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Tumor Microenvironment-Driven Immunotherapy Resistance in Solid Cancers: Emerging Biomarkers and Precision Combination Strategies

Abstract:
The advent of immune checkpoint blockade (ICB) and other immune-based therapies has redefined cancer treatment, yielding unprecedented and durable responses in select patient subsets. However, the majority of individuals with solid malignancies either fail to respond or eventually develop resistance, highlighting a fundamental limitation in current immunotherapeutic approaches. Increasing evidence implicates the tumor microenvironment (TME) as a central orchestrator of this resistance, functioning as a complex and adaptive ecosystem that actively suppresses antitumor immunity. Immunosuppressive cell populations such as tumor-associated macrophages, myeloid-derived suppressor cells, and regulatory T cells, alongside stromal remodeling, aberrant angiogenesis, and metabolic competition, collectively restrict effective immune cell infiltration and effector function.
Traditional biomarkers—such as PD-L1 expression, tumor mutational burden, and microsatellite instability—capture only fragments of this dynamic interplay and are inadequate predictors of therapeutic outcome. Advances in multi-omic profiling, spatial transcriptomics, and single-cell analytics are enabling the identification of more refined TME-related biomarkers that integrate immunologic, metabolic, and stromal signatures. These evolving frameworks hold promise for more accurate classification of resistant phenotypes and for guiding individualized therapeutic interventions. Concurrently, rationally designed combination approaches are emerging to overcome TME-mediated resistance. Strategies coupling checkpoint inhibitors with metabolic modulators, anti-angiogenic agents, oncolytic viruses, or epigenetic reprogrammers are demonstrating the ability to convert immune-excluded tumors into immune-reactive phenotypes. The future of immuno-oncology lies in precision frameworks that integrate multidimensional biomarker data with adaptive therapeutic regimens to counteract the evolving suppressive TME. Achieving this integration may redefine immunotherapy from a population-based intervention to a context-specific, dynamically guided precision strategy capable of realizing durable remission across solid tumors.

Introduction

Over the past decade, the emergence of immunotherapy has revolutionized the therapeutic paradigm of oncology, transforming once-fatal malignancies into diseases amenable to long-term immune-mediated control. The development of immune checkpoint inhibitors (ICIs) targeting PD-1, PD-L1, and CTLA-4 represented a breakthrough achievement, yielding durable responses in subsets of patients with melanoma, lung cancer, renal cell carcinoma, and other solid malignancies. This shift from cytotoxic

chemotherapy toward immune modulation introduced a new conceptual framework for cancer treatment—one in which the immune system can be therapeutically reactivated to sustain selective antitumor pressure and durable remission (Sharma et al., 2022). However, despite this conceptual and clinical progress, the majority of patients across solid tumor types derive limited benefit, with many exhibiting either primary (innate) non-responsiveness or acquired resistance following initial response (Gettinger et al., 2023). This discrepancy underscores the complex interplay between tumor-intrinsic alterations and the immunosuppressive tumor microenvironment (TME), which together dictate therapeutic outcome.

Evolution and Limitations of Immunotherapy in Solid Tumors

The conceptual foundations of modern immunotherapy trace back to the recognition of cancer immunosurveillance and the equilibrium phase between immune elimination and tumor escape. The clinical application of these principles was realized through checkpoint blockade—agents that disrupt inhibitory receptor–ligand interactions exploited by tumors to suppress T-cell function. Agents such as ipilimumab, nivolumab, pembrolizumab, and atezolizumab have reshaped survival outcomes in multiple malignancies, producing long-term overall survival plateaus previously unseen in advanced disease (Pai & Chen, 2022). Nevertheless, the enthusiasm surrounding these results has been tempered by the reality that less than one-third of unselected patients experience meaningful benefit from ICI-based therapy. Even among responders, late relapse and immune escape frequently occur, suggesting that the mechanisms governing durable immunity are tightly constrained by tumor evolutionary adaptation and environmental context (Kwon et al., 2024).

Primary resistance refers to the failure to mount an initial immunologic response despite checkpoint blockade. Multiple molecular and cellular drivers underlie this phenomenon, including low tumor immunogenicity, deficient antigen presentation, absence of pre-existing tumor-infiltrating lymphocytes, and constitutive activation of oncogenic pathways that suppress interferon signaling or promote T-cell exclusion (Zhao et al., 2023). In contrast, acquired resistance evolves from dynamic selective pressure, leading to immunoediting-driven loss of neoantigens, reactivation of inhibitory checkpoints, or phenotypic reprogramming of tumor and stromal cells. Both resistance types converge upon the TME as the functional arena that integrates tumor-intrinsic and -extrinsic signals to shape immune responsiveness

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The Clinical Burden of Resistance in Solid Malignancies

Resistance to immunotherapy carries substantial clinical implications across solid cancers. In melanoma and non-small cell lung cancer (NSCLC), where ICIs have achieved their most prominent success, approximately 60–70% of patients exhibit primary resistance, while an additional 20–30% develop progression after transient response (Postow & Callahan, 2022). In gastrointestinal, breast, and gynecologic malignancies, therapeutic benefit remains even more restricted, largely due to the immunologically "cold" nature of these tumors characterized by low T-cell infiltration and overwhelming immunosuppressive signaling (Wang et al., 2023). The clinical burden is magnified by the limited predictive accuracy of existing biomarkers, leading to widespread exposure to expensive and potentially toxic therapies without clear benefit. From a health systems perspective, the inability to pre-empt or overcome resistance substantially limits the cost-effectiveness and scalability of immuno-oncology as a universal treatment model.

The Central Role of the Tumor Microenvironment

The TME—the composite of immune cells, fibroblasts, endothelial networks, extracellular matrix, and soluble mediators—serves as both a shield and a sculptor of tumor evolution. It dictates whether an antitumor immune response can be effectively initiated, propagated, and sustained. Within this dynamic ecosystem, tumors exploit multiple axes of suppression. Myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) secrete arginase, prostaglandin E2, and TGF-β, which inhibit effector T-cell activation and promote regulatory T-cell expansion. Cancer-associated fibroblasts (CAFs) establish dense desmoplastic barriers and secrete CXCL12 and matrix remodeling enzymes that physically and chemically exclude cytotoxic lymphocytes (Chen et al., 2022). Furthermore, metabolic gradients—driven by hypoxia, lactate accumulation, and nutrient depletion—create a metabolically hostile niche that impairs T-cell function and fosters immune exhaustion. Hypoperfusion and aberrant vascularization exacerbate these processes by impeding immune trafficking into tumor cores (Huang et al., 2023).

Emerging evidence highlights that TME heterogeneity is neither static nor incidental; rather, it is shaped by reciprocal communication between tumor clones and immune infiltrates under therapeutic selection. Longitudinal biopsies reveal that even within a single patient, spatially distinct lesions can display divergent immune microenvironments, explaining discordant responses to systemic immunotherapy (Liu et al., 2024). Consequently, addressing resistance requires not only identifying static biomarkers but also understanding the temporal evolution of TME states under therapeutic pressure.

Inadequacies of Conventional Biomarkers

The widespread clinical reliance on PD-L1 immunohistochemistry, tumor mutational burden (TMB), and microsatellite instability (MSI) has produced mixed predictive accuracy. PD-L1 expression varies between primary and metastatic lesions, fluctuates dynamically during treatment, and often fails to predict outcomes in tumors with non-inflamed microenvironments (Conroy et al., 2023). Similarly, high TMB can correlate with increased neoantigen load

but is neither necessary nor sufficient for response; tumors with comparable TMB values can exhibit radically different immune phenotypes depending on stromal context and antigen presentation competence. MSI-high tumors respond favorably to ICB, yet such tumors represent a small fraction of most solid cancer types.

