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Digital immune twins and ai-integrated multi-omic biomarkers: Redefining personalized immunotherapy in non-small cell lung cancer

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ABSTRACT

Non-small cell lung cancer (NSCLC) remains one of the leading causes of global cancer mortality despite advances in immunotherapy. While immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis have transformed clinical outcomes for selected patients, response rates remain highly variable due to tumor heterogeneity, immune escape mechanisms, and evolving biomarker complexity. The need for dynamic, integrative biomarkers that better predict treatment response and guide personalized therapy is increasingly critical. This narrative review synthesizes recent advances (2023–2025) in genomic, transcriptomic, proteomic, metabolomic, and liquid-biopsy-based biomarkers relevant to NSCLC immunotherapy. Key databases, including PubMed, Scopus, and Web of Science, were screened, with emphasis on emerging artificial intelligence (AI) and digital twin–based frameworks supporting precision immuno-oncology. Across studies, single biomarkers such as PD-L1 or tumor mutational burden (TMB) demonstrate limited standalone predictive value. Multi-omic signatures incorporating circulating tumor DNA (ctDNA) fragmentomics, exosomal PD-L1, T-cell receptor (TCR) repertoire diversity, DDR alterations, metabolic checkpoint activity, and spatial immune profiling demonstrate improved accuracy and clinical relevance (clinical and preclinical evidence). AI-based multimodal models and digital immune twins further enhance predictive capacity by mapping resistance trajectories and simulating individualized therapeutic responses (computational/model-based evidence).The transition from static biomarkers toward integrated multi-omic and AI-driven decision frameworks represents a paradigm shift in NSCLC immunotherapy. These emerging platforms support a future of adaptive, anticipatory, and personalized treatment strategies with strong translational potential.

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Introduction

With 5-year survival rates for advanced illness rarely reaching 20-25%, non-small cell lung cancer (NSCLC) continues to dominate worldwide cancer mortality, accounting for over 85% of lung cancer cases and resisting advancements. Even while immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1 and CTLA-4 have completely changed the treatment landscape, only a small percentage of patients have long-lasting improvements, and primary and acquired resistance are still common. According to recent meta-analyses, the primary ICI resistance rate in NSCLC ranges from approximately 21% to 27% in first-line settings, rising to over 50% with successive lines (1). This clinical deficiency highlights the complex biology of NSCLC, in which treatment failure is driven by metabolic immunosuppression, immune escape via alternative checkpoints (LAG-3, TIM-3, TIGIT), evolving tumor heterogeneity, and dynamic stromal–immune interactions (2, 3). Thus, multidimensional customization has replaced one-size-fits-all checkpoint blockage in the immunotherapy sector. It is increasingly acknowledged that single biomarkers, such as tumor mutational burden (TMB) or

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PD-L1, are essential yet insufficient; respondents who are dissonant question the established paradigm (4, 5). Rather, precision immuno-oncology seeks to characterize every tumor as a dynamic ecosystem shaped by its tumor immune microenvironment (TIME), which may range from "cold" immune deserts and "excluded" phenotypes to "hot" T-cell-inflamed landscapes (6, 7). Multi-omic integration has gained prominence in the last two years (8). More intricate maps of immune–tumor interactions have been outlined by studies that integrate transcriptomics, epigenomics, proteomics, metabolomics, and microbiome data. It was recently shown that a 23-gene multi-omics signature may accurately predict the prognosis of NSCLC, immunological infiltration, and responsiveness to treatment (clinical and preclinical evidence)(9). Additionally, in 2025, metabolite-protein panels in plasma (such as immunoglobulin heavy variable 1-45 and anthranilic acid) were shown to correlate with ICI sensitivity in advanced NSCLC (clinical evidence)(10). Immune gradients are now localized relative to stromal barriers by the combination of spatial single-cell and imaging-omics, opening up new ideas for "immune corridors" and therapeutic access points (11). Predictive modeling has advanced as a result of the growing convergence of multi-omics and artificial intelligence (AI). Deep-learning radiomic models have been reported to predict PD-L1 expression and immunotherapy response with strong discriminatory performance in retrospective cohorts. However, performance varies across datasets and requires further external validation (e.g., computational or model-based evidence)(12, 13). Transformer-based multimodal survival predictors have demonstrated promising discriminatory capability in retrospective immunotherapy cohorts, although large-scale validation remains limited (computational/model-based evidence) (14). Meanwhile, latent traits uniquely linked to immune suppression, metabolic reprogramming, and resistance gradients across cancer types are being revealed by pioneering work in disentangled multi-omic modeling (15). Lastly, new counterfactual AI frameworks allow treatment simulation in addition to prediction, providing believable alternative regimens based on a patient's multi-omic fingerprint (16). Therefore, this review makes the case for a daring rephrasing: AI-driven immune graphs and digital immunological twins' virtual avatars, trained on high-dimensional longitudinal data, may replace strict PD-L1 thresholds to mimic therapeutic outcomes, predict resistance trajectories, and guide adaptive measures (17, 18). In 2025 and beyond, this change reframes NSCLC immunotherapy as a living, self-learning system in which patient-specific immune intelligence, rather than static biomarkers, guides treatment toward anticipatory oncology, therapeutic choices adjust in real time, and biology and algorithmic understanding work together to improve (19, 20).

Method

This review was conducted using a structured narrative synthesis approach to identify and examine emerging biomarker-driven and AI-enabled strategies in precision immunotherapy for NSCLC. The methodological framework was designed to ensure transparency, reproducibility, and thematic consistency across molecular, computational, and clinical evidence domains. Eligible literature was evaluated based on scientific relevance, conceptual novelty, and translational potential. To improve transparency, findings discussed throughout the review are contextualized by evidence level and classified as preclinical**,** clinical**,** or computational/model-based, where applicable.

Search strategy and selection criteria

A comprehensive literature search was conducted in PubMed, Scopus, Web of Science, and Google Scholar, covering peer-reviewed publications from January 2023 to February 2025. Search terms included standardized MeSH terminology and ontology-based expressions combined using Boolean operators, including: "Non-Small-Cell Lung Cancer," "PD-L1," "Immunotherapy," "Biomarkers," "Tumor Mutational Burden," "Liquid Biopsy," "ctDNA," "TCR repertoire," "Spatial omics," "Artificial Intelligence," "Machine Learning," and "Digital Twins."

Studies were included if they met one or more of the following criteria:
(1) addressed biomarkers associated with response or resistance to NSCLC immunotherapy;
(2) incorporated multi-omic or liquid biopsy–based predictive platforms;
(3) applied AI, machine learning, or digital immune twin modeling;
(4) demonstrated relevance to clinical translation, treatment stratification, or adaptive immunotherapy frameworks.

Exclusion criteria included duplicate records, non-English manuscripts, conference abstracts without full text, commentary-only publications, and non–peer-reviewed preprints. After screening titles, abstracts, and full texts, eligible studies were narratively synthesized. Due to methodological variability and lack of uniform outcome reporting, no statistical pooling or meta-analysis was conducted.

Evidence classification framework

To improve transparency and distinguish between validated findings and emerging hypotheses, all included references were classified into three evidence tiers during full-text screening:

• *Preclinical evidence*

Studies based on in-vitro systems (cell lines, organoids), animal models, molecular perturbation experiments, or mechanistic pathway analyses.

• *Clinical evidence*

Human studies, including randomized controlled trials, observational cohorts, biomarker validation studies, and meta-analyses relevant to NSCLC immunotherapy.

• *Computational / theoretical evidence*

AI frameworks, machine-learning prediction models, digital twin simulations, in-silico validation studies, and mathematical modeling without direct biological experimentation.

Each reference was tagged during screening and synthesis, and evidence levels were explicitly considered when reporting conclusions to avoid overstating preliminary or hypothesis-driven findings. Computational and preclinical observations were treated as exploratory unless supported by human clinical validation.

PD-L1 expression: Strengths, limitations, and context-dependence

Mechanistic role of PD-L1 in immune evasion

More than just a passive surface ligand, PD-L1 (also known as CD274/B7-H1) is a dynamic node of immunological, metabolic, and signaling interaction that tumor cells use to circumvent host immunity. By recruiting phosphatases SHP-2 (and to a lesser extent SHP-1), which dephosphorylate signaling intermediates in the TCR cascade (e.g., CD3ζ, ZAP70, PI3K), PD-L1 canonically engages PD-1 on activated T cells. It transmits inhibitory signals, dampening T-cell proliferation, cytokine production, and cytolytic function. Cancer uses this ligand-receptor axis to produce T-cell fatigue and act as a "brake" on effector immunity (21). Nevertheless, new research from 2024 to 2025 has shed light on tumor-intrinsic and non-canonical aspects of PD-L1 biology, strengthening its function in immune evasion:

PD-L1 may signal within the tumor cell (a process known as "reverse signaling") to alter survival pathways in addition to inhibiting T lymphocytes in trans. For example, tumor cells that overexpress PD-L1 may activate the RAS/MAPK and PI3K/AKT/mTOR axis, which promotes growth, metabolic adaptability, and stress tolerance. Certain cytoplasmic tail motifs, such as RMLDVEKC and DTSSK, have been linked to reducing interferon-induced apoptosis and fostering tolerance to DNA damage (22). This dual role makes PD-L1 both a signal suppressor and a pro-tumor support hub.

Dynamic regulation via post-translational modifications and degradation control

The stability, localization, and function of PD-L1 are tightly regulated by a suite of post-translational modifications (PTMs). As of 2024-25, key PTMs include:

- **Ubiquitination/deubiquitination**: PD-L1 turnover is regulated by E3 ubiquitin ligases and deubiquitinases; for instance, regulators such as CMTM6, HIP1R, and TRAPPC4 help protect PD-L1 from proteasomal or lysosomal degradation, stabilizing cell-surface expression. When these modulators are lost, PD-L1 is less available for PD-1 binding (22).
- **Glycosylation and phosphorylation**: Glycosylated PD-L1 is more stable and resistant to proteasomal degradation. Events involving phosphorylation may indicate PD-L1 internalization or trafficking (23).
- **Palmitoylation**: According to a recent study, palmitoylation, the attachment of fatty acid chains, for example, by DHHC family enzymes at cysteine residues, such as Cys272, is a new alteration that improves stability and function by shielding PD-L1 from ubiquitination and destruction. In preclinical models, disrupting palmitoylation makes tumors more vulnerable to immune response (preclinical evidence)(24).
- **m6A RNA modification**: Upstream regulation is also present: PD-L1 mRNA's m6A methylation, which is regulated by METTL3 and IGF2BP3, stabilizes transcripts and may be associated with immune evasion, particularly under inflammatory stress (24). In evaluations from the 2025 timeframe, interest in PD-L1-targeted proteolysis techniques (such as PROTAC chimeras) has increased due to predicted advances in our knowledge of PD-L1 degradation processes (ubiquitin-independent routes, autophagy pathways, and proteasome crosstalk)(25).

