppresses effector function and promotes Treg induction; and TGF-β suppresses CTL cytotoxicity, proliferation, and differentiation across the board (Chen Z *et al*., 2021; Pastore DRE *et al*., 2022) [21,22]. IDO1 expression in hypoxic niches further induces tryptophan catabolism and kynurenine-AhR signaling to suppress effectors and induce Tregs (Salminen A, 2022) [23].

Cytokine and chemokine dysregulation completes this suppressive circuitry. IL-6 acts on STAT3 to induce tumor survival, inhibit dendritic cell maturation, and increase myeloid suppressors (Jing B *et al*., 2020) [24]. IL-10 suppresses dendritic cell activation, diminishes Th1 responses, and fosters Treg expansion, whereas tumor-derived TGF-β is a master regulator of immune evasion through repression of CD8+ CTLs, prevention of IFN-γ secretion, induction of exhaustion, and

expansion of Tregs, TAMs, and MDSCs (Ye F *et al*., 2023) [25]. Chemokine gradients (e.g., CXCL12, CCL2, CCL22) guide spatial exclusion of effector T cells and preferentially recruit suppressive populations; in particular, CAF-S1 fibroblasts secrete CXCL12 that recruits CD4+CD25+ T cells and induces differentiation into CD25^High FOXP3^High cells (Costa A *et al*., 2018) [26]. Clinically, high FOXP3+ Treg infiltration is associated with worse survival in all tumor types, highlighting their importance in immune escape (Shen Z *et al*., 2010) [18]. Tumor-secreted factors GM-CSF and IL-6 additionally recruit MDSCs to accumulate and inhibit T-cell growth through arginase-1 and reactive oxygen species (Gabrilovich DI *et al*., 2009), whereas M2-polarized TAMs assist with angiogenesis and metastasis (Wang S *et al*., 2024) [19,20].

Synch, tumor-intrinsic metabolic reprogramming and extrinsic TME remodeling create a mutually supportive network that downregulates antigen presentation, excludes or inactivates effectors, and increases regulatory/suppressive populations. Interrupting these metabolic, hypoxic, cytokine, and chemokine axes—coupled with checkpoint blockade—may reveal strong immune infiltration and long-lasting anti-tumor immunity [10-26].

3. MECHANISMS OF RESISTANCE TO CHECKPOINT INHIBITION THERAPY

3.1. Primary (Innate) Resistance

Resistance Mechanisms to Checkpoint Inhibition Therapy

Tumor-intrinsic initial resistance to immune checkpoint inhibitors (ICIs) typically happens in TIL-poor or TIL-scarce tumors that are typically referred to as "cold tumors". These tumors inherently possess immunologically non-responsive phenotypes due to inefficient priming of T-cells by the tumor, defective antigen presentation, or lack of adequate chemokine-mediated trafficking (Kalbasi A *et al*., 2020) [27]. Failure to accommodate pre-existing cytotoxic $CD8^+$ T-cell infiltration significantly attenuates reactivity to treatment with PD-1, PD-L1, or CTLA-4 blockers. Cold tumors have been shown to be linked with reduced mutational burden, poor antigenic neoepitopes, or APM abnormality, all of which together prevent spontaneous T-cell priming and tumor microenvironmental recruitment. Such non-inflamed tumors also usually have immunosuppressive stroma barriers, including CAFs' dense extracellular matrix deposition, that physically inhibit T-cell infiltration and create spatial immune exclusion. Therapeutic methods for reprogramming cold tumors into inflamed phenotypes—e.g., vaccines, intratumoral injections of cytokines, or agonistic CD40 antibodies—are consequently aggressively investigated to render these tumors sensitive to checkpoint blockade (Liu YT *et al*., 2021) [28].

Defective Interferon-gamma (IFN-γ) Signaling and JAK/STAT Pathway Mutations

A second principal mechanism of primary resistance relies on interferon-gamma (IFN-γ) signaling pathway defects. IFN-γ, secreted predominantly by activated NK cells and T cells, is the central cytokine inducing an escalation of antitumor immunity by enhancing antigen presentation, MHC class I expression, and generating a pro-inflammatory tumor environment (Wawrzyniak P., 2025) [29]. Mutated cancer cells involving the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, especially JAK1, JAK2, or STAT1 mutations, become insensitive to IFN-γ stimulation, halting their immunogenic function. For instance, JAK1/JAK2 loss-of-function mutations in melanoma patients with primary or secondary resistance to anti-PD-1 therapy, resulting in insensitivity of tumor cells towards IFN-γ-induced growth inhibition and immune activation. Similarly, Nguyen TT *et al*. (2021) depicted that JAK/STAT mutations confer resistance against checkpoint inhibitors by inhibiting IFN-γ-mediated growth inhibition of tumor cells and upregulation of antigen presentation. These findings underscore the paramount importance of functional IFN-γ signaling in immune checkpoint blockade sensitivity, rendering JAK/STAT pathway integrity a predictive biomarker for ICI responsiveness as well as a potential therapeutic target for combinatorial therapy [30].