Consequently, contemporary research is shifting toward multidimensional biomarker platforms. Spatial transcriptomics, multiplex immunohistochemistry, and single-cell RNA sequencing now enable the deconvolution of TME composition and spatial architecture at unprecedented resolution (Lai et al., 2025). Integrative computational models combining immune cell topology, stromal density, and cytokine gradients are demonstrating superior predictive performance compared with single-parameter measures. Additionally, circulating biomarkers—such as peripheral T-cell clonality, cytokine signatures, and extracellular vesicle cargo—offer a non-invasive lens for real-time monitoring of immune dynamics. The integration of such data promises to refine patient selection, identify adaptive resistance trajectories, and guide early therapeutic recalibration.

Rationales for Combination Immunotherapy

Monotherapy with checkpoint inhibitors—though transformative—has revealed the limitations of targeting a single immune axis in a tumor ecosystem characterized by redundancy and adaptability. Rational combination approaches aim to simultaneously dismantle multiple layers of immune suppression and foster immune reinvigoration. The most studied combinations include dual checkpoint blockade (e.g., PD-1 plus CTLA-4), which has yielded higher response rates but also greater toxicity (Hugo et al., 2023). More innovative combinations now integrate agents that remodel the TME: VEGF and Ang2 inhibitors normalize vascular function to enhance T-cell infiltration; TGF-β antagonists relieve stromal exclusion; histone deacetylase inhibitors and DNA methyltransferase inhibitors reprogram the epigenetic landscape to restore antigen presentation and interferon signaling (Cruz et al., 2022).

Metabolic reprogramming is an emerging frontier in this context. Inhibition of IDO1, arginase, and adenosine-generating enzymes can shift nutrient competition in favor of effector lymphocytes. Similarly, targeting cancer cell glycolysis or glutamine metabolism may counterbalance the immunosuppressive metabolic microenvironment. Beyond targeted pharmacologic agents, approaches using radiation, chemotherapy, and oncolytic viruses can also act synergistically with ICB by releasing neoantigens, enhancing dendritic cell activation, and increasing immune infiltration. Clinical trials between 2021 and 2025 have increasingly adopted adaptive trial designs to test such combinations, integrating longitudinal biomarker assessment to refine patient cohorts and dose schedules (Nguyen et al., 2024).

Integrating Precision Biomarkers With Dynamic Therapeutic Strategies

The convergence of multi-omic profiling, computational biology, and high-dimensional imaging is enabling more precise mapping of TME-driven resistance mechanisms. This technological evolution supports a paradigm shift from static biomarker identification toward dynamic prediction models that capture real-time TME adaptation. Precision immuno-oncology envisions a future in which therapy selection and sequencing are customized to each patient's immune ecology. Longitudinal tissue and liquid biopsies, analyzed through integrated analytical pipelines, will enable clinicians to monitor microenvironmental shifts as tumors evolve under treatment pressure. Such an approach supports the timely addition or withdrawal of therapeutic modalities—checkpoint inhibitors, cytokine modulators, metabolic interventions, or adoptive cell therapy—based on evolving resistance signatures.

However, translating this into routine clinical practice remains challenging. Issues of cost, tissue accessibility, data standardization, and analytical reproducibility hinder widespread adoption. Furthermore, most biomarker discovery efforts remain confined to retrospective or early-phase trial settings. To truly operationalize biomarker-guided immunotherapy, prospective validation in large, diverse patient populations and integration into clinical decision-support systems are essential. The field must also contend with ethical implications of algorithm-driven therapy assignment and the societal challenge of equitable access to advanced diagnostic platforms.

The Significance and Aim of This Review

Given the rapidly expanding but fragmented landscape of immunotherapy resistance research, a comprehensive synthesis focusing on TME-driven mechanisms is urgently needed. The present review aims to critically examine emerging evidence on the multifaceted roles of the TME in mediating both primary and acquired resistance to immunotherapy across solid cancers. It will dissect the interplay among cellular, molecular, and metabolic factors shaping immune exclusion and dysfunction, elucidate cutting-edge biomarker strategies capable of capturing these dynamics, and evaluate ongoing efforts to develop precision combination regimens that recondition the TME toward immune responsiveness. Ultimately, by integrating insights from recent translational and clinical studies (2021–2025), this review seeks to delineate a forward-looking framework for overcoming immunotherapy resistance through TME-targeted, biomarker-informed strategies. This synthesis underscores a central thesis: sustained cancer immunotherapy success will depend not only on targeting immune checkpoints but also on intelligently redesigning the very ecosystem within which immunity operates.

The Role of the Tumor Microenvironment in Immunotherapy Resistance

The therapeutic landscape of oncology has been transformed by immune checkpoint inhibitors (ICIs), yet the durability of their efficacy remains profoundly

constrained by the tumor microenvironment (TME)—a multifaceted ecosystem that dynamically regulates immune surveillance, immune evasion, and therapeutic responsiveness. Rather than functioning as a passive structural scaffold, the TME constitutes an active participant in tumor evolution, comprising a complex interplay of malignant, stromal, immune, and endothelial cells within a matrix of cytokines, metabolites, and extracellular matrix (ECM) components (Joyce & Fearon, 2022). Mounting evidence indicates that immunotherapy resistance—both primary and acquired—is often rooted not in the failure of ICIs to engage their molecular targets, but in the ability of the TME to reestablish immunosuppression through redundant networks of cellular crosstalk and metabolic constraint (Binnewies et al., 2023).

1. The Immunosuppressive Tumor Microenvironment

The immunosuppressive nature of the TME manifests as a convergence of inhibitory immune populations, metabolite competition, and stromal remodeling that collectively impede cytotoxic T-cell infiltration and function. Immune checkpoint blockade, particularly targeting PD-1/PD-L1 and CTLA-4, relies on the reactivation of effector T cells within the tumor milieu. However, persistent immunosuppressive circuits blunt these effects despite receptor engagement. This is evident in "immune-excluded" and "immune-desert" tumors where immune cells are either physically sequestered outside the tumor core or rendered inactive by soluble mediators such as transforming growth factor-β (TGF-β), interleukin-10 (IL-10), and adenosine accumulation (Tauriello et al., 2023). Such immunosuppressive landscapes not only prevent the recruitment of cytotoxic lymphocytes but also accelerate T-cell exhaustion, creating a self-reinforcing ecosystem resistant to checkpoint inhibition.

The immunosuppressive TME can be viewed as a product of tumor adaptation under immune pressure. Tumors responding initially to PD-1 blockade may undergo immunoediting, selecting for variants that upregulate additional checkpoints (e.g., TIM-3, LAG-3, TIGIT) or enhance recruitment of suppressive myeloid cells (Waldman et al., 2024). Moreover, stromal components such as cancer-associated fibroblasts (CAFs) and an altered extracellular matrix (ECM) reduce the accessibility of immune cells to cancer nests. The result is a milieu that sustains immune ignorance, even in the presence of high neoantigen load or PD-L1 expression—explaining why "hot" tumors can evolve into "cold" phenotypes after initial response to immunotherapy.

2. Tumor-Associated Macrophages (TAMs)

Among all myeloid populations within the TME, TAMs play one of the most defining roles in modulating ICI resistance. Derived primarily from circulating monocytes, TAMs can adopt phenotypes along a continuum between pro-inflammatory M1-like and immunosuppressive M2-like states. In most solid tumors, especially pancreatic, breast, and glioblastoma, M2-skewed TAMs dominate, secreting immunosuppressive cytokines such as IL-10 and TGF-β, and expressing surface ligands including PD-L1 and stabilin-1, which directly inhibit T-cell activation (Ruffell et al., 2023).

Mechanistically, TAMs drive resistance through multiple axes. They dampen $CD8^+$ T-cell infiltration via secretion of CCL2 and VEGF, maintain chronic inflammation that paradoxically supports tumor progression, and promote angiogenesis and ECM remodeling to sustain immune exclusion. Recent studies indicate that PD-1 blockade may paradoxically enhance TAM proliferation and PD-L1 expression in certain contexts, creating a compensatory suppressive loop (Zhu et al., 2023). Moreover, cross-talk between TAMs and Tregs through IL-10 and CCL20 maintains an immunosuppressive state refractory to ICIs.