Soluble and exosomal PD-L1 as decoys and systemic suppressors

Systemic immunosuppressive mediators include membrane-bound PD-L1, soluble PD-L1 (sPD-L1), and exosomal PD-L1 (exo-PD-L1). Higher plasma levels of sPD-L1 are associated with a higher burden of illness and a worse prognosis; sPD-L1 may bind to PD-1 on T cells, neutralizing circulating T cells or sequestering therapeutic antibodies (clinical evidence)(26). Packed into tumor-derived vesicles, exosomal PD-L1 may reach peripheral T cells or distant lymphoid organs, precondition metastatic niches, and inhibit lethal activity beyond the tumor microenvironment. Resistance to PD-1/PD-L1 blockage is increasingly linked to this axis (emerging clinical and preclinical evidence)(23).

Microenvironmental induction and spatial heterogeneity

The expression of PD-L1 is very context-dependent and malleable. Through JAK/STAT→IRF1 signaling, pro-inflammatory cytokines, particularly IFN-γ from T or NK cells, strongly upregulate PD-L1 in the tumor microenvironment (TME). Immune assault triggers PD-L1, which in turn creates a counter-resistance barrier. This is an adaptive feedback loop (27). Due to stromal regulation, metabolic gradients, and local oxygen tension, there is spatial heterogeneity: areas of low-expression or repressed PD-L1 coexist with pockets of high PD-L1 (also known as "immune-activated niches"). According to certain 2025 investigations of tumor VEC PD-L1 overexpression, endothelial and stromal cells themselves may upregulate PD-L1, creating a vascular barrier of immunosuppression (preclinical and early clinical evidence)(28). Tumors develop as a result of immunological pressure. Immune editing and the selection of immune-resistant clones are made possible by subclones that dynamically co-opt PD-L1 upregulation, lose antigenicity, or downregulate MHC. Subclones' varying levels of PD-L1 expression lead to fractional ICI responses, with non-PD-L1 clones evading immune assault (29). All of these processes work together to make PD-L1 a complex immunologic and oncologic node. In tumor immune dynamics, PD-L1 is a responsive, controlled, and changing agent rather than a static eligibility indicator. By 2025, PD-L1 therapies will shift from antibody blocking to PTM destabilization, exosomal PD-L1 interception, and coupling with AI-generated models that monitor PD-L1 dynamics over time. To develop next-generation immunotherapy techniques that surpass tumor adaptation, it is essential to comprehend the molecular subtleties of PD-L1 in immune evasion (30). Lessons Learned: Current evidence demonstrates that PD-L1 is not a static biomarker but a dynamic, context-dependent signal regulated by immune pressure, spatial heterogeneity, and treatment exposure. While emerging platforms such as exosomal PD-L1 profiling and spatial analysis offer promising predictive capacity, variability in assay standardization and limited clinical validation remain major barriers. Future research integrating longitudinal monitoring with harmonized diagnostic frameworks may enable PD-L1 to function as a clinically actionable kinetic biomarker rather than a standalone eligibility metric.

Diagnostic platforms and standardization challenges

When it comes to NSCLC and PD-1/PD-L1 axis–

targeted immunotherapy, the lack of strict standardization among diagnostic platforms such as immunohistochemistry (IHC), multiplex immunofluorescence, RNA and protein sequencing, circulating (liquid biopsy) assays, exosomal PD-L1 detection, and digital or AI-augmented scoring systems makes it difficult to translate mechanistic insights into reliable clinical biomarkers. Although PD-L1 IHC remains the clinical reference method, its readout is influenced by factors such as assay selection, staining workflow, fixation and retrieval conditions, and scoring strategy, all of which may shift interpretation at clinically meaningful thresholds. Collaborative comparisons have shown that while some assays demonstrate broadly aligned staining patterns, others yield consistently reduced signals, underscoring that full interchangeability cannot be assumed. Consequently, ongoing harmonization efforts by professional bodies continue to refine standardization and improve consistency across laboratories (31), and Mandal *et al.* describe how guidelines are being consolidated to lessen inter-assay variance across various clones and scoring methods (32). A recent development study of a novel PD-L1 IHC assay utilizing the CAL10 clone demonstrated the challenge: concordance with SP263 at the 50% TPS cutoff exhibited acceptable agreement, yet at the 1% threshold, the confidence intervals widened significantly, underscoring the sensitivity of low-level expression boundaries to procedural details (32). In addition to IHC, multiplex immunofluorescence or spatially resolved protein/RNA panels provide enhanced phenotypic resolution (e.g., quantification of PD-L1 co-expression with IDO, CD8, and Treg markers); however, these platforms are often custom-made, lack widely accepted reference standards, and pose challenges for cross-platform normalization. In the realm of liquid biopsies, tumor (or immune) cell-free RNA, circulating tumor DNA (ctDNA) methylation signatures, and exosomal PD-L1 have surfaced as promising noninvasive biomarkers; however, each presents additional layers of variability exosome isolation yield, capture antibody efficiency, normalization against housekeeping cargo, and preanalytic biases in sample handling and there is presently no agreed-upon framework for calibration or clinical cutoff determination (33). The concurrent streams of multiomics (transcriptomic, epigenomic, proteomic) compound the issue: integrative biomarker discovery is increasingly prevalent (34). True cross-site reproducibility is hampered by the infrequent repeatability of the analytical pipelines (normalization, batch correction, missing-data imputation, and feature selection) used at a single institution. Aguilar *et al.* evaluated PD-L1 testing trends in the US Oncology Network in real-world settings and found that, despite several companion diagnostics that have received FDA/CE clearance, there remains significant variation in assay use and reporting interpretation, which compromises the validity of biomarker-driven treatment decisions (35). Clinically, this variability leads to inconsistent patient classification and warrants caution in cross-study meta-analyses, as it undermines the positive and negative predictive values and the consistency of trial outcomes. The field need a tiered standardization plan to address this: (1) universal reference reagents capable of calibrating instrument responses, such as exosomal PD-L1 reference standards, isotype-matched controls, and recombinant PD-L1–expressing cell lines embedded in formalin blocks; (2) Programs for external quality assessment (EQA) and interlaboratory proficiency testing tailored to PD-L1 IHC in cytology and histology (an unmet need identified in studies of cytologic PD-L1 IHC) (36); (3) standardized data models and metadata to support assays (preanalytic variables, tissue processing logs, imaging parameters) in order to make computational normalization easier later on; (4) Open, modular software ecosystems for digital scoring that preserve traceability, versioning, and explainability, preferably AI/ML models (e.g., universal IHC analyzers trained across clones and tumor types, such as the UIHC concept)(37); and (5) regulatory frameworks that transition from platform-centric approvals to function-based validation (i.e. delineating minimum performance, concordance tolerances, calibration standards) to enable emerging platforms (liquid, multiplex, exosomal) to integrate into a validated biomarker ecosystem. Only through a comprehensive, standardized framework encompassing reagents, assays, data models, and regulatory guidance can the diagnostic assessment of PD-L1 and multiomic biomarkers provide reliable, interoperable precision immunotherapy decision support in NSCLC. Lessons Learned: Liquid biopsy platforms, such as ctDNA dynamics, exosomal PD-L1, and TCR repertoire profiling, provide minimally invasive methods for real-time tracking of tumor evolution and immunotherapy responses. However, assay variability, limited standardization, and lack of regulatory approval remain key challenges. Broader validation and harmonization efforts could accelerate their transition into clinical decision frameworks.

Temporal and spatial plasticity of PD-L1 expression

Emerging findings indicate that PD-L1 functions as a dynamic biosignal rather than a fixed binary classifier, influenced by tumor evolution, immune responses, and treatment pressure. Spatial analyses reveal heterogeneity in expression across tumor regions, with higher levels sometimes observed at the invasive front, a pattern associated with interferon-γ signaling and macrophage-derived TNF-α activity. These observations primarily come from retrospective spatial profiling studies and remain exploratory (clinical evidence)(38). Spatial transcriptomics and multiplex immunofluorescence imaging reveal that PD-L1-positive niches are concentrated at immune-stromal interfaces (preclinical and translational evidence), where tumor cells enhance PD-L1 expression via STAT1-IRF1 and HIF-1α-mediated transcriptional pathways (39, 40). Inter-lesional heterogeneity between primary and metastatic locations adds further complexity. Analyses of matched lung and nodal metastases in EGFR-mutant NSCLC reveal uneven PD-L1 concordance, due to site-specific cytokine environments and differential oncogenic signaling (PI3K–AKT, MAPK, ALK fusion activation)(41). Temporal plasticity exacerbates these difficulties. PD-L1 levels fluctuate due to immunoediting and treatment-induced reprogramming. Baseline biopsies frequently misrepresent post-therapy conditions. Platinum chemotherapy, radiotherapy, and targeted EGFR/ALK inhibitors can temporarily elevate PD-L1 via DNA-damage-activated STING–NF-κB–IRF1 pathways, whereas prolonged IFN-γ exposure induces JAK-STAT–mediated upregulation followed by SOCS1-driven negative feedback, resulting in oscillatory PD-L1 expression patterns (42). Immunotherapy alters PD-L1 dynamics: in patients receiving pembrolizumab

or atezolizumab, resistant clones often re-express PD-L1 after initial downregulation, indicating adaptive immune evasion. Longitudinal biopsy studies indicate that PD-L1 expression may change during treatment, with some patients converting from PD-L1–negative to PD-L1–positive status, a dynamic process described as 'PD-L1 seroconversion.' These observations remain variable across cohorts and require further validation (clinical evidence)(33). Liquid biopsy and exosomal PD-L1 measurement further support the conclusion that circulating PD-L1 exhibits temporal fluctuations that correspond to immune activation and treatment response patterns (clinical evidence)(32). Significantly, non-tumor compartments, including tumor-associated macrophages (TAMs), endothelial cells, and dendritic subsets, demonstrate temporal plasticity of PD-L1; for example, macrophages progressively elevate PD-L1 expression as they polarize toward an M2-like suppressive phenotype in response to chronic exposure to IL-10 and lactic acid (43). These temporospatial dynamics reclassify PD-L1 as an ecosystemic variable rather than a tumor-intrinsic characteristic. Thus, static tests (individual IHC snapshots) provide limited predictive capability unless situated within regional maps or longitudinal temporal data. Innovative frameworks promote 4D PD-L1 modeling, which combines multiplex spatial proteomics, radiomic imaging, and serial exosomal profiling to reconstruct real-time PD-L1 trajectories (computational/model-based evidence). Integrators based on machine learning, such as spatiotemporal Bayesian tensor models, are being tested to interpolate PD-L1 states across spatial and temporal dimensions utilizing minimally intrusive sampling (computational/model-based evidence)(35). The clinical vision for 2025 regards PD-L1 not as a static eligibility criterion but as a dynamic biosensor, consistently indicating the interaction among genetic alterations, immunological influences, and treatment adjustments. Standardizing temporospatial assessment via harmonized multiplex methods, reference calibration for exosomal assays, and AI-assisted spatial analytics will be crucial in transforming PD-L1 from a static threshold into a dynamic precision-immunotherapy guide (Figure 1).