Non-inflamed Tumors and T-cell Exclusion via WNT/β-catenin Signaling

CTL exclusion from tumor beds is a critical component of primary resistance, and one of the key signaling pathways involved in coordinating such exclusion is the WNT/β-catenin pathway. Tumor-intrinsic activation of WNT/β-catenin has been associated with impaired dendritic cell recruitment and reduced secretion of chemokines, such as CCL4, thus inhibiting $CD8^+$ T-cell invasion (Luke JJ *et al*., 2019) [31]. In a groundbreaking paper, Spranger *et al*. (2015) demonstrated that β-catenin-oncogenic melanoma tumors were severely devoid of T-cell invasion and refractory to anti-PD-L1/PD-1 checkpoint blockade. Mechanistically, β-catenin activation suppresses the essential chemokine expression that is needed for

dendritic cell migration, where it specifically causes decreased expression of chemokresponsible for attracting T cells and APCs, such as CCL4 and CXCL10 (Luke *et al*., 2019). Thus, WNT/β-catenin-activated cancers are immunologically barren regions directly related to ICI resistance across a number of cancers including melanoma, colorectal, and hepatocellular carcinoma. Therefore, pharmacological inhibition of WNT or combinations that involve strategies for targeting this pathway are possible candidates for breaking immune exclusion and converting non-inflamed tumors into susceptible immunotherapy targets [31].

3.2. Checkpoint Inhibition Therapy-Induced Acquired Resistance

Adaptive Resistance Processes After Checkpoint Blockade

Tumors initially responsive to ICIs may develop resistance through adaptive mechanisms that guarantee immune escape during continuous treatment. One of the key adaptive reactions is the compensatory overexpression of other immune checkpoint receptors on T cells, such as T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), and T cell immunoreceptor with Ig and ITIM domains (TIGIT), functionally rehabilitating T-cell exhaustion profiles (Wang L *et al*., 2023) [32]. For instance, Koyama *et al*. (2016) demonstrated in melanoma mouse models and patient tumor samples that treatment with anti-PD-1 induced upregulation of TIM-3 on T cells, correlated with disease progression and therapeutic resistance. In line with these findings, experiments by Woo SR *et al*. (2012) showed that simultaneous blockade of PD-1 and LAG-3 exerted potent effects to restore T-cell effector functions in patients showing acquired resistance to PD-1 monotherapy, indicating co-targeting other checkpoints as an important mechanism for adaptive immune evasion [33].

Furthermore, a hyperprogressive immune effect can occur on checkpoint inhibitor therapy, in which rapid tumor growth and escalated metastatic dissemination occurs. Hyperprogressive disease (HPD) in approximately 9% of patients treated with anti-PD-1 or anti-PD-L1 antibodies in different types of cancer. Although the biological drivers of hyperprogression are obscure, it is theorized to be associated with aberrant activity of immunosuppressive circuits, changes in regulatory T cell populations, and myeloid-derived suppressor cell (MDSC) disruption, together to skew the tumor microenvironment in favor of immune-permissive proliferation and metastasis. Research by De Clercq S *et al*. (2010) also demonstrated that overexpression of the MDM2/MDM4 genes or EGFR pathway mutations can predispose patients to hyperprogressive disease, with significant clinical implications for biomarker-based patient selection [34].

Epigenetic Changes and Acquired Resistance

Epigenetic modifications have a key role in tumoral evolution and have a crucial role in acquired resistance mechanisms against checkpoint blockade treatment. Epigenetic reprogramming in the form of abnormal patterns of DNA methylation, histone marks, and chromatin modification controls immune recognition and antigen presentation gene expression patterns and modulates responsiveness to immunotherapy. For instance, Tumor cells with adapted resistance to checkpoint inhibitors expressed extensive DNA hypermethylation of antigen processing machinery (APM)-encoding, chemokine-encoding, and MHC class I molecule-encoding genes, leading to impaired antigen presentation as well as T-cell infiltration of the tumor tissue. Similarly, histone changes, for example, increased histone deacetylase (HDAC) activity, can be employed to suppress the transcription of critical immune-related genes, such as IFN-γ-inducible elements, to reduce the tumor's immunogenicity and checkpoint blockade sensitivity (Seto E *et al*., 2014) [35].