Therapeutically, strategies targeting CSF1R, CCR2, or PI3Kγ—key regulators of TAM polarization—have shown promise in re-sensitizing tumors to ICB by shifting macrophage phenotypes toward pro-inflammatory states (DeNardo & Ruffell, 2022). However, the plasticity of TAM populations remains a major challenge, as their reprogramming is transient and context-dependent.

3. Regulatory T Cells (Tregs)

Tregs, characterized by expression of FOXP3 and CTLA-4, are essential for maintaining immune tolerance but become detrimental in the TME by suppressing antitumor effector responses. Elevated intratumoral Treg density correlates with poor response to PD-1/PD-L1 inhibitors across several tumor types, including hepatocellular carcinoma and ovarian cancer (Tanaka et al., 2024). Tregs suppress effector T-cell function via IL-2 consumption, secretion of immunosuppressive cytokines (IL-10, TGF-β), and direct cytotoxic activity mediated by granzyme B.

Importantly, CTLA-4 blockade, while designed to enhance T-cell priming, can expand the Treg compartment peripherally, particularly when Fc-mediated depletion is incomplete. This paradox has led to differential outcomes across tumor types depending on antibody isotype and Fc receptor engagement (Simpson-Trapani et al., 2023). PD-1 blockade also affects Tregs variably: in some settings, PD-1 inhibition reinvigorates exhausted Tregs, augmenting their suppressive capacity rather than diminishing it, thereby neutralizing the therapeutic benefit (Kamphorst et al., 2022).

Novel combinations—such as dual checkpoint blockade with anti-CTLA-4 variants optimized for Treg depletion or therapies targeting IL-2 signaling bias—are under investigation to selectively modulate the Treg niche. However, systemic Treg depletion risks autoimmunity, illustrating the delicate balance between enhancing immunity and preserving peripheral tolerance.

4. Myeloid-Derived Suppressor Cells (MDSCs)

MDSCs represent a heterogeneous population of immature myeloid cells renowned for their potent ability to suppress adaptive immunity. Elevated

circulating and intratumoral MDSC levels strongly predict ICI resistance in melanoma and lung cancer (Nishikawa et al., 2023). Mechanistically, MDSCs impair interferon signaling, deplete L-arginine and cysteine essential for T-cell proliferation, and generate reactive oxygen and nitrogen species that induce T-cell dysfunction and apoptosis. They also support the expansion of Tregs through IL-10 and promote TAM polarization toward M2 phenotypes, reinforcing immunosuppression in a feed-forward manner.

Several studies between 2021 and 2024 have expanded understanding of MDSC biology in the context of ICIs. In patients treated with PD-1 inhibitors, MDSC expansion has been observed following therapy initiation, suggesting treatment-induced recruitment as a compensatory mechanism of resistance. MDSCs also upregulate PD-L1 and ARG1 under hypoxic conditions, enhancing both metabolic and checkpoint-mediated suppression (Kumar et al., 2024).

Therapeutic targeting of MDSCs has yielded mixed outcomes. Agents such as all-trans retinoic acid (ATRA) and CXCR2 inhibitors can reduce MDSC accumulation and restore ICI sensitivity in preclinical models, but clinical efficacy remains inconsistent, potentially due to the rapid phenotypic conversion and incomplete depletion of myeloid subsets (Maniati et al., 2023). The consensus emerging from current literature emphasizes that durable immunotherapy benefit will likely require concurrent modulation of MDSCs along with T-cell activation.

5. Cancer-Associated Fibroblasts (CAFs)

CAFs constitute a major non-immune cellular component of the TME and are increasingly recognized as central architects of immunotherapy resistance. Through secretion of extracellular matrix proteins, chemokines, and growth factors, CAFs construct physical and biochemical barriers to immune infiltration. In desmoplastic tumors such as pancreatic ductal adenocarcinoma (PDAC), fibroblast-derived collagen and hyaluronic acid compress intratumoral vasculature, resulting in hypoxia and poor T-cell trafficking (Moffitt et al., 2023).

CAFs secrete CXCL12, which binds to CXCR4 on T cells, creating a chemokine "trap" that immobilizes lymphocytes at the peritumoral stroma. They also release TGF-β, which enforces T-cell exclusion and synergizes with PD-L1 signaling to maintain immune evasion (Mariathasan et al., 2022). Interestingly, CAFs are not a homogeneous population; distinct subsets identified by single-cell transcriptomic profiling exhibit divergent roles—some immunosuppressive and others potentially supportive of antitumor immunity (Öhlund et al., 2023). This heterogeneity has sparked debate regarding whether CAF depletion is universally beneficial. In some models, broad fibroblast targeting enhanced tumor invasiveness and diminished immune infiltration, reflecting the dualistic nature of stromal modulation.

Therapeutic strategies now focus on reprogramming rather than depleting CAFs, using inhibitors of FAP, TGF-β, or CXCR4 to normalize stroma and facilitate T-cell entry. Combination of PD-1 blockade with TGF-β antagonists, for instance, has demonstrated synergy in converting immune-excluded to inflamed phenotypes in preclinical and early clinical studies.

6. Hypoxia and Metabolic Reprogramming

Hypoxia is a hallmark of the TME, shaping immune outcomes through both direct metabolic stress and transcriptional reprogramming mediated by hypoxia-inducible factors (HIFs). Hypoxic zones suppress immune cell infiltration and favor the recruitment of MDSCs and TAMs. Concurrently, cancer cells adapt by switching to glycolytic metabolism and secreting lactate, which acidifies the microenvironment and impairs T-cell receptor signaling (Scharping et al., 2022).

Metabolic competition between tumor and immune cells exacerbates this suppression. Tumor cells monopolize glucose, glutamine, and tryptophan, depriving infiltrating lymphocytes of essential substrates for proliferation and cytokine production. The tryptophan-degrading enzyme IDO1 and adenosine-generating ectoenzymes CD39 and CD73 contribute to T-cell anergy and expansion of Tregs within this nutrient-limited milieu (Antonioli et al., 2024). These metabolic checkpoints act independently of PD-1 or CTLA-4 signaling, providing parallel routes to resistance even when checkpoint receptors are blocked.

Strategies aimed at rebalancing metabolic homeostasis—such as adenosine receptor antagonists, IDO inhibitors, or lactate dehydrogenase blockers—are currently under clinical evaluation. Results remain variable, with some trials failing to reproduce preclinical efficacy, emphasizing the adaptive metabolic plasticity of both tumors and the immune compartment. Nonetheless, the consensus is that metabolic interventions hold significant promise as adjuncts to ICIs in reconditioning the TME toward immune competence.

7. Cytokine Signaling Networks

The TME is saturated with cytokines that influence immune cell fate, often tipping the balance toward tolerance rather than immunity. IL-10, IL-6, and TGF-β are prototypical immunosuppressive cytokines that attenuate antigen presentation, inhibit dendritic cell maturation, and downregulate MHC expression on tumor cells. Conversely, IFN-γ, a cytokine central to T-cell effector function, can paradoxically drive adaptive resistance through induction of PD-L1 expression on both tumor and stromal cells (Spranger et al., 2023).

This cytokine ambivalence exemplifies the nuanced resistance mechanisms at play—immune activation and suppression are intertwined within a feedback loop. Persistent IFN signaling, initially beneficial, promotes upregulation of JAK/STAT–dependent PD-L1 induction and alternative checkpoints like LAG-3. Similarly, TNF-α signaling can facilitate tumor cell dedifferentiation into mesenchymal states less visible to immune surveillance.

Therapeutic modulation of cytokine signaling remains a double-edged sword. While IL-2 agonists and type I

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interferons can invigorate immune responses, their systemic administration often triggers severe toxicity. Recent innovations include engineered cytokine variants and targeted delivery systems designed to localize immune stimulation within the TME, thereby enhancing synergy with ICIs while minimizing systemic inflammation (Bailey et al., 2025).