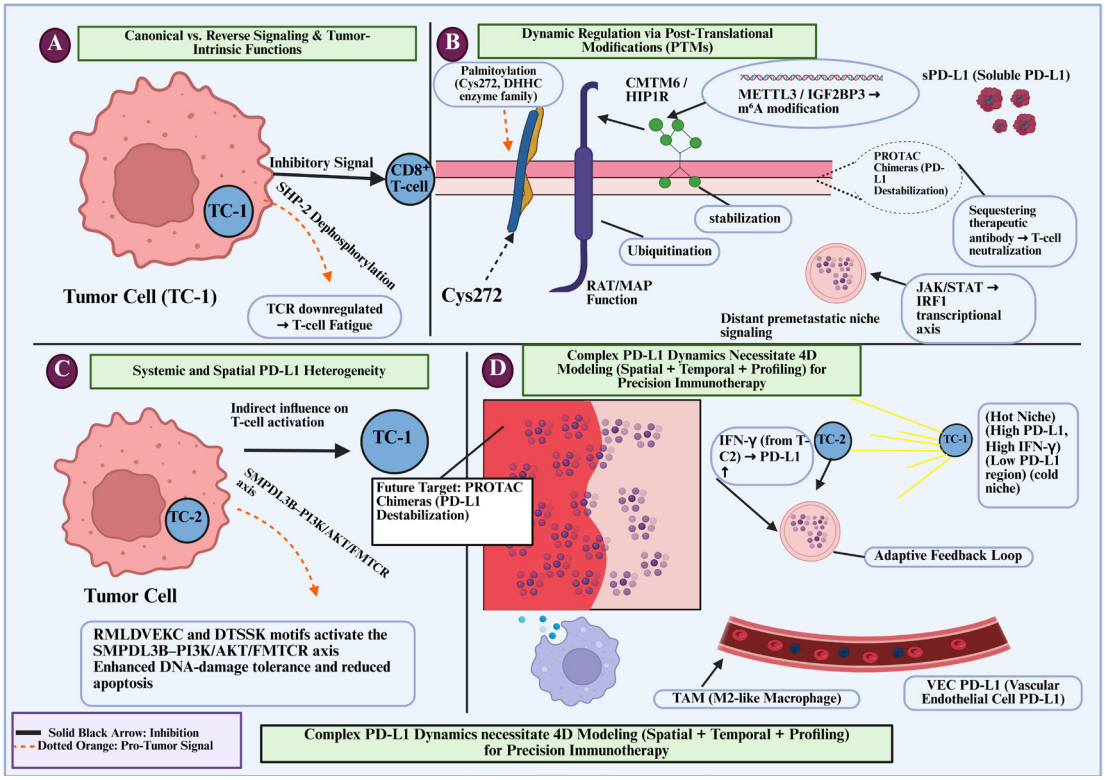

Figure 1. Four-dimensional PD-L1 dynamics as a digitally tractable immuno-ecosystem in non-small cell lung cancer (NSCLC)
This conceptual map unifies molecular, spatial, and temporal PD-L1 heterogeneity into a computationally interpretable framework that underpins next-generation digital immune-twin simulations.
(A) Canonical-to-reverse signaling continuum: Tumor cell TC-1 couples PD-1 ligation to SHP-2–dependent TCR dephosphorylation, inducing exhaustion loops in $CD8^+$ T cells, while the cytoplasmic tail motifs RMLDVEKC and DTSSK initiate autonomous RAS/MAPK and PI3K/AKT/mTOR activation. This bidirectionality transforms PD-L1 from a passive ligand into a dual-state signal transducer integrating immune and oncogenic circuitry (44).
(B) Post-translational logic layer: PD-L1 persistence is rewritten through palmitoylation at Cys272 (DHHC enzymes), m^6A-dependent transcript stabilization (METTL3–IGF2BP3), and ubiquitin-shielding by CMTM6/HIP1R modules. Proteolysis-targeting chimeras (PROTACs) have emerged as programmable "degraders," integrating PD-L1 into synthetic feedback control. Soluble and exosomal PD-L1 vectors propagate long-range immunosuppression and remodel the pre-metastatic niche, forming a liquid immune Internet of inhibitory cues (23).
(C) Spatial-systemic PD-L1 polymorphism: Interclonal dialogue between TC-1 and TC-2 subpopulations via the SMPDL3B–PI3K/AKT/TM/TCR axis establishes metabolic zonation of PD-L1, driving differential T-cell accessibility and DNA-damage tolerance. This spatial coding defines a therapeutic frontier for PROTAC-induced PD-L1 destabilization and PTM-specific drugging of immune-resistant microdomains (45).
(D) Temporal-adaptive circuitry: Cytokine-mediated cross-talk (IFN-γ pulses from TC-2 to TC-1) generates oscillatory PD-L1 waves and bifurcates the tumor into "hot" (PD-L1^high/IFN-γ^high) and "cold" (PD-L1^low/IFN-γ^low) niches, maintained by M2-polarized TAMs and endothelial (VEC) PD-L1 barriers. These self-organizing networks represent a chronospatial immuno-field suitable for 4D modeling that merges single-cell proteomics, radiomic texture mapping, and AI-based temporal tensors (46,47). In sum, the figure reframes PD-L1 as a dynamic, algorithm-learnable biosignal rather than a static eligibility marker, an evolving molecular dialect through which tumors negotiate immune pressure. Capturing these nonlinear kinetics enables anticipatory, feedback-aware immunotherapy design within digital-twin architectures capable of simulating PD-L1 perturbation, therapeutic adaptation, and immune-ecosystem rewiring *in silico*. This schematic was conceptually designed and illustrated by the author

Dissecting PD-L1's predictive paradox: TMB, neoantigen dynamics, and the shifting landscape of immunoediting

The paradox of PD-L1 as a predictive biomarker in NSCLC immunotherapy arises from a fundamental discord between adaptive immune resistance and intrinsic tumor immunogenicity, resulting in instances where PD-L1 positivity does not predict response, while PD-L1-negative tumors may exhibit responsiveness, thus necessitating the interpretation of PD-L1 through the multifaceted perspectives of TMB, neoantigen quality, and the dynamic influences of immunoediting. TMB is extensively used because of its general correlation with the number of neoepitopes; nevertheless, elevated TMB alone may not ensure immunogenicity if the mutations are not well processed, displayed, or detected. Zhang *et al*. demonstrated that the incorporation of MHC-I neoantigen-presentation mechanisms (such as HLA expression, proteasome activity, and TAP transporters) with TMB and CD8+ T cell infiltration significantly enhances the correlation with responses to PD-1/PD-L1 inhibition compared to TMB alone (r = 0.865 vs 0.783)(48). Sun *et al*. contend that TMB and PD-L1 are complementary; TMB assesses the capacity for immune recognition, while PD-L1 indicates the tumor's inducible checkpoint resistance within a specific immune milieu (49). However, that superposition remains fragile in clinical practice due to neoantigen clonality and subclonal evolution altering the landscape under therapeutic pressure. Subclonal neoantigens may not attain enough allelic frequency to initiate or maintain T cell responses; only clonal neoantigens serve as reliable predictive anchors. During immunoediting, T cells selectively eradicate high-immunogenic clones, resulting in the survival of escape variants characterized by reduced antigenicity or MHC deletion, hence diminishing the predictive efficacy of both TMB and PD-L1. Progress in neoantigen prediction, integrating immunogenomic sequencing and immunopeptidomics, represents significant advancements; yet, challenges persist regarding HLA polymorphism, context-dependent peptide processing, and the identification of low-frequency antigens in spatially diverse tumors (50). The situation is further complicated by the dynamic upregulation or downregulation of PD-L1 as tumors respond to persistent T cell pressure: in inflamed ("hot") tumors, IFN-γ from tumor-infiltrating lymphocytes may induce elevated PD-L1 expression even in moderate tumor mutational burden contexts, whereas "cold" tumors with high tumor mutational burden but inadequate immune infiltration may remain PD-L1–negative and unresponsive. Consequently, PD-L1 sometimes functions as a proxy for previous immune activation, whereas TMB reflects baseline antigenic potential. This distinction elucidates contradictory examples. Clinical trials, including CheckMate-227, indicate that patients with both high TMB and PD-L1 positivity derive the greatest benefit from immune checkpoint inhibitor (ICI) combinations. However, patients with high TMB and negative PD-L1 status also receive benefits, while those with low TMB and positive PD-L1 status frequently do not, illustrating this paradoxical dissociation (49). Furthermore, the paradox is exacerbated by temporal variations in neoantigen landscapes and PD-L1 expression during treatment: immune pressure can promote the emergence of antigen-escape clones (e.g., through HLA loss, antigen truncation, or epigenetic silencing) and the modulation of PD-L1 expression via feedback mechanisms (e.g., SOCS induction, JAK–STAT regulation), resulting in changes in biomarker status from baseline to resistance. A patient originally designated as PD-L1-positive/high TMB may transition to a condition of reduced responsiveness, or conversely. In this context, the predictive paradox of PD-L1 is not a failure but an indication of biomarker variability. The solution lies in multidimensional, dynamic biomarker modeling: composite signatures integrating tumor mutational burden, clonal neoantigen load, antigen-presentation integrity, tumor-infiltrating lymphocyte phenotypes, and inducible PD-L1 regulation kinetics (based on computational and emerging clinical evidence). Novel computational frameworks, such as physiologically disentangled multi-omic deep learning models, show promise in elucidating latent immune-resistance networks and in forecasting response trajectories beyond static biomarkers (computational/model-based evidence)(15). In summary, the conundrum of PD-L1 lies in its function as a dynamic indicator of immunological strain, rather than a static regulator: it can only be made predictive, rather than perplexing, by contextualizing it within the evolving landscape of neoantigen development and immune editing (Table 1). Lessons Learned: TMB provides important insight