Besides, epigenetic plasticity contributes to diminished T-cell receptor (TCR) heterogeneity and activity in tumor-infiltrating lymphocytes, a mechanism associated with therapeutic resistance. Saadey AA *et al*. (2022) demonstrated that chronic antigen exposure during checkpoint blockade therapy triggers global epigenetic reprogramming of T cells, such as widespread DNA methylation and histone modification of exhaustion-associated gene loci such as PD-1, CTLA-4, and TIM-3 [36]. These epigenetic changes stably impose T-cell exhaustion phenotypes, which disrupt their cytolytic function and proliferative potential, reducing TCR repertoire diversity and immune surveillance capacity. Epidriven resistance could be treated using combinatorial treatment approaches that incorporated checkpoint inhibitors with epigenetic drugs such as DNA methyltransferase inhibitors (azacitidine) or histone deacetylase inhibitors. It was promising in preclinical models, which suggested epigenetic therapies as potential therapeutic additions for the avoidance of immunotherapy resistance [36] (Figure **1**).

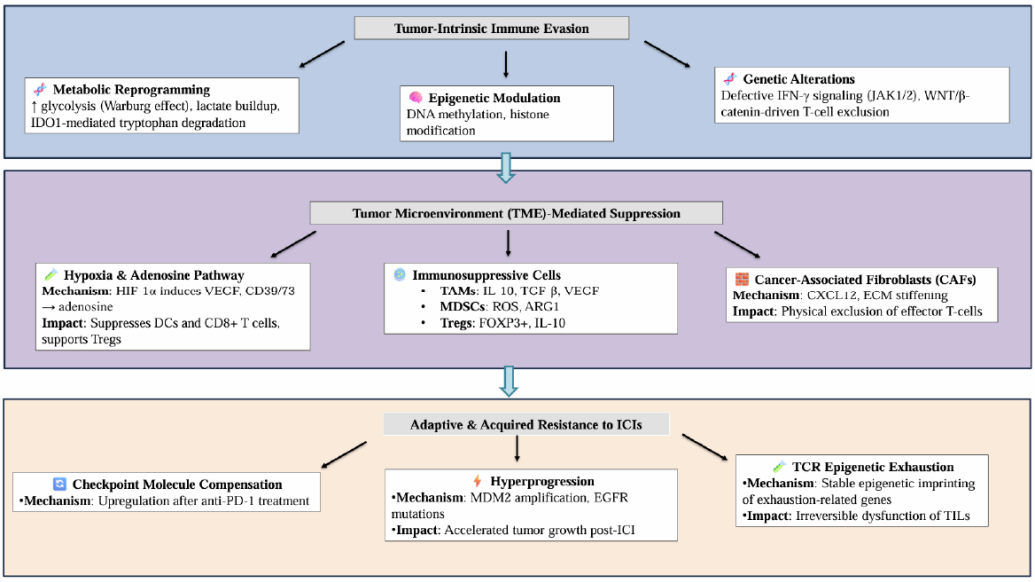

Figure 1: Mechanisms of Immune Evasion and Checkpoint Inhibition Resistance in Cancer [10-36].

3.3. Role of the Microbiome in Immunotherapy Resistance

Gut Microbiota and its Influence on Response to PD-1 Blockade

The gut microbiome has become a key regulator of host immunity, playing a crucial role in patient responsiveness and resistance to immune checkpoint inhibitors (ICIs), especially anti-PD-1 and anti-PD-L1 therapies. There is emerging evidence to indicate that different gut microbiota compositions will either enhance or inhibit immunotherapy efficacy through changing tumor-localized and systemic immune responses (Yang W *et al*., 2021) [37]. The gut microbiome influences antitumor immunity through mechanisms including improved antigen presentation, heightened effector T-cell infiltration, and regulation of systemic inflammatory conditions. Metagenomic sequencing of melanoma patients who were responding well to anti-PD-1 therapy had a unique microbiota with greater microbial diversity, enriched with specific bacterial taxa with the ability to enhance Th1 immunity and T-cell activation. In contrast, lower microbial diversity or dysbiosis was highly associated with therapy resistance, and the gut microbiome emerged as a potential predictive biomarker and therapeutic target in oncology.

Clinical Evidence of Bacteroides fragilis and Akkermansia muciniphila Affecting Outcomes

Some certain bacterial strains, particularly Bacteroides fragilis and Akkermansia muciniphila, have attracted great interest because of their significant effect on immune checkpoint blockade therapy results. Deng H *et al*. (2016) initially revealed the immunomodulatory activity of B. fragilis and showed that oral supplementation with the bacterium enhanced tumor infiltration with IFN-γ-producing $CD8^+$ T-cells and significantly augmented responses to CTLA-4 blockade in melanoma models of mice. Clinical correlation has now validated these preclinical observations to show that patients with Bacteroides-enriched microbiota have good clinical responses and prolonged survival after anti-PD-1 antibody treatment [38].