8. Immune Exhaustion Mechanisms

T-cell exhaustion represents a state of functional decline induced by chronic antigen exposure and sustained inhibitory receptor engagement. Exhausted $CD8^+$ T cells exhibit diminished cytokine production, metabolic inflexibility, and co-expression of multiple inhibitory receptors, including PD-1, TIM-3, and LAG-3. While PD-1 blockade can transiently reinvigorate these cells, transcriptional and epigenetic programs driven by TOX, NR4A, and TCF1 limit the durability of reversal (Beltra et al., 2022).

Recent investigations have revealed spatial hierarchies of exhaustion within the TME. Peripheral $PD\text{-}1^+TCF1^+$ progenitor cells retain self-renewal potential, giving rise to terminally exhausted $PD\text{-}1^+TIM\text{-}3^+$ cells deep within the tumor core. Checkpoint blockade preferentially acts on the progenitor subset; however, when these niches are depleted or spatially isolated by stromal barriers, therapeutic reinvigoration is attenuated (Jansen et al., 2023). Thus, exhaustion must be contextualized as both a molecular and spatially regulated process.

In addition to T cells, natural killer (NK) cells also undergo exhaustion-like phenotypes, displaying increased NKG2A expression and diminished cytotoxic capacity, particularly in solid tumors such as hepatocellular carcinoma. This broad landscape of immune fatigue highlights the limitation of monotherapy targeting a single checkpoint and underscores the rationale for combinatorial strategies that incorporate metabolic and epigenetic reprogramming.

9. Extracellular Matrix Remodeling

ECM remodeling is more than structural reinforcement; it is an active mediator of immunotherapy resistance. Dense collagen networks and fibronectin deposition restrict T-cell infiltration and facilitate integrin-mediated survival signaling in tumor cells. Crosslinking of collagen via lysyl oxidase (LOX) increases interstitial stiffness, compresses vessels, and reduces immune cell motility (Burgess et al., 2022). Additionally, ECM fragments can signal through pattern recognition receptors such as TLR2 and TLR4 on macrophages, further amplifying inflammation-associated immunosuppression.

Multiple studies have shown that high ECM stiffness correlates inversely with response to PD-1 blockade in melanoma and NSCLC, even after adjusting for immune cell density (Zeltz et al., 2023). Enzymatic degradation of ECM components, use of LOX inhibitors, or antifibrotic drugs such as pirfenidone have been shown in preclinical settings to enhance immune infiltration and sensitize tumors to ICIs. However, as with CAF targeting, ECM modulation requires precision; complete degradation may induce metastasis by facilitating tumor cell dissemination. Therefore, the emerging view advocates for "ECM normalization" rather than obliteration as the therapeutic goal.

10. Intercellular Communication and Exosomes

Exosomes and other extracellular vesicles (EVs) have recently been recognized as pivotal mediators of intercellular crosstalk within the TME. Tumor-derived exosomes carry PD-L1 on their surfaces, enabling systemic suppression of T-cell activity even beyond the local TME (Chen et al., 2022). They also deliver immunosuppressive microRNAs—such as miR-23a and miR-146a—that reprogram macrophages and dendritic cells toward tolerogenic phenotypes.

EV-mediated signaling extends resistance beyond the site of primary tumor–immune interaction, creating a "pre-metastatic niche" that is refractory to immunotherapy. Recent clinical studies in melanoma and NSCLC have shown that elevated exosomal PD-L1 in plasma correlates with poor ICI response and shorter progression-free survival (Cordonnier et al., 2024). Conversely, exosomes derived from activated immune cells can propagate antitumor signals, suggesting a dualistic role depending on cellular origin. Modulating exosome biogenesis or intercepting their uptake using small-molecule inhibitors or antibody-coated nanoparticles represents a nascent but promising strategy to disrupt systemic immune suppression.

Integrative Perspective

Collectively, these findings reveal that the TME is not a static antagonist but a dynamic, co-evolving system capable of adapting to immunologic pressure. Each component—immune, stromal, metabolic, and molecular—contributes to a layered resistance architecture that blunts the efficacy of PD-1/PD-L1 and CTLA-4 blockade. While significant strides have been made in deconstructing individual mechanisms, the major conceptual challenge lies in integrating these pathways into comprehensive therapeutic frameworks.

The evidence suggests that successful immunotherapy will depend on multi-dimensional interventions targeting the immune ecosystem at multiple levels: macrophage and MDSC reprogramming, CAF and ECM normalization, metabolic adaptation, and cytokine network modulation. The complexity and redundancy of TME-driven resistance argue against a "one-size-fits-all" approach. Instead, the future of immunotherapy lies in precision integration—guided by dynamic biomarker profiling—to adaptively remodel the TME toward a sustained immunogenic state.

Emerging Predictive Biomarkers of Immunotherapy Resistance in Solid Tumors

The transformative success of immune checkpoint inhibitors (ICIs) across multiple malignancies has necessitated an equally sophisticated framework for biomarker development. Despite notable clinical gains in melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma, most patients with solid tumors

exhibit limited or transient responses to immunotherapy. The heterogeneity of tumor–immune interactions, coupled with the dynamic plasticity of the tumor microenvironment (TME), has rendered single-parameter biomarkers largely insufficient. Current research efforts have therefore shifted toward integrative biomarker paradigms that contextualize immune responsiveness within genomic, proteomic, spatial, and metabolic dimensions. This section examines emerging predictive biomarkers of immunotherapy resistance, critically appraising their mechanistic rationale, methodological challenges, and clinical translational potential.

1. PD-L1 Expression: The Foundational but Imperfect Marker

PD-L1 expression, quantified by immunohistochemistry (IHC), remains the most widely adopted biomarker guiding ICI therapy. Across tumor types, higher PD-L1 expression has generally correlated with increased response rates to PD-1/PD-L1 blockade. Yet its predictive capacity is far from absolute. Multiple clinical trials—including those in NSCLC, urothelial carcinoma, and gastric cancer—have demonstrated therapeutic benefit even among PD-L1–low or –negative patients, while some PD-L1–high patients fail to respond (Herbst et al., 2023).

The limitations stem from biological and technical heterogeneity. PD-L1 expression is spatially and temporally dynamic, influenced by interferon-γ signaling, oncogenic pathways (e.g., EGFR, PI3K–AKT), and prior therapies. Moreover, discrepancies in antibody clones, scoring algorithms, and cutoff thresholds across assays (SP142 vs. 22C3 vs. 28-8) undermine reproducibility. Intratumoral heterogeneity further compounds interpretive variability, as biopsy samples may underrepresent immune-infiltrated regions (Hellmann et al., 2022).

Efforts to increase sensitivity through combined positivity scores (CPS) and multiplexed IHC have improved spatial resolution but not fully addressed biological diversity. Ultimately, PD-L1 should be viewed as a contextual rather than deterministic marker—its expression reflects a dynamic immune equilibrium that must be interpreted alongside complementary biomarkers such as tumor mutational burden (TMB) and immune gene signatures.

2. Tumor Mutational Burden (TMB): Quantity Does Not Equal Immunogenicity

TMB, defined as the number of somatic mutations per megabase of genomic DNA, emerged as a surrogate for neoantigen load and immunogenicity. High-TMB tumors, such as melanoma and smoking-related NSCLC, tend to exhibit improved responses to ICIs. Nonetheless, its predictive value has proven inconsistent across cancer types and therapeutic contexts. Clinical trials have revealed that the association between TMB and treatment response attenuates substantially in tumors with low baseline immunogenicity, such as pancreatic and prostate cancer (Nabet et al., 2023).