Table 1. Framework summarizing the evolving PD-L1 evidence layers in non-small cell lung cancer (NSCLC) immunotherapy

Analytical Layer	Key Insight	Interaction With PD-L1	Translational Relevance	Evidence Type	References
Mutational Burden & Neoantigens	Transition from total mutation counts to neoantigen quality	Interferon signaling may couple mutation load with inducible PD-L1	Guides patient stratification+vaccine/ICI combinations	Clinical+Computational	(51)
Tumor Microenvironment	Spatial immune niches define checkpoint expression	PD-L1 enriched in immune–stromal interfaces	Enables biopsy targeting+radiomic mapping	Clinical	(52)
Circulating / Liquid Biomarkers	Noninvasive PD-L1 and immune signatures emerging	Exosomal PD-L1 contributes to systemic immune suppression	Supports longitudinal monitoring and adaptive dosing	Emerging clinical	(33)
Temporal Dynamics	PD-L1 is fluctuating rather than static	IFN-γ and therapy pressure induce oscillatory regulation	Supports dynamic biomarker frameworks	Translational	(53)
AI-Driven Predictors	Multimodal models integrate genomics, imaging, and immune profiling	Learns causal immuno-regulatory networks	Enables digital-twin–based personalization	Computational	(12)

into tumor antigenicity, but high mutation burden does not uniformly translate into immunotherapy responsiveness. Neoantigen quality, clonality, and antigen presentation machinery significantly influence predictive value. Large prospective studies and multi-omic integration are still needed before TMB can reliably guide treatment strategies in routine clinical practice.

Beyond PD-L1: Expanding the biomarker landscape

Tumor mutational burden (TMB) and its predictive value in immunotherapy responsiveness.

Based on the premise that a higher load of somatic mutations (particularly nonsynonymous) increases the likelihood of generating immunogenic neoantigens that T cells can recognize, TMB has become one of the most studied "beyond-PD-L1" biomarkers in immunotherapy over the past ten years. The translation from mutational load to therapeutic efficacy is mediated by a number of conditional steps, including subsequent antigen processing/loading, MHC presentation, T cell receptor recognition, and host immune competence. In mechanistic terms, high TMB enhances the neoantigen generation phase (i.e., the raw substrate from which immune surveillance can detect "nonself" peptides)(49). Higher TMB (typically ≥10 mut/Mb, by standard hybrid-capture panels) is associated with better response rates, progression-free survival (PFS), and overall survival (OS) across ICI therapies in NSCLC and melanoma, according to numerous clinical meta-analyses and large real-world cohorts (54) and (55) confirm TMB as a meaningful correlative biomarker in next-generation immunotherapy trials. In extensive real-world NSCLC datasets, TMB ≥10 mut/Mb is a commonly used stratification threshold and maintains predictive efficacy for ICI benefit (55). Nonetheless, TMB's performance is significantly flawed: numerous technological, biological, and clinical limitations reduce its predictive accuracy. Technical factors such as tumor purity variability, sequencing depth, panel design, variant calling procedures, and germline filtering contribute to inaccuracies in TMB estimations (56). The disparities between tissue-based and blood-based tumor mutational burden (i.e., circulating tumor DNA) further complicate standardization. Biologically, not all mutations contribute equally to the immunogenic neoantigen burden; the "quality" of mutations (e.g., insertions and deletions, frameshifts, mutations in expressed genes, mutations producing high-affinity MHC-binding peptides) may have proportionally larger significance than mere mutation count (56). Furthermore, tumor heterogeneity and subclonality reduce effective neoantigen exposure, since subclonal mutations may exist in only small proportions of tumor cells, hence limiting their ability to generate strong T cell clones. Simultaneously, immunoediting may eliminate highly immunogenic clones over time, so dissociating baseline tumor mutational burden from persistent immunogenicity after treatment. The predictive power of TMB is context-dependent and inconsistent across tumor types. Zgura *et al.* observe that TMB accurately predicts ICI benefit in lung cancer and melanoma; however, its efficacy is much more variable in breast and prostate cancers (54). Complementary biomarkers (e.g., PD-L1, microsatellite instability, gene-signature scores) often help refine stratification.

NSCLC, particular oncogenic settings influence the relationship between TMB and treatment response; for instance, KRAS G12C–mutant tumors have been correlated with enhanced outcomes from ICIs, while research (57) indicates that this is primarily influenced by tobacco-related mutational signatures (SBS4) and the consequent elevated TMB rather than the inherent biology of G12C. In contrast, NSCLC tumors with STK11 or KEAP1 co-mutations often exhibit immunosuppressive microenvironments and decreased susceptibility to immune checkpoint inhibitors, even with high tumor mutational burden (58). Furthermore, immune parameters like the dynamics of peripheral lymphocyte count may influence the TMB signal: in a 2025 JCO abstract, Saleh *et al.* discovered that both TMB ≥10 and lymphocyte slope (LS) were independently correlated with improved survival on ICI, suggesting that systemic immune fitness interacts with mutational burden (59)leading to the pan-cancer FDA approval of the PD-1 inhibitor pembrolizumab in tumors with a TMB ≥10 mut/Mb. Lymphocyte count dynamics (lymphocyte stability LS. The use of sophisticated modeling techniques underscores TMB's constraints, as evidenced by a deep learning model in NSCLC reported in JAMA Oncology (60), which utilized integrated imaging, genomic, and clinical data to predict immune checkpoint inhibitor response, exceeding the efficacy of individual indicators such as tumor mutational burden or PD-L1 alone. Collectively, the emerging perspective is that TMB is a robust yet imprecise tool: it provides a broad indication of immunogenic potential but lacks mechanistic specificity. The next route involves "biologically calibrated TMB," which weights mutation load based on projected neoantigen production, clonality, antigen expression, and immune-editing history (56) and incorporates TMB into multi-omic integrative models that measure tumor–immune interactions across time. TMB has a crucial but subordinate position in the biomarker landscape: it is beneficial for general triage and enrichment, but inadequate as a sole criterion for precise patient-level decision-making in customized immunotherapy.

Microsatellite instability (MSI), DNA damage repair (DDR) mutations, and immunogenic signatures

In tumors characterized by microsatellite instability (MSI) resulting from defective mismatch repair (dMMR), recurrent frameshift insertions and deletions in short tandem repeats produce dense arrays of novel neoepitopes. These neoepitopes significantly reconfigure local immune environments through a high TMB and a plethora of antigenic peptides presented on MHC-I/II complexes (e.g., via indel neoantigens). This molecular mechanism elucidates the pronounced sensitivity of MSI-H tumors to immune checkpoint blockade (ICB)(61). Nevertheless, new data from 2025 complicate that concept by demonstrating that not all MSI-H patients produce consistently "hot" immune microenvironments: the presence or absence of DDR pathway mutations beyond MMR may affect immunogenicity, alter mutational signatures, and impact clinical outcomes. A 2025 pan-cancer analysis of 264 DDR genes from The Cancer Genome Atlas demonstrated that specific DDR gene mutations correlate positively or negatively with the expression of immune stimulators, inhibitors, and MHC pathway genes, thereby linking DDR defects to immunoregulatory alterations beyond simple mutational load (62). A recent research on brain metastases identified a mutational profile associated

with impaired DNA damage repair, which correlated with diminished survival and, surprisingly, lower immunogenicity despite increased TMB, suggesting that certain DNA damage repair-related signatures may inhibit immune recognition (63). Mechanistically, defects in homologous recombination (HR) or nucleotide excision repair within the DNA damage response (DDR) can result in the accumulation of cytosolic DNA, activation of the cGAS-STING pathway, type I interferon signaling, and dendritic cell activation, thereby initiating a feed-forward loop of antitumor immunity provided that downstream immunosuppressive mechanisms (e.g., checkpoint upregulation, Treg recruitment) do not prevail (64). The interaction between MMR and HR deficiencies has recently been framed as a non-mutually exclusive axis, whereby tumors may possess hybrid combinations of MSI markers and HR deficit, resulting in unique synthetic vulnerabilities and hybrid mutational footprints (65). In clinical settings, the mutation status of DNA damage response (DDR) in colorectal and other malignancies, beyond MSI, is now being explored as a biomarker. A 2025 review in Cancer Biomedicine indicates that MSI-stable CRC patients with DDR mutations have heightened susceptibility to immune checkpoint inhibitors and distinct immunological profiles compared to those without DDR abnormalities, suggesting a possible extension of immunotherapy beyond MSI status alone (66). A DDR-related immune activation (DRIA) signature, derived from the expression of DDR-associated immune genes, has shown predictive efficacy for immunotherapy response, amalgamating transcriptomic and mutational data into a cohesive representation of immunological-DDR interaction (67). These converging lines indicate a sophisticated conceptual framework: MSI status establishes a foundational measure of mutational antigenic potential; nevertheless, the integration of DDR mutation patterns shapes the true immunogenic visibility of the tumor by modifying antigen processing, neoantigen quality, immune-modulatory gene expression, and checkpoint landscapes. Consequently, the integration of high-resolution mutational signature deconvolution (such as distinguishing pure MSI indel signatures from DDR-associated signatures), DDR genotypes (HR, NHEJ, base excision repair, etc.), transcriptomic immune signatures, and functional assays of antigen presentation may provide a mechanistic and clinically actionable criterion for ICI selection. In forthcoming translational procedures, malignancies may be classified not just as "MSI-H" or "MSS," but along a multidimensional axis: neoantigen load × DDR-signature impact × immune gene rewiring, allowing precision immunotherapy informed by mechanistic rather than simply statistical insights.