Likewise, the mucin-degrading bacterium Akkermansia muciniphila has also been identified as a predictive biomarker and therapeutic target for regulating immunotherapy response. Zhu Z *et al*. (2024) demonstrated that the relative abundance of A. muciniphila in the gut microbiota was positively correlated with clinical response to anti-PD-1 immunotherapy in patients with non-small-cell lung cancer (NSCLC), renal cell carcinoma (RCC), and melanoma [39]. Mechanistically, A. muciniphila enhances antitumor immunity through enhanced intestinal barrier function, attenuation of systemic inflammation, and promotion of IL-12-mediated dendritic cell activation, hence initiating strong cytotoxic T-cell responses in the tumors. These findings collectively emphasize the significant therapeutic role of microbiome composition on the efficacy of checkpoint blockade.

Microbiome-Modulating Strategies to Overcome Immunotherapy Resistance

FMT, in turn, is a promising strategy backed by clinical evidence. Baruch *et al*. (2021) also reported

that FMT from responders to immunotherapy into melanoma patients who were initially refractory to anti-PD-1 therapy caused considerable microbiome changes, leading to clinical responses in initially refractory patients [40]. Zhao W *et al*. (2023) further demonstrated that FMT derived from responders reprogrammed the microbiota of gut in melanoma patients, increasing $CD8^+$ T-cell infiltration into tumors and overcoming resistance to anti-PD-1 therapy. These pioneering studies validate the causal link between microbiome composition and response to immunotherapy and set FMT as a potential treatment method to boost the efficacy of checkpoint blockade [41].

Probiotic and prebiotic therapies have also been explored as safer, more controllable methods of microbiome modulation. Injection of specific probiotic strains (e.g., Bifidobacterium) may increase $CD8^+$ T-cell-mediated tumor regression, complementing the efficacy of anti-PD-L1 therapy. While clinical trials evaluating probiotics and prebiotics are still in process, initial results indicate potential therapeutic benefits in boosting antitumor immunity through restoration of microbiota, decreased inflammation, and enhanced gut barrier function.

PD-L1 EXPRESSION AND PERSONALIZED IMMUNOTHERAPY

4.1. PD-L1 as a Predictive Biomarker: Advantages and Limitations

Programmed death-ligand 1 (PD-L1) is an important predictive biomarker for immune checkpoint blockade, especially for PD-1/PD-L1 pathway-targeting agents. Its expression by tumor cells and infiltrating immune cells can predict clinical response to immune checkpoint inhibitors (ICIs) [42]. Nevertheless, the usefulness of PD-L1 is limited by some intrinsic and extrinsic factors. Spatial inter- and intra-tumoral heterogeneity produces enormous variability based on the site and timing of the biopsy, which may not accurately represent the global immune landscape of the tumor. This heterogeneity is also temporal, with PD-L1 expression being extremely dynamic and influenced by past treatments, stress in the tumor microenvironment, and immune engagement.

Analytical variation also makes PD-L1's role even more complex. The application of varying immunohistochemistry (IHC) assays—22C3 (Dako), SP263 (Ventana), and SP142 (Ventana)—each with unique antibody clones, detection platforms, and scoring algorithms—causes varying classification of PD-L1 status. Research, including the Blueprint PD-L1 IHC Assay Comparison Project, has also demonstrated poor concordance among these assays, especially in immune cell staining. Moreover, PD-L1 expression could also be shaped by intrinsic oncogenic signaling (e.g., EGFR, ALK alterations), further complicating its use as a credible stand-alone biomarker [43].**(**Table **1)**

4.2. Beyond PD-L1: Comprehensive Biomarker Strategies

Understanding the limitations of PD-L1, new biomarker strategies pursue a more diverse strategy for stratifying patients. Tumor Mutational Burden (TMB), measured in terms of nonsynonymous somatic mutations per megabase of tumor DNA, is correlated with increased neoantigen production and thus potentially tumor immunogenicity. Elevated TMB has been predictive of relevance across several tumor types, including NSCLC and melanoma [52]. Yet, TMB's predictive capability is platform-dependent, and standardization across sequencing platforms is an obstacle to clinical application. Microsatellite Instability (MSI) and mismatch repair deficiency (dMMR) are strong predictive biomarkers, specifically for pembrolizumab sensitivity in colorectal, endometrial, and gastric malignancies. MSI-high tumors contain abundant frameshift mutations, yielding truncated, antigenic peptides. Notably, MSI/dMMR status is also an FDA-approved indication for immune checkpoint blockade, irrespective of tumor site, foreshadowing a tumor-agnostic biomarker approach [53].

RNA immune signatures provide a window into the tumor immune microenvironment. The interferon-gamma (IFN-γ) gene signature, including CXCL9, IDO1, and HLA-DR, indicates a T-helper type 1 (Th1)-dominant microenvironment consistent with active cytotoxic T-cell infiltration. T-cell inflamed gene expression profiles (GEPs), established by high-throughput transcriptomic analyses, have been shown to possess strong predictive utility in stratifying responders to PD-1 blockade in multiple cancers. Liquid biopsies and circulating tumor DNA (ctDNA) are revolutionizing the landscape of immunotherapy biomarker development. ctDNA provides a dynamic, noninvasive window on the changing tumor genome and mechanisms of resistance. New research has shown the ability of ctDNA kinetics, e.g., early clearance or tracking of mutations, to predict therapeutic response, minimal residual disease, and relapse before imaging modalities [54].