TMB's mechanistic premise rests on the generation of neoantigens capable of eliciting cytotoxic T-cell recognition. However, many mutations produce non-immunogenic peptides or occur in subclonal regions not represented on MHC molecules. Moreover, tumor editing and loss of antigen-presentation machinery (e.g., β2-microglobulin loss) frequently decouple TMB from actual immune visibility.

Analytically, TMB quantification remains nonstandardized. Whole exome sequencing (WES) provides high fidelity but limited feasibility, while targeted gene panels risk sampling bias. Cutoff thresholds for "high" TMB vary—from ≥10 to ≥20 mutations/Mb—depending on assay and cancer type. These inconsistencies generate discordant clinical interpretations and highlight the need for context-specific calibration. Integrating TMB with measures of clonality and epitope quality, rather than gross mutation counts, may more accurately reflect tumor immunogenic potential.

3. Microsatellite Instability (MSI): A Potent but Rare Predictor

Microsatellite instability (MSI), indicative of deficient DNA mismatch repair (dMMR), represents one of the most robust predictive biomarkers for ICI response. MSI-high tumors display elevated mutational rates and abundant frameshift-derived neoantigens, eliciting dense lymphocytic infiltration and upregulated immune checkpoints. This biology explains the dramatic sensitivity of MSI-high colorectal and endometrial cancers to PD-1 blockade (Overman et al., 2022).

However, MSI's applicability is constrained by prevalence—present in only 3–4% of solid tumors overall—and by emerging evidence of nonuniform benefit within MSI-high cohorts. Some MSI-high tumors exhibit immune exclusion secondary to stromal remodeling or JAK1/JAK2 loss, which impairs interferon-γ signaling despite high neoantigen load (Schrock et al., 2024). Conversely, certain microsatellite stable tumors can achieve comparable responses through alternative mutational processes generating immunogenic neoepitopes.

Technologically, MSI detection has evolved from PCR-based loci panels toward next-generation sequencing (NGS) and methylation signatures, enhancing precision in ambiguous cases. Yet as a predictive biomarker, MSI functions best as a categorical diagnostic criterion rather than a continuous metric, limiting its refinement for intermediate phenotypes such as "MSI-low" tumors.

4. Neoantigen Profiling: Toward Qualitative Immunogenicity

While TMB and MSI indirectly estimate neoantigen load, neoantigen profiling directly interrogates the repertoire of tumor-specific peptides presented on MHC molecules. Advances in immunopeptidomics and machine learning–driven epitope prediction are enabling high-fidelity mapping of immunogenic epitopes with functional relevance (Yaeger et al., 2025). Critically, recent work has demonstrated that the *quality* and *presentation* of neoantigens outweigh sheer quantity. Neoepitopes derived from clonal, rather than

subclonal, mutations persist throughout tumor progression and serve as stable immune targets. Loss of these epitopes via chromosomal deletion or impaired antigen processing has been implicated in acquired resistance to PD-1 inhibitors in melanoma and NSCLC (Richard et al., 2023).

Despite these insights, neoantigen profiling remains technically challenging. It requires integration of WES, RNA sequencing, and mass spectrometry, often limited by tissue availability and the need for extensive bioinformatics pipelines. Additionally, the immunogenicity of computationally predicted neoantigens must still be validated experimentally, underscoring the translational gap between discovery and clinical deployment. Nevertheless, neoantigen-based stratification offers exceptional promise for designing personalized neoantigen vaccines and combinatorial regimens that preempt resistance.

5. Circulating Tumor DNA (ctDNA): A Window Into Dynamic Resistance

Circulating tumor DNA analysis provides a minimally invasive approach to monitoring tumor evolution and immune escape. ctDNA levels often decrease following successful ICI therapy, correlating with radiologic response, whereas re-emergence heralds early relapse (Ota et al., 2024). Beyond quantitative tracking, ctDNA sequencing identifies emergent resistance mutations—such as JAK1/2 inactivation, B2M deletions, or PTEN loss—that impair interferon signaling or antigen presentation.

ctDNA also captures spatial heterogeneity overlooked by tissue biopsies. In metastatic melanoma, ctDNA-derived mutational profiling has revealed coexisting resistant subclones across metastatic sites that were undetected histologically. However, ctDNA's sensitivity varies by tumor burden and vascularization, limiting its reliability in non-shedding tumors like gliomas.

From a clinical standpoint, standardized thresholds for ctDNA positivity remain undefined. Distinguishing treatment-induced immune "flare" from true progression requires longitudinal sampling and contextual interpretation with imaging and clinical outcomes. While not yet a standalone predictive biomarker, ctDNA complements tissue-based assays by providing real-time surveillance of evolving resistance mechanisms—a paradigm increasingly adopted in adaptive trial designs.

6. Single-Cell Sequencing Biomarkers: Dissecting Cellular Heterogeneity

Single-cell RNA sequencing (scRNA-seq) has fundamentally redefined biomarker discovery by resolving intratumoral heterogeneity at unparalleled resolution. By profiling the transcriptional states of individual immune and tumor cells, scRNA-seq allows identification of rare but functionally critical subpopulations contributing to immune evasion.

For instance, scRNA-seq studies have delineated exhausted $CD8^+$ T-cell subsets expressing TOX, TIM-3, and CXCL13 that predict poor response to PD-1 blockade in melanoma and renal carcinoma (Luoma et al., 2022). Concurrently, spatial transcriptomics has revealed immune-excluded niches characterized by fibroblast-derived TGF-β signatures, which correlate strongly with ICI failure (Mariathasan et al., 2022). In parallel, profiling of myeloid and stromal compartments has uncovered macrophage states expressing SPP1 and C1QC that modulate local immune tone.

These discoveries have catalyzed interest in cell-state–based biomarkers—distinct from static expression markers—reflecting the *functional circuitry* of the immune response. However, the translation of single-cell biomarkers into clinical assays remains limited by cost, computational complexity, and technical variability in tissue processing. Moreover, the need for fresh samples restricts retrospective validation. Nevertheless, as sequencing costs decline and analytic pipelines mature, single-cell–derived signatures are poised to inform precision immunotherapy at both diagnostic and monitoring levels.

7. Transcriptomic and Proteomic Signatures: Beyond Genomic Metrics

Bulk transcriptomic profiling has generated robust immune-related gene expression signatures predictive of therapeutic response. The "IFN-γ–related gene signature," comprising CXCL9, CXCL10, IDO1, and other interferon-stimulated genes, consistently correlates with response across melanoma and NSCLC cohorts (Ayers et al., 2023). Conversely, transcriptional programs driven by TGF-β and WNT/β-catenin signaling associate with immune exclusion and ICI resistance.

Proteomic analyses complement these findings by quantifying soluble mediators in the plasma or TME microenvironment. Elevated baseline levels of IL-6, VEGF, and soluble PD-L1 have been linked to early progression under PD-1 blockade, reflecting systemic immunosuppression (Taguchi et al., 2024). Likewise, mass cytometry (CyTOF)–based profiling of circulating immune subsets allows dynamic monitoring of T-cell activation and myeloid suppression signatures predictive of outcomes.

However, both transcriptomic and proteomic biomarkers face reproducibility challenges. Variability in tissue composition, RNA degradation, and sampling bias confounds analysis. Furthermore, most gene expression signatures are retrospective, developed from small cohorts with limited power for multi-cancer validation. Moving forward, leveraging standardized multiplex platforms and machine learning models for cross-cohort harmonization will be crucial to establish clinically actionable thresholds.

8. Microbiome-Associated Biomarkers: Systemic Modulators of Immunity

The intestinal microbiota has emerged as a systemic regulator of immunotherapy outcomes. Fecal microbiome analyses from melanoma and NSCLC patients demonstrate distinct bacterial compositions between responders and non-responders to ICI therapy. Enrichment of commensals such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and

Bifidobacterium longum correlates with enhanced antigen presentation, elevated effector T-cell infiltration, and improved clinical response (Frankel et al., 2022).