Peripheral and tissue-based immune signatures ($CD8^+$ T cells, IFN-γ, cytokine profiles)

We propose a comprehensive mechanistic model that integrates $CD8^+$ T cell phenotypes, IFN-γ–centric signaling pathways, and contextual cytokine environments to form spatially defined immunological signatures that predict disease states and therapeutic responses. Recent atlases of human organ donor tissues have shown that immune composition is significantly site-specific, with blood and mucosal or parenchymal tissues exhibiting distinct transcriptome and epigenomic patterns across T cell lineages and other immune compartments (68). In these atlases, circulating memory $CD8^+$ T cells exhibit elevated levels of canonical cytotoxic molecules (e.g., perforin, granzyme B) but gradually downregulate these as they transition to tissue residency, which inversely correlates with surface markers such as CD69 and CD103, indicating a transcriptional reprogramming influenced by local signals (69). In tissues, a novel population of precursor exhausted tissue-resident memory (pf-Trm) $CD8^+$ cells identified by PD-1^+ Tim-3^- $CD103^+$ has been differentiated from completely terminal exhausted (Tex) $CD8^+$ T cells (PD-1^+ Tim-3^+ $CD103^+$) in colorectal cancer (70). These cells inhabit a mechanistic niche: they maintain partial effector potential and may react to checkpoint inhibition, while exhibiting a "primed exhaustion" cytokine signature. In tumor microenvironments, the equilibrium of IFN-γ, IL-2, TNF-α, and immunosuppressive cytokines (such as IL-10 and TGF-β) influences the destiny of $CD8^+$ cells, determining whether they are reinvigorated or rendered profoundly exhausted (71). We propose that in peripheral blood, the presence of antigen (or bystander cytokine signals) stimulates the proliferation of IL7R^low effector memory $CD8^+$ T cells, which are enriched in GZMB, GZMK, PRF1, and ISG signatures, and whose production of IFN-γ contributes to a positive feedback loop of cytokine amplification (72). $CD8^+$ subsets may infiltrate tissues in response to gradients of CXCR3/CCR5 ligands and upregulate CD49d/VLA-4 to penetrate the endothelium. Upon localization, tissue signals (e.g., TGF-β, hypoxia, matrix stiffness) inhibit conventional cytotoxic gene expression while enhancing the production of alternative granzymes (A, H, K, M) and epigenetic regulators, resulting in a localized "functional rewiring" phenomenon (69). Within inflamed tissues, $Gzmk^+$ $CD8^+$ T cells have been observed to localize prominently (73). They may contribute by noncytotoxic processes such as complement activation, extracellular matrix remodeling, or proinflammatory substrate cleavage, therefore enhancing local IFN-γ–mediated cytokine cascades (73). Tissue-resident $CD8^+$ T cells exhibiting pf-Trm signatures detect local antigens via minimal MHC I expression and secrete IFN-γ pulses, which then enhance the local expression of CXCL9/10 and attract more $CXCR3^+$ T cells, therefore perpetuating a "local T cell seeding" circuit. This conceptual framework suggests the establishment of a tissue-periphery cytokine coupling index, such as the ratio of peripheral IFN-γ-producing $CD8^+$ cells to the local tissue IFN-γ signature (obtained from transcriptomic or multiplex cytokine assays), which could categorize patients based on their anticipated response to immunotherapy or immunomodulation. In autoimmune conditions, peripheral IL7R^low EM $CD8^+$ T cells that secrete IFN-γ and TNF have been shown to promote neutrophil extracellular trap formation (NETosis), suggesting a mechanistic connection between blood $CD8^+$ signatures, cytokine production, and end-organ damage (72). In dermatological disorders, individuals with psoriasis exhibit modified peripheral $CD8^+$ signatures: upon stimulation, $CD8^+$ T cells from these patients generate distinct cytokine profiles (IL-17, IFN-γ) in contrast to healthy controls, and their expression of trafficking receptors (e.g., CCR4, CCR10) correlates with skin infiltration (74). Likewise, extracellular cytokine profiles in biological fluids (e.g., serum, cerebrospinal fluid) have been used to sub-

classify immunological neuropathies (75). The mechanistic innovations presented include 1) the conceptualization of bidirectional coupling between peripheral $CD8^+$ T cell IFN-γ output and local tissue cytokine environments, 2) the integration of transcriptional reprogramming of cytotoxic machinery as $CD8^+$ T cells migrate from blood to tissue, and 3) the introduction of quantitative indices that can be validated in patient cohorts to forecast responsiveness to checkpoint blockade or cytokine therapies. To empirically validate, one may do dual-site single-cell RNA and TCR sequencing of peripheral blood alongside matching tissue biopsies, integrating cytokine receptor/ligand modules and flux-modeling predicted IFN-γ gradients. Longitudinal sampling before and after treatment would evaluate whether changes in the coupling index more accurately forecast clinical outcomes than peripheral or tissue indicators alone. This integrated mechanistic-conceptual framework may facilitate the development of a new generation of spatially informed immunological biomarkers based on $CD8^+$ T cell–IFN-γ–cytokine circuitry.

Liquid biopsy biomarkers: circulating tumor DNA (ctDNA), exosomal PD-L1, and T-cell receptor (TCR) diversity

In the advancing field of precision oncology, liquid biopsy biomarkers, including ctDNA, exosomal PD-L1, and T-cell receptor (TCR) diversity, are coalescing into a cohesive molecular and clinical framework that offers dynamic surveillance of tumor-immune interactions. ctDNA, released as fragmented, tumor-derived cell-free DNA into the bloodstream by apoptotic or necrotic tumors and circulating tumor cells, contains not only mutation and copy number information but also fragmentomic, methylation, and nucleosome footprint signatures. By 2025, ultrasensitive assays will routinely detect variant allele fractions at parts-per-million levels, enabling measurable residual disease (MRD) detection and early relapse prediction well before imaging. This was exemplified in the VICTORI trial in colorectal cancer, where ctDNA positivity post-resection predicted recurrence a median of approximately 198 days earlier than scans, with detection in 87% of recurrent cases (76). Comprehensive meta-analyses relating baseline ctDNA to OS and PFS under immune checkpoint inhibitor treatment in urothelial carcinoma show comparable predictive power across tumor types (HR ~2.75 for PFS) (77). Mechanistically, ctDNA quantification reflects the equilibrium among tumor cell turnover, DNase-mediated degradation, and phagocytic elimination. In contrast, variations in fragment size distribution and terminal motif patterns convey biophysical characteristics of nuclease-mediated DNA processing and apoptotic mechanisms (fragmentomics). Recent multi-analyte reviews underscore that integrating variant detection with methylation and fragmentomics improves both sensitivity and specificity in early detection and immunotherapy monitoring (78). Alongside ctDNA, exosomal PD-L1 (exoPD-L1) contained within tumor-derived extracellular vesicles has emerged as a systemic immunoregulatory biomarker. Cancer cells encapsulate PD-L1 into intraluminal vesicles through ESCRT machinery (e.g., Rab27a, nSMase2-dependent generation) and release exosomes that travel away from the primary tumor. In this process, exoPD-L1 interacts with PD-1 on circulating T cells, transmitting inhibitory signals via SHP2-mediated dephosphorylation of proximal TCR signaling nodes, thereby inhibiting T cell activation in distant lymphoid or metastatic environments (33). Clinically, higher plasma exoPD-L1 levels are associated with suboptimal responses to ICB in melanoma, NSCLC, head and neck malignancies, and gastric cancers, whereas dynamic reduction of exoPD-L1 may indicate the effectiveness of immunotherapy; however, the uniformity of exosome extraction and quantification remains a challenge (33). ExoPD-L1 offers an immunological context that complements ctDNA: while ctDNA monitors tumor burden and genomic evolution, exoPD-L1 assesses functional immune suppression. The third axis, TCR diversity, represents the host's adaptive immune response: comprehensive sequencing of TCR β or α/β chains in peripheral blood yields metrics such as Shannon diversity, clonality, and clusters of functionally analogous clonotypes (repertoire functional units, RFUs) that indicate clonal expansion or contraction in response to tumor antigen exposure. A recent lung cancer study that integrated graph-based TCR repertoire clustering with ctDNA analysis demonstrated improved early-stage detection performance compared with ctDNA-only platforms. In parallel, the DeepCaTCR model showed promising behavior in early computational analyses using peripheral TCR alterations. Both findings remain preliminary and require external verification before clinical adoption (computational and emerging clinical (33, 79). Mechanistically, these alterations signify antigen-driven proliferation of tumor-reactive T cell clones and the reduction of non-relevant clones, influenced by thymic output, homeostatic turnover, and immunological fatigue or suppression (e.g., by exoPD-L1). Clinically, alterations in TCR diversity during treatment, such as increased clonality or targeted expansion, have been linked to positive responses or potential relapse. At the same time, dynamic immune repertoire profiling may predict immune-related adverse events due to off-tumor activation, as indicated by Shannon and Simpson indices (80). The integrated biomarker paradigm aims to combine ctDNA (tumor burden and mutational progression), exoPD-L1 (immune suppression dynamics), and TCR diversity (adaptive immune response) into a mechanistic "tumor–immune systems graph." This approach facilitates temporally resolved decision-making: for instance, an isolated increase in ctDNA without alterations in TCR or exoPD-L1 suggests the presence of a silent escape clone, while a rise in ctDNA accompanied by an increase in exoPD-L1 and a decrease in TCR indicates imminent immune evasion, necessitating combination therapy. In the age of immunotherapy, multiplex liquid biopsy signatures may inform adaptive treatment escalation, prompt early transition to alternative immunological modalities, or facilitate the reduction of toxin exposure, representing an advancement beyond static tissue biomarkers. Moreover, Bayesian hierarchical models of ctDNA sensitivity across various cancer types (e.g., for MCED tests) are being implemented in 2025 to calibrate diverse shedding priors (81, 82). Analogous Bayesian integration of immunological markers may enhance individualized risk assessment. Advancements in ultra-low input exosome capture, single-molecule barcoding of cfDNA fragments, and multiplex TCR-antigen mapping through innovative generative TCR prediction frameworks will be crucial for implementing

this triad in multicenter trials and subsequent clinical workflows. Parallel advances in nanomedicine are converging with these liquid-biopsy frameworks to close the loop between molecular detection and targeted drug delivery. In a recent 2025 study, Al Khatib *et al.* developed spray-dried lipid nanoparticles encapsulating pimozide with optimized aerodynamic behavior and enhanced tropism toward non-small-cell lung cancer cells (83). Beyond pharmacokinetic improvements, these nanoformulations exhibited preferential uptake in PD-L1-high tumor niches, linking drug localization to immunometabolic vulnerability (83). Such integration of nanoscale pharmacology with exosomal and liquid-immune monitoring exemplifies a new therapeutic feedback architecture where diagnostic biomarkers and nanocarrier design evolve synchronously to reinforce precision immunotherapy efficacy (83).