Table 1: Predictive Biomarkers for Immune Checkpoint Inhibitor Response in Cancer Immunotherapy

Biomarker	Biological Basis	Detection Method	Predictive Value	Clinical Application	Limitations	References
PD-L1 Expression	Overexpression on tumor cells leads to T-cell inhibition via PD-1 interaction.	Immunohistochemistry (IHC) assays (e.g., 22C3, SP263, SP142).	Moderate; higher expression often correlates with better response to ICIs.	FDA-approved companion diagnostic for selecting patients for pembrolizumab and other ICIs in various cancers.	Variability in assay platforms; spatial and temporal heterogeneity in expression.	[44, 45].
Tumor Mutational Burden (TMB)	High number of somatic mutations increases neoantigen load, enhancing tumor immunogenicity.	Next-generation sequencing (NGS) platforms assessing mutations per megabase (mut/Mb).	High; elevated TMB is associated with improved response to ICIs across multiple cancer types.	FDA-approved for pembrolizumab in tumors with TMB ≥10 mut/Mb, regardless of tumor type.	Lack of standardized cut-off values; variability across different NGS platforms.	[46, 47].
Microsatellite Instability (MSI)/Mismatch Repair Deficiency (dMMR)	Defective DNA mismatch repair leads to accumulation of mutations, resulting in high MSI.	Polymerase chain reaction (PCR) or IHC for mismatch repair proteins (MLH1, MSH2, MSH6, PMS2).	High; MSI-high/dMMR tumors respond favorably to ICIs.	FDA-approved indication for pembrolizumab in MSI-high or dMMR solid tumors, irrespective of origin.	MSI/dMMR is rare in many tumor types; testing requires specific expertise.	[48, 49].
IFN-γ Gene Expression Signature	Reflects active Th1-mediated immune response within the tumor microenvironment.	RNA sequencing or quantitative reverse transcription PCR (qRT-PCR).	Moderate; presence of an IFN-γ signature correlates with better ICI response.	Investigational; potential to identify responders among patients with low PD-L1 expression.	Requires fresh or well-preserved tissue samples; not yet standardized in clinical practice.	[50].
Circulating Tumor DNA (ctDNA)	Fragments of tumor-derived DNA circulating in the bloodstream reflect tumor burden and mutational landscape.	Liquid biopsy using digital PCR or NGS.	Emerging; changes in ctDNA levels may predict response or resistance to ICIs.	Minimally invasive monitoring tool for treatment response and early detection of relapse.	Limited sensitivity; not yet widely validated for routine clinical use.	[51].

5. STRATEGIES TO OVERCOME RESISTANCE AND IMPROVE CHECKPOINT INHIBITION

5.1. Combination Strategies for Enhanced Efficacy

With immune checkpoint inhibitors (ICIs) now a cornerstone of cancer treatment, a large percentage of patients have either intrinsic resistance or eventually become resistant to PD-1/PD-L1 blockade. Combination approaches have arisen as a logical means to overcome these constraints through the simultaneous disruption of multiple immunosuppressive mechanisms. These approaches involve dual checkpoint blockade, combination of ICIs with small molecule inhibitors, and metabolic manipulation to reprogram the tumor microenvironment (TME) for broader anti-tumor immunity.

Dual Checkpoint Blockade: PD-1/PD-L1 + CTLA-4, LAG-3, TIM-3

Dual checkpoint blockade has quickly taken hold as a therapeutic approach to break through tumor immune evasion by simultaneously targeting multiple inhibitory signaling pathways that blunt T cell activity. The combination of PD-1/PD-L1 inhibitors with CTLA-4 blockade (e.g., nivolumab + ipilimumab) has demonstrated greater efficacy compared to monotherapy in various malignancies, such as melanoma, lung cancer, and renal cell carcinoma [55]. In a seminal study, combined PD-1 and CTLA-4 blockade led to increased effector T cell activation, decreased regulatory T cells (Tregs), and improved overall survival in advanced melanoma patients compared to monotherapy.

Outside of CTLA-4, other inhibitory receptors like LAG-3 (lymphocyte-activation gene 3) and TIM-3 (T-cell immunoglobulin and mucin-domain containing-3) have surfaced as important mechanisms of resistance to PD-1 blockade. LAG-3 is commonly upregulated on PD-1-exhausted T cells after PD-1 therapy, and preclinical research has indicated that LAG-3 blockade cooperates with PD-1 inhibition to restore T cell function. This resulted in the approval of relatlimab (LAG-3 inhibitor) with nivolumab for advanced melanoma. Likewise, TIM-3 is increased in patients who become resistant to ICIs, and combination approaches using PD-1 + TIM-3 inhibitors (e.g., sabatolimab) are in clinical trials with encouraging early results [56].