Conversely, dysbiosis—whether due to antibiotic exposure, diet, or tumor progression—impairs immunotherapy efficacy. Microbiome-derived metabolites, including short-chain fatty acids and polyamines, influence epigenetic and metabolic programming of immune cells. For instance, propionate and butyrate enhance memory T-cell differentiation, whereas indole derivatives suppress dendritic cell activation.

Translationally, microbiome-based biomarkers face major hurdles in reproducibility and causality. Cohort-specific variations in diet, geography, and sequencing methodology yield inconsistent taxonomic associations across studies. Moreover, it remains unclear whether microbial signatures reflect intrinsic host immune competence or directly modulate it. Nonetheless, the growing success of fecal microbiota transplantation (FMT) trials to restore ICI responsiveness provides compelling proof-of-concept for microbiome-informed precision immunotherapy (Derosa et al., 2024).

9. AI-Assisted Biomarker Discovery: From Complexity to Clinical Decision

The explosive growth of multi-dimensional immuno-oncology data—integrating genomics, imaging, and clinical metrics—has outpaced traditional analytic frameworks. Artificial intelligence (AI) and machine learning have thus become indispensable tools for biomarker discovery. Using high-dimensional datasets, AI algorithms can uncover nonlinear relationships among biomarker features that correlate with therapeutic outcomes.

Recent studies have leveraged deep learning models trained on histopathology slides to predict PD-L1 expression, TMB, and even spatial immune architectures indicative of immunotherapy responsiveness (Saillard et al., 2023). Integration with radiomic data—CT or PET-derived imaging features—has yielded noninvasive "radiomic-immunomic" signatures that reflect tumor inflammation and predict response to PD-1 blockade.

However, AI-assisted biomarker development faces issues of generalizability, transparency, and data bias. Most models are trained on limited, single-center datasets, risking overfitting. Moreover, the "black box" nature of many neural networks impedes biological interpretability. Ongoing efforts to integrate explainable AI frameworks and prospective clinical trials incorporating algorithmic decision support may bridge this translational divide. The ultimate value of AI lies not merely in prediction accuracy but in its capacity to uncover mechanistic pathways underlying immunotherapy resistance.

10. Multi-Omics Integration: The Path Toward Precision Immuno-Oncology

No single biomarker adequately captures the multifactorial determinants of immunotherapy response. Consequently, the research frontier has pivoted toward multi-omics integration—combining genomic, transcriptomic, proteomic, metabolomic, and spatial data within unified analytical frameworks. This holistic approach seeks to generate *immune ecosystems maps* that delineate how tumor intrinsic factors and the surrounding microenvironment jointly shape therapeutic outcomes (Huang et al., 2025).

For example, integrated analyses have demonstrated that TMB coupled with microbiome-derived signatures and T-cell receptor (TCR) clonality predicts response to PD-1 blockade more accurately than TMB alone in NSCLC. Similarly, combining proteomic cytokine profiles with ctDNA dynamics has improved early detection of acquired resistance in patients undergoing combination immunotherapy for melanoma. Multi-omics atlases now provide unprecedented insight into resistance phenotypes—such as concurrent activation of myeloid suppression and metabolic exhaustion pathways—that single-layer biomarkers fail to resolve.

Translational implementation of multi-omics biomarkers, however, remains complex. Computational integration demands harmonized data preprocessing, functional interpretation, and cross-platform validation. From a practical standpoint, cost and assay availability limit routine use. Nevertheless, the integration of multi-omics data into adaptive clinical trial designs—where biomarker feedback informs real-time therapeutic adjustment—heralds a paradigm shift toward dynamic precision immuno-oncology.

Integrative Outlook

The pursuit of predictive biomarkers for immunotherapy resistance in solid tumors reflects the field's maturation from reductionist to systems-level thinking. PD-L1, TMB, and MSI established the conceptual groundwork but lack the granularity to fully explain therapeutic heterogeneity. The evolution toward neoantigen profiling, ctDNA monitoring, single-cell characterization, and microbiome assessment underscores an emerging consensus: immunotherapy resistance arises from complex, temporally evolving networks that bridge tumor genomics and immune ecology.

Yet several obstacles impede clinical translation: inconsistent assay standardization, tumor sampling limitations, data interpretation variability, and shortages of prospective validation studies. The next generation of biomarkers will thus need to be *integrative, adaptive, and clinically pragmatic*. Partnerships between academia, industry, and regulatory agencies are essential to benchmark performance metrics—sensitivity, specificity, and predictive value—across cancer types.

Ultimately, the convergence of advanced analytics, AI-driven pattern recognition, and multi-omics data integration offers the clearest route toward overcoming the current biomarker ceiling. By transforming massive data complexity into clinically actionable insights, the field may finally achieve what has long been elusive: reliable prediction of immunotherapy resistance and rational design of personalized immunotherapeutic strategies that sustain long-term tumor control across solid cancers.

Precision Combination Strategies to Overcome Immunotherapy Resistance

The therapeutic revolution fueled by immune checkpoint inhibitors (ICIs) has decisively altered the trajectory of cancer treatment. However, the majority of patients with solid malignancies exhibit intrinsic resistance or eventually develop adaptive evasion mechanisms. These limitations underscore the inadequacy of monotherapy approaches in addressing the multifactorial immunosuppressive milieu of the tumor microenvironment (TME). Rational combination strategies have thus emerged as a central theme in precision immuno-oncology—aiming to simultaneously target parallel resistance pathways, enhance effector immune cell infiltration, and recondition the TME toward immune responsiveness. The following sections critically evaluate major categories of combination therapies—each grounded in unique molecular rationales—through the lens of recent mechanistic insights, clinical evidence, and translational challenges.

1. Immunotherapy and Chemotherapy: Reprogramming Immunogenic Cell Death

Chemotherapy, once viewed solely as a cytotoxic intervention, is now recognized for its profound immunomodulatory potential. Specific chemotherapeutics such as oxaliplatin, cyclophosphamide, and anthracyclines can elicit *immunogenic cell death* (ICD), characterized by calreticulin exposure, HMGB1 release, and ATP secretion that collectively enhance dendritic cell maturation and antigen cross-presentation (Galluzzi et al., 2023). This cascade amplifies neoantigen release, converting poorly immunogenic tumors into immune-active ones receptive to ICIs.

Clinically, combination regimens such as pembrolizumab plus platinum-based chemotherapy in NSCLC (KEYNOTE-189 trial) and atezolizumab with nab-paclitaxel in triple-negative breast cancer (IMpassion130) have demonstrated robust survival advantages over chemotherapy alone (Schmid et al., 2022). These results highlight synergistic dynamics rather than additive effects. Importantly, chemotherapy can transiently deplete immunosuppressive myeloid and regulatory T-cell populations, augmenting the therapeutic window for ICIs.

Toxicity remains a challenge, as overlapping adverse effects—particularly myelosuppression and pneumonitis—limit dose flexibility. Moreover, the immunogenic potential of chemotherapeutics is context-dependent; agents such as gemcitabine may suppress immune function when administered at high intensity. Future directions emphasize optimizing sequencing, dosing schedules, and biomarker-driven selection, ensuring that chemotherapeutic stress enhances immunogenicity rather than immune exhaustion.

2. Immunotherapy and Radiotherapy: The Spatial Immunologic Synergy

Radiotherapy (RT) induces localized tumor cell death, yet its systemic immune sequelae—most notably the "abscopal effect"—have invigorated its combination with ICIs. Mechanistically, RT damages tumor DNA, releasing double-stranded DNA fragments that activate the STING–IFN pathway, yielding robust type I interferon responses that drive dendritic cell recruitment and T-cell priming (Tang et al., 2023). This immunostimulatory milieu can overcome checkpoints of immune neglect in "cold" tumors.

Preclinical studies have shown that fractionated low-dose RT optimally promotes T-cell infiltration and enhances PD-1 blockade efficacy. This is being validated clinically: in metastatic NSCLC, concurrent nivolumab with stereotactic body RT yielded striking improvements in abscopal responses compared with immunotherapy alone (Theelen et al., 2024). Likewise, trials in head and neck squamous cancers linking durvalumab with RT have revealed enhanced locoregional immune activation.