Integrating multi-parametric biomarkers into composite response indices

The next advancement in precision medicine involves the incorporation of multi-parametric biomarkers into cohesive composite response indices, representing a significant change that surpasses the constraints of unidimensional biomarker models and facilitates mechanistic, predictive, and therapeutically relevant outcomes. A composite index integrates orthogonal biomarker modalities (e.g., proteomic, imaging, immunologic, genetic, metabolic) via algorithmic weighting to provide a latent "response score" that accurately reflects disease biology, therapeutic efficacy, and progression. Meyre *et al.* developed a composite cardiovascular risk index using D-dimer, GDF-15, IL-6, NT-proBNP, and high-sensitivity troponin T, with each biomarker contributing to a synergistic composite hazard predictor for cardiovascular events (mortality, stroke, myocardial infarction) within a Cox model framework (84). Saha *et al.* created an elastic-net composite biomarker utilizing structural MRI, diffusion MRI, quantitative susceptibility mapping, demographics, and genetics to forecast the severity of Friedreich ataxia, achieving an R^2 of 0.79 and a longitudinal sensitivity (d=1.12) that surpasses traditional clinical scales (85) inherited progressive movement disorder for which there is currently no cure. The field urgently requires more sensitive, objective, and clinically relevant biomarkers to enhance the evaluation of treatment efficacy in clinical trials and to speed up the process of drug discovery. This study pioneers the development of clinically relevant, multidomain, fully objective composite biomarkers of disease severity and progression, using multimodal neuroimaging and background data (i.e., demographic, disease history, genetics. Mechanistically, each biomarker represents certain biological dimensions such as immunological activation, mitochondrial dysfunction, and tissue remodeling, while the composite score applies covariance weighting to enhance the signal-to-noise ratio across these dimensions. To implement this, one might initiate with candidate biomarker panels encompassing dimensions such as inflammatory cytokines, microRNA signatures, imaging texture features, and metabolic flux indices, normalize each to z-scores, and subsequently utilize regularized regression or machine learning techniques (LASSO, elastic net, gradient boosting) to allocate coefficients that optimize the prediction of a gold-standard clinical outcome (e.g., progression, remission, survival). D'Angelo *et al.* recently proposed a logic-indicator modification of the Youden index to optimally dichotomize dual-biomarker combinations, demonstrating that "either both positive or one positive" criteria may surpass single-biomarker thresholds in patient stratification (86). By iterating via combinations and cross-validation folds, a composite decision boundary is constructed that incorporates continuous biomarkers into a robust index. The systemic immune-inflammation index (SII=platelets×neutrophils/lymphocytes) has recently been validated as a composite cellular biomarker in autoimmune diseases, reflecting inflammatory and immunological balance in rheumatoid arthritis, systemic lupus erythematosus, and vasculitis. It is associated with disease activity and prognosis (87). These simpler composites exemplify the connection between proof-of-concept and advanced multi-omics integration. Clinically, composite indicators provide more dependable early stopping criteria, improved differentiation between responders and nonresponders, and the certification of surrogate endpoints. For example, in lung disease associated with rheumatoid arthritis, Wan *et al.* demonstrated that the incorporation of tumor markers (e.g., CA19-9, CYFRA21-1), inflammatory indices (CRP, NLR), and disease activity (DAS28-CRP) into a multidimensional index resulted in an AUC of 0.857 for predicting RA-ILD, surpassing the efficacy of unimodal markers (88). At the molecular systems level, meta-analyses of proteomic biomarker sets in cancer identify similar upstream regulators across modalities (e.g., TNF, p38-MAPK, miR-34a) that support the combined weighting of biomarkers across fluid, tissue, and exosomal tests to represent pathway crosstalk (88). Implementing composite response indices requires meticulous calibration (thresholds, dynamic ranges), clear interpretability (e.g., coefficient maps), external validation in separate cohorts, and regulatory approval. The outcome is an advanced biomarker framework: mechanistically based, statistically sound, clinically relevant, and conceptually novel, introducing compound "response indices" that may function as surrogate endpoints, adaptive trial indicators, and decision-making aids across many illnesses (Figure 2) (Table 2).

Multi-omic biomarker networks in NSCLC immunotherapy

Genomic and transcriptomic correlates of immune checkpoint response

Over the past year, the discipline has progressed from simplistic mutational burden metrics to a mechanistic multi-omic analysis elucidating why only certain tumor subsets exhibit sustained responses to checkpoint blockade: integrated evaluations now demonstrate that genomic alterations, transcriptomic profiles, and epitranscriptomic regulation collaboratively influence efficacy by shaping antigen presentation, interferon signaling, metabolic pathways, and suppressive microenvironments. Chen *et al.* conducted a comprehensive pan-cancer analysis of PD-1 transcriptomic landscapes, revealing that elevated PDCD1 RNA expression (after adjusting for TIL infiltration) was associated with improved checkpoint outcomes, but solely in conjunction with preserved JAK/STAT1/IRF1 signatures, indicating that the "licensing" of PD-1 transcription necessitates upstream interferon responsiveness (96). In patients with NSCLC undergoing anti-PD-1/PD-L1 therapy, Ravi *et al.* demonstrated that TERT amplification and ATM alterations (compared to wildtype) differentiate poor

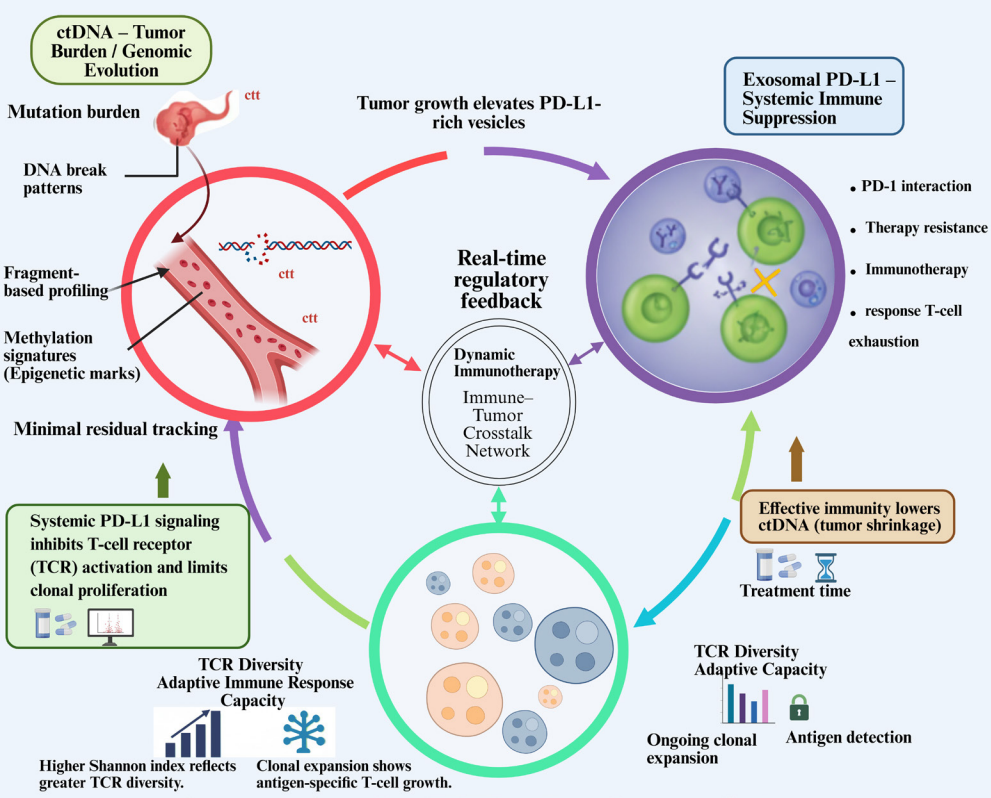

Figure 2. AI-integrated liquid-immune feedback architecture linking ctDNA fragmentomics, exosomal PD-L1 vesicular signaling, and T-cell receptor (TCR) repertoire entropy in adaptive immunotherapy

This diagram depicts a closed-loop model in which cfDNA, exosomal PD-L1, and TCR diversity coevolve. These biomarkers help reflect the dynamic immune control in NSCLC (33). Fragmentomic asymmetry and methylation topology of ctDNA provide high-resolution telemetry of tumor genome flux and residual disease; vesicular PD-L1 released via Rab27a/nSMase2-regulated exocytosis broadcasts systemic immunosuppression, attenuating SHP2-mediated TCR signaling; and entropy metrics of the peripheral TCR repertoire quantify adaptive immune plasticity. These orthogonal dimensions converge into an AI-driven digital-twin model that reconstructs longitudinal immune–tumor trajectories, correlating ctDNA variant-allele kinetics with exosomal PD-L1 oscillations and TCR clonal re-expansion (34,89). The resulting composite response index transforms liquid-biopsy snapshots into a continuously learning biosystem capable of forecasting therapeutic resistance, identifying immune rejuvenation windows, and guiding precision-timed checkpoint modulation. Collectively, the figure reframes liquid-immune biomarkers as dynamic network variables within a self-correcting immuno-oncologic cybernetics paradigm. This schematic was conceptually designed and illustrated by the author

responders from favorable responders, with the latter group exhibiting increased expression of immunoproteasome subunits (PSMB8/9) and a "induced antigen processing" transcriptomic program (97). Ye *et al.* recently integrated 34 single-cell and bulk RNA-sequencing datasets to establish an EGFR-related transcriptional signature. Higher EGFR activity was associated with reduced responsiveness to immune checkpoint inhibitors, although the predictive reliability of this signature remains variable across datasets. Several Hub-EGFR genes were also functionally linked to immune escape pathways, supported by CRISPR screening evidence (98). A 2025 review on m^{6}A RNA methylation in the epitranscriptomic domain emphasized how modified m^{6}A "writers" (e.g., METTL3/METTL14), "erasers" (e.g., ALKBH5), and "readers" affect the stability of STAT1/IRF1 transcripts, regulate interferon gamma loops, and consequently impact CD8$^+$ T-cell infiltration and PD-1/PD-L1 axis dynamics (99). ICBatlas has aggregated 1,515 ICI-