ICIs + Small Molecule Inhibitors

Another promising strategy includes combining ICIs with intracellular signaling pathway small molecule inhibitors that are important for tumor growth and immune suppression. Among them, the MEK inhibitors (trametinib, cobimetinib), IDO inhibitors (epacadostat), and epigenetic modulators (DNMT and HDAC inhibitors) have shown the potential to improve checkpoint blockade efficacy.

- •MEK Inhibitors + ICIs: The MAPK pathway is also often activated in tumors with KRAS or BRAF mutations and causes immunosuppression through cytokine modulation and antigen presentation defects. MEK inhibitors (e.g., trametinib) have been reported to increase MHC-I expression and T cell infiltration and restore sensitivity to ICIs. Cobimetinib + atezolizumab (MEK + PD-L1 blockade) demonstrated encouraging activity in microsatellite-stable (MSS) colorectal cancer (Md Entaz Bahar et al., 2023) [57].

- IDO Inhibitors + ICIs: Indoleamine 2,3-dioxygenase 1 (IDO1) is an immunosuppressive enzyme that depletes tryptophan and generates kynurenine, inhibiting T cell proliferation and inducing Treg differentiation. Preclinical research revealed that IDO1 inhibition replenishes the ability of effector T cells, and when combined with PD-1 blockade, synergistic effects are seen (Pallotta MT et al., 2022) [58].

- Epigenetic Modulation + ICIs: Epigenetic dysregulation facilitates cancer immune evasion through silencing TAAs, inhibiting MHC expression, and inducing T cell exhaustion. The DNMT inhibitor (e.g., azacitidine) and HDAC inhibitor (e.g., entinostat) have been proved to enhance immunogenicity in tumors and boost the efficacy of ICIs. In clinical experiments, DNMT inhibitors upregulated PD-L1 expression and increased T cell trafficking, which suggested combination therapy for NSCLC and melanoma (Huang KC et al., 2021) [59].

ICIs + Metabolic Modulators

Metabolic reprogramming of the tumor microenvironment (TME) is an important mechanism of immune evasion. Tumors establish a metabolically toxic niche that exhausts critical nutrients (glucose, arginine, tryptophan), generates immunosuppressive metabolites (lactate, kynurenine), and remodels T cell metabolism. Inhibition of these metabolic vulnerabilities is a new strategy to overcome ICI resistance and improve T cell function.

- Targeting Lactate Metabolism: Cancer cells exhibit aerobic glycolysis (Warburg effect), resulting in lactate buildup and acidosis in the TME. This inhibits $CD8^+$ T cell activity and induces regulatory T cell (Treg) differentiation. Lactate dehydrogenase-A (LDHA) and monocarboxylate transporters (MCTs) inhibitors are being explored to enhance T cell fitness and increase PD-1 blockade efficacy (Doherty JR *et al*., 2013) [60].

- Treg Metabolism and A2AR Antagonists: Regulatory T cells (Tregs) have strong immunosuppressive activity by using high glucose consumption and generating adenosine that inhibits the activation of effector T cells through A2A adenosine receptor (A2AR) signaling. Preclinical research proved that A2AR antagonists (such as CPI-444) are synergistic with PD-1 blockade to reverse immunosuppression and induce long-lasting anti-tumor immunity (Emens LA *et al*., 2017) [61].

- Arginine Metabolism Modulation: MDSCs secrete arginase-1 (ARG1), which consumes extracellular arginine, a key amino acid needed for T cell activation and proliferation. ARG1 inhibitors (INCB001158) combined with ICIs are under investigation to circumvent this metabolic obstacle and reactivate T cell functionality in refractory tumors (Raber P *et al*., 2012) [62].

5.2. Next-Generation Immunotherapies

Bispecific and Trispecific Antibodies

Bispecific and trispecific antibodies are new-generation biologics designed to bind multiple immunological targets simultaneously, in essence, linking immune cells and tumor cells together to enhance antitumor immunity. Bispecific T-cell engagers (BiTEs), like blinatumomab (anti-CD19/CD3), have shown activity in hematological malignancies by recruiting cytotoxic T cells directly to tumor cells, inducing effective tumor cell lysis in an MHC-independent manner (Goebeler et., 2024) [63]. Applying this concept, novel trispecific antibodies target other immunomodulatory targets—like checkpoint receptors (PD-L1, CTLA-4)—to maximize efficacy and avoid resistance.