However, the dual-edged nature of RT must be considered. Excessive radiation can augment TGF-β signaling and vascular damage, inducing fibrosis that impedes T-cell trafficking. Additionally, determining the optimal sequencing and dose fractionation remains unresolved. Adaptive approaches integrating RT with immunotranscriptional biomarkers—such as radiation-induced interferon gene signatures—are under exploration to tailor therapy to tumor immune contexture dynamically.

3. Dual Immune Checkpoint Blockade: Targeting Parallel Exhaustion Pathways

Dual ICI combinations aim to overcome resistant immune exhaustion phenotypes sustained by redundant inhibitory receptors. The archetype—ipilimumab (anti-CTLA-4) plus nivolumab (anti-PD-1)—has established efficacy in melanoma, renal cell carcinoma, and microsatellite instability-high (MSI-H) colorectal cancer by enhancing both priming and effector phases of T-cell activation (Postow et al., 2022). Mechanistically, CTLA-4 blockade expands repertoire diversity within the tumor-draining lymph nodes, while PD-1 blockade reactivates exhausted effector cells in situ.

However, the spectrum of immune-related adverse events (irAEs)—ranging from colitis to endocrinopathy—exceeds that seen with monotherapies. Grade 3–4 toxicities approach 55% in melanoma trials (Hodi et al., 2023). Thus, narrower dual targeting—such as PD-1 combined with LAG-3 or TIGIT inhibitors—is gaining traction to preserve efficacy with improved tolerability. The RELATIVITY-047 trial demonstrated that nivolumab plus relatlimab (anti–LAG-3) produced superior progression-free survival to nivolumab alone with fewer severe irAEs, validating LAG-3 as a viable co-target.

A key limitation is the heterogeneity of inhibitory receptor expression across tumor types and immune compartments. Dynamic profiling of checkpoint signatures by single-cell sequencing could enable precision selection of dual blockade regimens rather

than empirical pairing—a shift toward biomarker-directed immunomodulation.

4. CAR-T and Adoptive Cell Therapies: Rewriting the Rules of Immune Specificity

Adoptive T-cell therapies (ACT), including chimeric antigen receptor (CAR)-T and T-cell receptor (TCR)–engineered lymphocytes, have redefined hematologic malignancy treatment but face formidable barriers in solid tumors. The TME imposes physical and immunologic constraints—hypoxia, nutrient competition, TGF-β dominance—that curtail CAR-T persistence and trafficking.

To counter these limitations, combinatorial approaches leveraging ICIs have shown potential. Preclinical studies demonstrate that PD-1 blockade enhances the function and survival of CAR-T cells, reversing exhaustion phenotypes (Liu et al., 2022). Engineering "armored CAR-Ts" expressing IL-12 or dominant-negative TGF-β receptors further boosts activity within suppressive microenvironments. Clinical translation remains nascent: early-phase trials combining pembrolizumab with HER2-targeted CAR-Ts in sarcomas have revealed encouraging safety and biological activity.

Adoptive transfer of tumor-infiltrating lymphocytes (TILs) also benefits from combination paradigms. Co-treatment with ICIs facilitates re-expansion of polyclonal repertoires and may overcome antigenic drift. Yet logistical complexity, cost, and manufacturing limitations hinder scalability. Moreover, on-target but off-tumor toxicity—especially with shared antigen targets—necessitates precision antigen mapping to ensure safety. Integrating T-cell engineering with spatial proteomics and computational epitope prediction offers a pathway to rational combination design.

5. Anti-Angiogenic Combinations: Vascular Normalization and Immune Facilitation

Tumor vasculature is inherently aberrant—disorganized, hypoxic, and immunosuppressive. Excess VEGF signaling not only sustains angiogenesis but also interferes with dendritic cell maturation and upregulates PD-L1 expression on endothelial and tumor cells (Huang et al., 2024). This dual function justifies anti-angiogenic and ICI combinations as complementary strategies.

The paradigm has been clinically validated: the combination of atezolizumab with bevacizumab significantly improved overall survival in hepatocellular carcinoma (IMbrave150 trial) compared with sorafenib, marking a benchmark in first-line therapy (Finn et al., 2023). Mechanistically, transient "vascular normalization" induced by VEGF blockade facilitates T-cell infiltration and reduces MDSC recruitment. Similar benefit has been observed in renal cell carcinoma with pembrolizumab–axitinib and in endometrial cancer with lenvatinib–pembrolizumab combinations.

Nevertheless, managing toxicity remains pivotal. Hypertension, hepatotoxicity, and hemorrhagic risk may curtail prolonged treatment. Moreover, the temporal window of vascular normalization is narrow; suboptimal timing can exacerbate hypoxia and counteract immune activation. Emerging evidence supports using dynamic perfusion imaging or hypoxia gene signatures to optimize scheduling. Expanding this strategy to refractory tumor types will require context-specific insights into angiogenic and immunologic cross-regulation.

6. Epigenetic Modulators: Reawakening Silenced Immunity

Epigenetic silencing of antigen presentation genes and interferon pathways constitutes a reversible mechanism of immune escape. Agents that modulate DNA methylation (DNMT inhibitors) and histone acetylation (HDAC inhibitors) can restore tumor immunogenicity by upregulating MHC class I expression, endogenous retroviral elements, and viral mimicry responses (Topper et al., 2023).

Combination trials have confirmed the translational potential of this approach. In NSCLC, low-dose azacytidine combined with nivolumab increased T-cell infiltration and expression of immune-related transcripts. Similarly, entinostat (HDAC inhibitor) combined with pembrolizumab in estrogen receptor–positive breast cancer reinvigorated exhausted $CD8^+$ clones in early-phase studies (Yost et al., 2024).

Despite these promising signals, epigenetic–immune combinations demand caution due to broad transcriptional effects that may also foster tumor plasticity or systemic toxicity. Distinguishing reprogramming from de-differentiation remains a fine balance. Future precision strategies are exploring locus-selective epigenetic drugs and CRISPR-based editing to fine-tune immune potentiation while minimizing off-target alterations.

7. Oncolytic Viruses: In Situ Vaccination Against Resistance

Oncolytic virotherapy represents a dual mechanism—direct lytic destruction of tumor cells and the generation of a proinflammatory milieu that converts immune-deserted tumors into immune-engaged states. Viral infection stimulates DAMP release, type I interferon signaling, and neoantigen presentation, creating local "in situ vaccination" effects that synergize with ICIs (Ribas et al., 2023).

Talimogene laherparepvec (T-VEC), an oncolytic herpesvirus expressing GM-CSF, exemplifies this paradigm. In melanoma, T-VEC plus pembrolizumab elicited response rates exceeding either monotherapy, attributed to increased intratumoral $CD8^+$ and dendritic cell recruitment. Similarly, adenoviral and vaccinia vectors encoding CXCL9 or IFN-β are under active investigation to remodel immune landscapes in cold tumors such as pancreatic and colorectal cancer.

Yet safety remains an enduring concern: systemic viral spread and preexisting antiviral immunity can limit efficacy and necessitate intratumoral administration. Engineering conditionally replicative or tumor-selective viral vectors—perhaps guided by tumor-specific protease promoters—could optimize therapeutic windows. Additionally, the combination of

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oncolytic viruses with metabolic modulators (e.g., adenosine pathway inhibitors) holds promise for augmenting depth and durability of response.

8. Nanoparticle-Based Delivery Systems: Localized Precision Immune Modulation

Nanomedicine provides an innovative platform to enhance the pharmacokinetics and targeting precision of immunotherapeutic agents. Nanoparticles can co-deliver antigens, adjuvants, and checkpoint inhibitors directly into the TME, focusing immune activation while mitigating systemic toxicity (Chen et al., 2024).