Table 2. Multi-axis, AI-integrated biomarker framework that unifies genomic, immune, and liquid profiles for precision adaptive immunotherapy

Biomarker Axis	Mechanistic Basis	Key 2025 Technologies	Clinical Impact	Advantages	Challenges / Limitations	References
Tumor Mutational Burden (TMB)	High mutation load → more neoantigens and T-cell visibility	AI-calibrated TMB models (DeepHLApan 2.0, NetMHCpan-2025)	Refines ICI stratification beyond PD-L1	Quantitative, broadly applicable	Variable assays, poor specificity	(90)
MSI / DDR Mutations	dMMR or DDR defects → frameshift indels+STING activation	DDR-gene panels, mutational-signature AI deconvolution (DRIA index)	Expands ICI eligibility beyond MSI-H	Mechanistic clarity; multi-tumor utility	Overlapping signatures, unclear cut-offs	(62, 91)
Peripheral & Tissue Immune Signatures	CD8$^+$ T-cell phenotypes+IFN-γ / cytokine circuits	scRNA/TCR-seq, spatial cytokine AI maps	Predicts ICI response via immune functional states	Dynamic and context-aware	Costly multi-omic profiling	(92, 93)
Liquid Biopsies (ctDNA, exoPD-L1, TCR)	ctDNA=tumor burden; exoPD-L1=systemic suppression; TCR=adaptive response	Ultra-sensitive fragmentomics, AI ExoFlow-ML, DeepCaTCR models	Enables real-time therapy monitoring	Minimally invasive, multi-dimensional	Isolation biases, data integration issues	(94)
Composite Response Indices	Weighted fusion of multi-modal biomarkers → latent response score	Elastic-net / Bayesian fusion, digital-twin simulation	Mechanistic and predictive surrogate endpoint	Integrates orthogonal signals; regulatory potential	Needs standardized cut-offs & external validation	(95)

treated tumors from 9 cancer types at the transcriptomic level, identifying approximately 4,782 differentially expressed genes that distinguish responders from nonresponders, with many converging on immune-metabolic, chemokine, and RNA splicing pathways (100). In hepatocellular carcinoma, Yang *et al.* used single-cell mapping to identify a dysfunctional $CD28^{+}PD\text{-}1^{+}$ Tc subpopulation, whose enrichment (by bulk deconvolution) correlates with worse outcomes and reduced expression of cytotoxic gene programs (GZMB, IFNG), suggesting a possible mechanistic link to resistance (101). Spatial transcriptomics in non-small cell lung carcinoma has identified specific groups of cancer-associated fibroblasts with transcriptional profiles indicative of immune exclusion, fatigue niches, and Treg enrichment in non-responsive phenotypes (102). Clinical longitudinal liquid biopsy studies in head and neck cancer have shown that alterations in circulating tumor DNA fragment size and variant allele fraction kinetics may predict checkpoint response before radiographic changes occur (103). Huang *et al.* have discovered GSDMD expression as a cross-tumor biomarker, with greater expression correlating with TMB, MSI, increased checkpoint ligand transcripts, and pro-immune infiltrates, providing both mechanistic and prognostic insights (104). Tariq and Fraenkel developed BDVAE, a biologically disentangled variational autoencoder that fuses genomic and transcriptomic signals to capture latent features linked to immune suppression and metabolic rewiring. Retrospective evaluation in a cohort of patients receiving immune checkpoint inhibitors indicated promising computational performance; however, external validation is currently lacking (computational/model-based evidence)(15). Collectively, these advancements establish a mechanistic framework: 1) driver mutations and copy number alterations regulate antigenic characteristics and interferon pathways; 2) transcriptomic states execute downstream effector programs or suppressive signatures; and 3) epitranscriptomic modulation influences mRNA stability and feedback mechanisms. The co-registration of these layers not only accurately predicts response but also indicates combinatorial therapies (e.g., targeting $m^{6}A$ modulators, CAF–immune interaction, or metabolic reprogramming) to sensitize resistant tumors.

Epigenomic reprogramming and its impact on immune escape (methylation of immune genes)

In the evolving domain of tumor-immune interactions, epigenomic reprogramming through DNA methylation serves as a critical mechanism by which tumors evade immune surveillance: numerous recent studies from 2025 have demonstrated that hypermethylation of promoters and enhancers of immune-regulatory genes (e.g., MHC class I/II loci, antigen-processing machinery, interferon signaling pathways, STING pathway effectors) suppresses their transcription, diminishes antigen presentation, obstructs chemokine signals, and consequently impairs T cell infiltration and cytotoxic activity. In triple-negative breast cancer, the global methylome landscape stratifies subtypes by distinct immunological microenvironments, suggesting that distal regulatory CpG methylation contributes to the silencing of immune-recognition pathways (105). In preclinical models, decitabine therapy reactivates suppressed MHC class I, PD-L1, and STING, consequently restoring the interferon response and synergizing with checkpoint blocking *in vivo*, consistent with the molecular re-opening of chromatin at immune loci (106). At the biochemical level, oncogenic signaling (e.g., through mutant KRAS, IDH, or EGFR) recruits DNMTs and associated methyl-CpG binding proteins to immune gene promoters, resulting in localized CpG island hypermethylation and trimethylation of histone H3K27 via Polycomb repressor complexes; this establishes a feedback loop that maintains immune genes in a repressed state (107). Furthermore, oxidative stress and redox imbalance, a common characteristic of cancer, interact with methylation dynamics: reactive oxygen species regulate the oxidation of 5-methylcytosine by TET enzymes, thereby affecting the methyl/demethyl turnover at immune genes and shifting the equilibrium towards hypermethylation and immune silencing (108). The idea of phase-locked methylation domains, recently modeled in 2025, indicates that localized synchronization of methylation turnover within chromatin domains may reinforce immunologically silent modules that are resistant to random reactivation, thereby facilitating sustained immune evasion (109). Tumors characterized by immune-silenced methylation profiles exhibit reduced responsiveness to PD-1/PD-L1 inhibitors and have lower TIL infiltration; these methylation signatures may serve as predictive biomarkers of resistance to immunotherapy (110). The therapeutic implications are significant: combining DNMT inhibitors (or locus-targeted epigenome editing) with immune checkpoint inhibition or STING agonists may reverse immune gene silencing, replenish effector T-cell niches, and restore tumor immunogenicity (107). Nonetheless, obstacles persist: Tumor heterogeneity promotes subclones with varying methylation profiles (some immune-silent, others permissive); off-target demethylation poses hazards of autoimmunity or ectopic gene activation; and compensation by histone modifiers or non-coding RNAs may reinstate immune gene insulation (111). Future mechanistic investigations must analyze the kinetics of methylation turnover on immune genes throughout tumor evolution, integrate spatial single-cell methylomics with chromatin conformational mapping (e.g., HiChIP) to associate distal methylation with immune gene promoters, and validate methylation-based biomarkers in prospective immunotherapy trials. This innovative mechanistic approach asserts that epigenomic reprogramming of immunological loci is not only an epiphenomenon but a catalyst for immune escape—one that may be reversed with targeted epigenetic-immunotherapy combinations.

Proteomic and metabolomic biomarkers: metabolic immune checkpoints (IDO1, arginase, tryptophan–kynurenine axis)

A contemporary frontier in immunometabolic research is emerging, wherein proteomic and metabolomic signatures collaboratively clarify the functioning of metabolic immune checkpoints, particularly IDO1, arginase, and the tryptophan–kynurenine axis, serving as both mechanistic regulators and prospective clinical biomarkers. In a mechanistic synthesis, immune-suppressive myeloid populations (e.g., MDSCs, tolerogenic macrophages) express IDO1, which catalyzes the oxidation of tryptophan (Trp) to N-formylkynurenine. This process drives the accumulation of kynurenine and subsequent AHR engagement in T cells, thereby inducing exhaustion