Engineered IL-2 and IL-15 Cytokine Therapy

Cytokine therapies have been known to be able to augment anti-cancer immune responses for a long time; however, endogenous cytokines like interleukin-2 (IL-2) are associated with considerable clinical toxicity.Next-generation engineered cytokines such as modified IL-2 ("superkines") and IL-15 fusion proteins have been engineered to increase antitumor activity with less toxicity. Charych *et al*. (2021) showed that engineered IL-2 variants specifically binding to CD122 (IL-2 receptor β-chain) substantially expanded $CD8^+$ effector T-cells and natural killer (NK) cells but not immunosuppressive $CD25^+$ regulatory T cells (Tregs), thereby showing superior effectiveness with lower systemic toxicity. Likewise, IL-15 superagonists like ALT-803 (N-803) have demonstrated robust NK cell and memory $CD8^+$ T-cell expansion with long-lasting antitumor activities in phase I/II clinical trials in various cancers (Margolin K *et al*., 2018) [64].

Nanoparticle-Based PD-L1 Blockade

The marriage of nanotechnology and immunotherapy has created new nanoparticle-based platforms that aim to increase checkpoint blockade efficacy while lowering systemic toxicity. Nanoparticle formulations facilitate greater biodistribution, tumor-targeting specificity, and controlled release of drugs, thereby improving therapeutic index over traditional antibodies. Nanoparticle PD-L1 blockade in preclinical tumor models, demonstrating that PD-L1 antibodies incorporated in biodegradable nanoparticles successfully enhanced intratumoral concentration, increased T-cell activation, and improved therapeutic effectiveness.

5.3. Personalized Neoantigen-Based Cancer Vaccines

mRNA-Based Cancer Vaccines

Recent breakthroughs in messenger RNA (mRNA) technology—emphasized by their swift clinical use for COVID-19 vaccines (Moderna/Pfizer)—have rekindled interest in patient-specific cancer vaccines against tumor-specific neoantigens. mRNA vaccines expressing patient-specific neoantigens, which are identified by whole-exome sequencing and computational epitope prediction tools, induce strong, polyclonal T-cell responses against tumor cells. Berraondo P *et al*. (2024) first developed personalized mRNA vaccines with notable T-cell responses and clinical benefit in melanoma patients. Such customized mRNA vaccines possess revolutionary promise by accurately tapping into individual immune repertoires against genetically different tumors [65].

Peptide Vaccines against Tumor-Specific Mutations

Peptide neoantigen vaccines are yet another powerful modality of precision oncology that leverages tumor-specific mutational burden to specifically prime and expand T-cells recognizing neoantigens. Peptide vaccines designed according to the mutational landscape of individual melanoma patients elicited robust $CD8^+$ and $CD4^+$ T-cell responses, triggering long-term tumor regression when combined with anti-PD-1 therapy. The personalized peptide vaccines successfully bypass central tolerance mechanisms, targeting extremely immunogenic neoepitopes that are not present in normal tissues, hence avoiding risks of autoimmunity. Latzer P *et al*. (2024) went on to affirm these results by noting how custom peptide vaccines initiated sustained immune responses associated with sustained survival in patients with glioblastoma and other resistant tumors [66].

5.4. CAR-T and TCR-T Cell Therapies for Solid Tumors

Chimeric antigen receptor T cell (CAR-T) and engineered T-cell receptor T cell (TCR-T) treatments have made impressive gains in hematologic malignancies, but translating these cell-based therapies to solid tumors poses a significant challenge owing to barriers such as compromised T-cell trafficking, a suppressive microenvironment, and antigen heterogeneity. Recent progress, guided by detailed mechanistic knowledge, has pushed the frontiers to break through these hurdles by innovative strategies, most importantly promoting CAR-T cell

trafficking to the tumor and intracellular antigen recognition by TCR-T cell engineering.

Improving CAR-T Cell Trafficking to Tumors

One of the major challenges constraining CAR-T activity in solid tumors is the inefficient homing of gene-engineered T cells to the tumor site. To counter this, scientists have investigated genetic engineering approaches that involve chemokine receptor mutations like CXCR4, CCR5, and CCR2, promoting CAR-T cell chemotaxis and tumor infiltration.

- Modifications of CXCR4: Moon et al. (2011) illustrated that CAR-T cells engineered to express CXCR4, the CXCL12 receptor richly expressed in most solid tumors, exhibited greatly enhanced trafficking and infiltration into tumor beds, leading to increased antitumor activity. Additional preclinical research by Zhao H et al. (2015) confirmed CXCR4 overexpression, showing significant accumulation of CAR-T cells in solid tumors, hence overcoming poor infiltration and exhibiting prolonged tumor regression in preclinical models of glioblastoma and pancreatic cancer [67].