Preclinical models of PD-L1 siRNA–loaded nanoparticles demonstrate effective receptor silencing within tumor tissues, restoring T-cell infiltration even in PD-L1–negative phenotypes. Liposomal formulations of TLR7/8 agonists have shown promise in "priming" exhausted microenvironments prior to ICI administration. Moreover, nanoparticle carriers enable controlled release kinetics, extending drug residence and ensuring sustained immune modulation.

From a translational standpoint, the bottleneck lies in manufacturing reproducibility, biodistribution variability, and immune-related off-target accumulation in the liver or spleen. While early-phase trials using nanovaccine–checkpoint co-delivery have revealed encouraging safety profiles, regulatory standardization is essential for scaling into broader clinical frameworks. Integrating imaging biomarkers to track nanoparticle localization in real time could guide personalized dosing, merging nanotechnology with adaptive precision medicine principles.

9. Personalized Medicine Approaches: Stratifying Resistance Landscapes

Personalized immuno-oncology seeks to align therapeutic combinations with individual tumor immune ecologies. Comprehensive genomic and immunophenotypic profiling identifies patients likely to benefit from specific modalities—such as MSI testing for PD-1 inhibitors or angiogenic signatures for anti-VEGF combinations.

Neoantigen prediction algorithms now enable personalized vaccine designs that complement ICIs by expanding patient-specific T-cell repertoires (Ott et al., 2023). Similarly, transcriptomic TME profiling using spatial transcriptomics allows detection of immune-excluded architectures that can be targeted with TGF-β or CAF-modulating agents. Integrative platforms, exemplified by the *Tumor Immune Dysfunction and Exclusion (TIDE)* model, quantify immunosuppression signatures and predict responsiveness to distinct combination classes.

Clinical translation demands logistical agility—rapid biopsy processing, high-throughput sequencing, and adaptive clinical trial structures. Basket trials anchored in biomarker-defined cohorts, rather than histology, are redefining the development pipeline. Nevertheless, real-world implementation remains constrained by cost, assay heterogeneity, and limited access to sequencing infrastructure in low-resource settings.

10. AI-Guided Therapeutic Selection: From Biomarker Discovery to Dynamic Optimization

Artificial intelligence (AI) has become integral to deciphering the multidimensional complexity of combination immunotherapy. Machine learning algorithms integrate diverse data modalities—genomic, epigenomic, proteomic, and radiomic—to predict optimal therapeutic combinations for individual patients. Deep learning models trained on large clinical datasets can forecast both efficacy and toxicity, thereby informing personalized treatment sequencing (Li et al., 2025).

For instance, AI-assisted image analysis of histopathology slides can quantify spatial immune contexture—$CD8^+$ proximity to tumor nests, stromal fibrosis indices, and lymphoid aggregates—correlating these features with response to dual ICI therapy. Similarly, predictive models linking cytokine kinetics and circulating tumor DNA dynamics to early resistance signatures enable adaptive switching from monotherapy to combination regimens before clinical progression is evident.

Critically, algorithmic interpretability remains essential for clinical acceptance. The refinement of explainable AI frameworks ensures that learned patterns correspond to biologically interpretable mechanisms. Hybrid models integrating mechanistic simulations with data-driven inference may deepen understanding of immune response evolution under therapy. As regulatory agencies embrace digital biomarkers, AI-guided design could accelerate the identification of synergistic combinations and streamline trial enrollment through predictive enrichment.

Integrative Perspective

The landscape of combination immunotherapy has evolved from empirical pairing toward mechanistically guided precision strategies informed by systems biology. Each modality—whether chemotherapy-induced immunogenic death, radiotherapy-triggered DNA sensing, or epigenetic reprogramming—converges on the central goal of reinvigorating antitumor immunity by dismantling distinct layers of TME-mediated resistance.

Despite significant progress, challenges remain. Synergistic efficacy often comes at the cost of compounded toxicity, necessitating integrative models that optimize benefit-to-risk ratios. Many clinical trials lack longitudinal biomarker analysis to confirm mechanistic hypotheses, and intertumoral heterogeneity continues to impede predictive reliability. To overcome these barriers, future frameworks should embrace *adaptive precision immunotherapy*—a continuously learning model where multi-omics, real-world data, and AI algorithms collectively inform therapeutic adjustments in real time.

The frontier of immuno-oncology now resides at the intersection of biology and computation. Precision combination strategies offer an evolving blueprint to transform transient immune activation into durable, systemic tumor control. By rationally modulating interconnected resistance circuits, these integrated approaches hold the promise of converting

immunotherapy from a probabilistic intervention into a consistently curative discipline across the full spectrum of solid malignancies.

Conclusion

Immunotherapy has redefined the therapeutic paradigm of oncology, yet its limitations in solid tumors underscore a fundamental truth: resistance is not a single molecular defect but an ecosystemic adaptation orchestrated within the tumor microenvironment (TME). The TME is neither a passive scaffold nor a static shield; it is a dynamic, self-regulating network of stromal, immune, metabolic, and vascular components that collectively modulate response to immune attack. Through immune exclusion, cytokine-mediated suppression, metabolic competition, and stromal remodeling, the TME establishes multiple layers of defense that blunt or rewire the effects of immune checkpoint blockade and other immune-based therapies (Joyce & Fearon, 2022). Understanding this complexity is essential for reconciling the disparity between immunotherapy's theoretical universality and its clinical selectivity.

Over the past decade, a deeper molecular appreciation of the TME has catalyzed a transformation in biomarker discovery. Traditional single-parameter indicators—such as PD-L1 expression, tumor mutational burden (TMB), or microsatellite instability (MSI)—have proven inadequate to capture the spatial and temporal heterogeneity of the immune landscape. Emerging biomarkers now integrate data from spatial transcriptomics, single-cell profiling, and circulating tumor–derived signals, offering a multidimensional view of immune activity and resistance evolution (Binnewies et al., 2023). These advances signal a shift from static prediction to dynamic monitoring, enabling clinicians to anticipate therapeutic escape and recalibrate treatment before clinical progression occurs. Crucially, biomarker-guided strategies are beginning to dissolve the binary notion of "responder" versus "non-responder," replacing it with continuous, context-dependent immunologic phenotypes that better reflect the fluidity of tumor–host interactions.

Parallel innovations in therapy design further strengthen this precision framework. Rational combination regimens—integrating ICIs with chemotherapy, radiotherapy, anti-angiogenic agents, oncolytic viruses, epigenetic modulators, or metabolic inhibitors—seek to dismantle the redundant feedback loops that sustain immune tolerance. Rather than pursuing maximal immune activation, contemporary strategies emphasize reprogramming the TME to restore physiological balance between immune surveillance and immunoregulation (Hegde et al., 2024). Early clinical results from multimodal regimens demonstrate that such synergy can transform immune-desert tumors into inflamed, therapy-responsive ecosystems. Nevertheless, toxicity management, biomarker validation, and the optimization of treatment sequencing remain ongoing challenges requiring translational rigor.

The integration of artificial intelligence and multi-omics analytics into clinical oncology represents the next decisive frontier. These technologies can synthesize genomic, proteomic, and spatial data to generate predictive models of immune responsiveness and resistance adaptation at patient-specific resolution (Li et al., 2025). In this vision, precision immuno-oncology becomes a learning system—one in which therapy selection, monitoring, and adjustment are continuously informed by computational insight and real-time biological feedback.

Ultimately, overcoming immunotherapy resistance demands a paradigm shift from targeting isolated pathways to managing an evolving immunological ecosystem. The future of cancer immunotherapy will rely on our ability to decode and modulate the TME with molecular precision—integrating biomarkers that map resistance, combination strategies that reconfigure the tumor niche, and adaptive analytical models that evolve with disease dynamics. In mastering the language of the microenvironment, oncology stands at the threshold of a new era—one defined not by transient response but by durable immune equilibrium and the prospect of genuine, system-level remission.

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