- CCR5 and CCR2 Modifications:Chemokine receptors CCR5 and CCR2 have also been utilized to enhance CAR-T trafficking to tumor-secreted chemokines (CCL5 and CCL2). Wang et al. (2017) demonstrated CCR2-modified CAR-T cells were found to migrate more favorably towards CCL2-dense tumor microenvironments, resulting in superior control of murine solid tumor models. Likewise, Tian Y et al. (2025) demonstrated improved tumor trafficking and persistence of CCR5-modified CAR-T cells in Hodgkin lymphoma xenografts that translated into enhanced therapeutic efficacy and survival benefits in preclinical models [68].

TCR-T Cell Engineering for Intracellular Antigen Recognition

Whereas CAR-T cells recognize extracellular surface antigens, TCR-T cells engineered to recognize peptides from intracellular tumor-specific proteins presented by MHC complexes offer a much broader range of targetable tumor antigens. Greater specificity and ability to target intracellular antigens make TCR-T therapy an attractive approach for solid tumors.

- High-Affinity TCR Engineering:Spear TT et al. (2019) led high-affinity TCR-transduced T cells against MART-1, gp100, and NY-ESO-1 with compelling clinical responses in metastatic melanoma patients [69].

- CRISPR-Based TCR Engineering and Neoantigen Recognition: Advances in emerging CRISPR/Cas9 technologies have enhanced TCR engineering accuracy to allow for customized targeting of patient-specific neoantigens. Stadtmauer et al. (2020) described the initial clinical trial involving CRISPR-engineered TCR-T cells targeting individual cancer mutation-specific neoantigens, and the findings showed safe editing of T-cells, persistent TCR expression, and indication of tumor-targeted immune responses in refractory cancers. The strategy allows for personalized tumor targeting and increases specificity and therapeutic potential of TCR-T therapy manyfold [70].

- Affinity Optimization and Safety Considerations: Safety is still of utmost concern in TCR-T cell therapies because off-target recognition poses a risk. TCR affinity thresholds, proving that optimized affinity is important for efficient tumor recognition while keeping cross-reactivity and off-target tissue injury at a minimum. Multifaceted TCR engineering methods, such as affinity-tuned receptors and safety switches, are still under development, minimizing risks and maximizing therapeutic index for clinical application [69,70].

CAR-T and TCR-T cell therapy optimization against solid tumors increasingly combines strategies, incorporating checkpoint inhibitors, cytokine modulation, or metabolic reprogramming to overcome immunosuppressive tumor microenvironments and improve persistence and function of the engineered T cells. Furthermore, the use of sophisticated delivery platforms like nanoparticles or intratumoral injection techniques also enhances efficacy through accurate localization, decreased systemic toxicity, and amplified local immune modulation.

CONCLUSION

Breaking immune evasion and checkpoint blockade resistance necessitates the intersection of next-generation strategies with primary and adaptive mechanisms of resistance. Tumor-intrinsic processes such as B2M mutations, JAK1/JAK2 mutations, and WNT/β-catenin-mediated T-cell exclusion restrict response, whereas adaptive resistance through TIM-3, LAG-3, and TIGIT upregulation demands dual checkpoint blockade. Epigenetic dysregulation (DNA methylation, histone deacetylation) and metabolic restriction (lactate, adenosine, arginine depletion) are

also underlying immune suppression. Developments in bispecific/trispecific antibodies (PD-L1/CD3, HER2 trispecifics), engineered cytokines (IL-2 superagonists, IL-15 variants), and nanoparticle-mediated PD-L1 blockade enhance efficacy. Microbiome modulation (Akkermansia muciniphila, FMT-based reprogramming) enhances ICI response, while personalized mRNA and peptide neoantigen vaccines have potential to induce long-term T-cell immunity. CAR-T and TCR-T engineering with CXCR4/CCR5 modifications and CRISPR-based TCR optimization can target solid tumors. Next-generation multi-omics-guided precision oncology, combining genomics, proteomics, and artificial intelligence-driven patient stratification, will maximize combinatorial regimens to induce long-term antitumor responses.

Establishing dynamic biomarkers (circulating tumor DNA, immune repertoire, and spatial single-cell readouts) to inform real-time treatment adaptation; designing safer, persistence-adjusted cell therapies that escape antigen loss without restricting on-target/off-tumor toxicities; rationally sequencing or cycling immunotherapies to pre-empt resistance evolution in heterogeneity-laden tumors; and standardizing microbiome-driven interventions for durable, reproducible effect. Underlying questions include how to permanently re-engineer the TME with minimal systemic toxicity, how to optimize the combination of metabolic and epigenetic modulators with checkpoint blockade, and how to architect adaptive, platform trials fueled by AI-powered patient stratification—alongside fair access and strong data-sharing—to bring these advances into steady, long-term cures.

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Received on 22-08-2025 Accepted on 19-09-2025 Published on 21-10-2025

https://doi.org/10.30683/1927-7229.2025.14.09