programs, promoting Treg differentiation, and inhibiting effector proliferation via GCN2, mTOR, and integrated stress-response pathways (112). Arginase isoforms (ARG1/ARG2) concurrently diminish extracellular L-arginine, depriving activated T cells of an essential substrate necessary for CD3ζ chain phosphorylation, mTORC1 activation, and subsequent glycolysis, while generating ornithine and urea that contribute to proline/polyamine synthesis in immunosuppressive stromal or tumor environments (Frontiers review on metabolic reprogramming)(113). Integrated proteome analyses of tumor interstitial fluid and plasma have commenced elucidating protein expression levels of IDO1, arginase, and co-regulated enzymes (e.g., TDO2, ARG2, iNOS) as measurable indicators associated with immunotherapy resistance. Recently, untargeted mass spectrometric proteomics, integrated with network-guided penalized regression, has identified hub proteins, including metabolic regulators, that predict immune phenotypes in CPTAC populations (114). In the realm of metabolomics, targeted LC-MS/MS quantification of serum tryptophan and its kynurenine derivatives (kynurenic acid, xanthurenic acid, 3-hydroxykynurenine) has demonstrated that diminished tryptophan levels and elevated kynurenine-to-tryptophan ratios are associated with increased disease activity (e.g., rheumatoid arthritis) and compromised immune equilibrium (115), In cancer populations, adaptive resistance to checkpoint blockade has been associated with elevated serum Kyn/Trp ratios and modified downstream metabolites (Metabolomic adaptations research)(116). The recently published review "Metabolic checkpoints in immune cell reprogramming" consolidates evidence that metabolite flux through the Trp-Kyn axis actively influences macrophage polarization, dendritic cell tolerogenic function, and Treg stability, establishing these metabolites as genuine signaling mediators rather than mere byproducts (113). A combined proteo-metabolomic index (e.g., IDO1 protein abundance×Kyn/Trp ratio×ARG1/arginine ratio) may clinically stratify patients for immune checkpoint inhibitor responsiveness, predict immune-related adverse events, and inform combination therapies (e.g., IDO1/TDO dual inhibitors, arginase inhibitors, AHR antagonists). In preclinical ovarian cancer models, IDO1 inhibition prompted compensatory metabolic reprogramming, characterized by increased alternative amino acid catabolism, indicating that targeting a particular route may lead to adaptive escape (117). Future innovation lies in spatial proteomics/metabolomics to delineate microregional gradients of Trp–Kyn and arginine metabolism in situ, integrating with single-cell transcriptomics to facilitate mechanistic deconvolution of the emergence of metabolic checkpoint zones within tumor niches. Consequently, proteomic and metabolomic biomarkers of IDO1, arginase, and the Trp–Kyn axis serve not only as indicators but also as functional modulators and decision-making instruments in precision immunometabolic therapy (117). Checkpoints. In glioblastoma, for instance, Al-Ameer *et al.* demonstrated that simultaneous siRNA-mediated silencing of the metabolic stress-response genes PFKFB4 and HMOX1 reprograms glycolytic flux and redox homeostasis (118), thereby collapsing the adaptive metabolic plasticity that underlies tumor immune evasion and chemoresistance. This dual-gene inhibition amplified the cytotoxic efficacy of doxorubicin and temozolomide and shifted tumor metabolism from a pro-survival Warburg phenotype toward oxidative vulnerability. Mechanistically, the PFKFB4-HMOX1 axis integrates hypoxia-induced glycolysis, NADPH regeneration (118), and heme catabolism pathways that are increasingly recognized as immunometabolic nodes coupling energy metabolism with immune checkpoint signaling. Such findings reinforce the emerging concept that targeted metabolic interference can both sensitize tumors to cytotoxic therapies and recalibrate the immune microenvironment, positioning combined metabolic-genetic modulation as a next-generation complement to immunometabolic precision oncology (118).

Spatial single-cell and multi-omic integration to dissect tumor–immune ecosystem heterogeneity

The rapidly advancing field of spatial omics, integrated with single-cell and multi-omic approaches, is ushering in a new mechanistic phase in the analysis of tumor–immune ecosystem heterogeneity. By integrating transcriptomic, proteomic, epigenomic, and spatial data at single-cell (or subcellular) resolution, we can elucidate the emergence of micro-niches characterized by immune exclusion, immunosuppression, or infiltration as dynamic, locally regulated domains within the tumor–immune interaction. Recent advancements in the integration of scRNA-seq with spatial transcriptomics (ST) in 2025 have shown that these synergies mitigate the loss of spatial context characteristic of dissociative single-cell assays, facilitating the reconstruction of ligand-receptor topologies mapped onto physical neighborhoods within tumor cross-sections (119). In lung cancer, integrative analysis of scRNA-seq and ST identified the MDK–NCL signaling axis as spatially concentrated at tumor–immune interfaces and mechanistically involved in localized immune suppression (120). In non-small cell lung carcinoma undergoing neoadjuvant chemoimmunotherapy, single-cell and spatial transcriptomic analyses demonstrated reorganization of tumor cell, macrophage, and T cell spatial densities following treatment across distinct immune hot and cold regions, accompanied by alterations in interferon response and checkpoint ligand gradients that correlate with pathological outcomes (121) and the combination of neoadjuvant immunotherapy with chemotherapy has emerged as the first-line treatment for NSCLC. Nevertheless, the efficacy of this therapeutic approach remains variable. The present study aims to examine the impact of chemoimmunotherapy in NSCLC patients, with a view to identifying key molecules, critical cell subpopulations, communication patterns and spatial distributions that potentially correlate with therapeutic sensitivity. A total of 16 lung cancer tissue samples were collected from a cohort of 12 NSCLC patients and subjected to single-cell RNA and spatial transcriptome sequencing. Our data demonstrated that the distribution of CD4+Treg T cells and mCAFs indicated an immunosuppressive tumor microenvironment, while the accumulation of CD4+Th17 T cells and iCAFs could act as a positive marker for the sensitivity to chemoimmunotherapy. Furthermore, a significant high level of SELENOP-macrophages was observed in tissues from positive responders, and a strong co-localization between SELENOP-macrophages and antigen-presenting cancer associated fibroblasts (CAFs. In metastatic melanoma, the prolonged use of a multimodal integration toolkit that combines CITE-seq (RNA and

epitopes) with highly multiplexed spatial imaging (such as PhenoCycler) uncovered the emergence of "immune-striving" peritumoral lymphoid aggregates, differential clonal expansions ($MITF^+SPARCL1^+$ and $CENPF^+$ subclones), and B cell-rich signatures that were spatially concentrated within tumor interiors in responders, while being restricted to margins in resistant cases (122). These investigations demonstrate that tumor-immune heterogeneity is spatially regulated and that mechanisms of resistance or responsiveness can be confined to certain niches (e.g., perivascular channels, stromal barriers, TLS-like aggregates). Recent advancements in computational methods, specifically Bayesian hierarchical models such as multivariate log-Gaussian Cox process approaches, facilitate the aggregation of spatial dependency structures across subjects in multiplex imaging space, thereby enabling the inference of conserved "immune-permissive" versus "immune-exclusive" spatial motifs across cohorts (123), and spatial topic models like TopSpace offer mixed-membership mapping of overlapping microenvironments (124). Tools like SPAC enhance spatial single-cell analytics by incorporating segmentation-to-phenotyping pathways that facilitate the merging of image- and molecular-derived data modalities (125). The "tumor microenvironment across four dimensions" paradigm posits that integrating scRNA-seq, spatial omics, temporal dynamics, and modality layering (e.g., epigenome and metabolome) produces a multidimensional atlas that enables the development of predictive biomarkers and geographically targeted therapies (126). This integrated paradigm could transform immunotherapy stratification by moving beyond simplistic "hot vs cold" classifications to create spatial vulnerability maps for each patient, administering checkpoint inhibitors or modulators into specific micro-domains of receptor-ligand imbalances, or selectively targeting stromal "barrier walls" identified through local chromatin-accessibility alterations. The forthcoming challenge involves scaling these integrations across various tumor types, managing batch and alignment noise across modalities, and translating niche-level mechanistic discoveries into spatially resolved therapeutic interventions. However, the rapid advancement of spatial, single-cell, and multi-omic fusion holds the potential to transform tumor-immune heterogeneity from an insurmountable enigma into a clinically actionable spatial atlas.

Systems-biology frameworks linking omic layers to functional immunophenotypes

In an integrative systems-immunology framework, innovative models now correlate high-dimensional omic layers (genome, epigenome, transcriptome, proteome, metabolome, and spatial omics) with emergent immunophenotypes through mechanistic network models, causal inference, and deep learning, elucidating how molecular variation influences cellular immune function and clinical outcomes. The recent mini-review "When Systems Biology Meets Immunology" delineates how network pharmacology, mechanistic modeling, and AI integrate multi-omics data with immune cell phenotyping to predict biomarkers and perturbation responses in the context of autoimmune and infectious diseases (127). One illustration demonstrates that multi-omics insights into immune cells in idiopathic membranous nephropathy connect 731 GWAS-derived immune-cell phenotypes through Mendelian randomization and single-cell transcriptomics, establishing a causal role for $CD39^+$ regulatory T cells (Tregs) in disease risk and prognosis, based on mechanistic gene-protein–metabolic circuits that regulate immune equilibrium (128). In summary, contemporary systems frameworks integrate omic layers into multiscale models, such as linking chromatin accessibility (scATAC-seq) to gene expression (scRNA-seq) and surface proteomics (CITE-seq) within a cohesive latent regulatory network, facilitating the inference of transcription factor–metabolite–cytokine circuits that influence immune cell fate transitions (129). Simultaneously, multi-omics serves as a conduit linking genotype and phenotype, highlighting how integrative pipelines harmonize regulatory, signaling, and metabolic modules to correlate genotype with functional phenotype, particularly in illness scenarios (130), Genomics and multiomics in precision medicine illustrate how a "genomics-first" framework can be systematically enhanced with transcriptomic, epigenomic, proteomic, and metabolomic data to improve molecular-clinical subtyping and immunological stratification (131). At the cohort level, emergent longitudinal meta-cohort initiatives like INSIS utilize "adversomics," the integration of transcriptomics, proteomics, epigenetics, and immunological profiling to delineate immune trajectories and correlate molecular perturbations with unfavorable immunologic outcomes (132). Mechanistically, these frameworks often employ causal network inference (e.g., Bayesian networks, structural equation models), perturbation simulations, and AI-enhanced feature attribution to analyze how a variant in epigenomic regulation leads to changes in gene expression, protein phosphorylation dynamics, metabolic flux alterations, cytokine secretion, and upstream receptor network reconfiguration, ultimately resulting in a specific immunophenotype (e.g., hyperinflammatory vs. tolerogenic). In translational studies of monogenic immune deficiency, multiomics analysis of human RAG deficiency utilized CITE-seq to link genotype to cell lineage-specific transcriptomic and proteomic signatures across varied clinical phenotypes, demonstrating phenomena such as TH2 skewing or type 1 signatures, thereby illustrating how integrative omics elucidate functional immunophenotypes in human disease (133). These frameworks produce clinically relevant insights: they facilitate mechanistic biomarker identification, patient stratification (e.g., responders versus nonresponders), *in silico* perturbation prediction, and the design of immunomodulatory therapies. Challenges persist: guaranteeing data quality and interoperability, preventing overfitting in AI models, experimentally testing mechanistic predictions, and scaling to population cohorts. The integration of mechanistic systems modeling with well-annotated single-cell and geographic omics offers a new generation of prediction immunophenotype maps based on causal molecular biology, linking genotype to functional immune states (Figure 3)(Table 3).

Tumor microenvironment (TME) and immunotherapy responsiveness

Classification of TME phenotypes: Inflamed, excluded, and desert types

The TME immunophenotype can be systematically categorized into a spectrum of inflamed, immune-

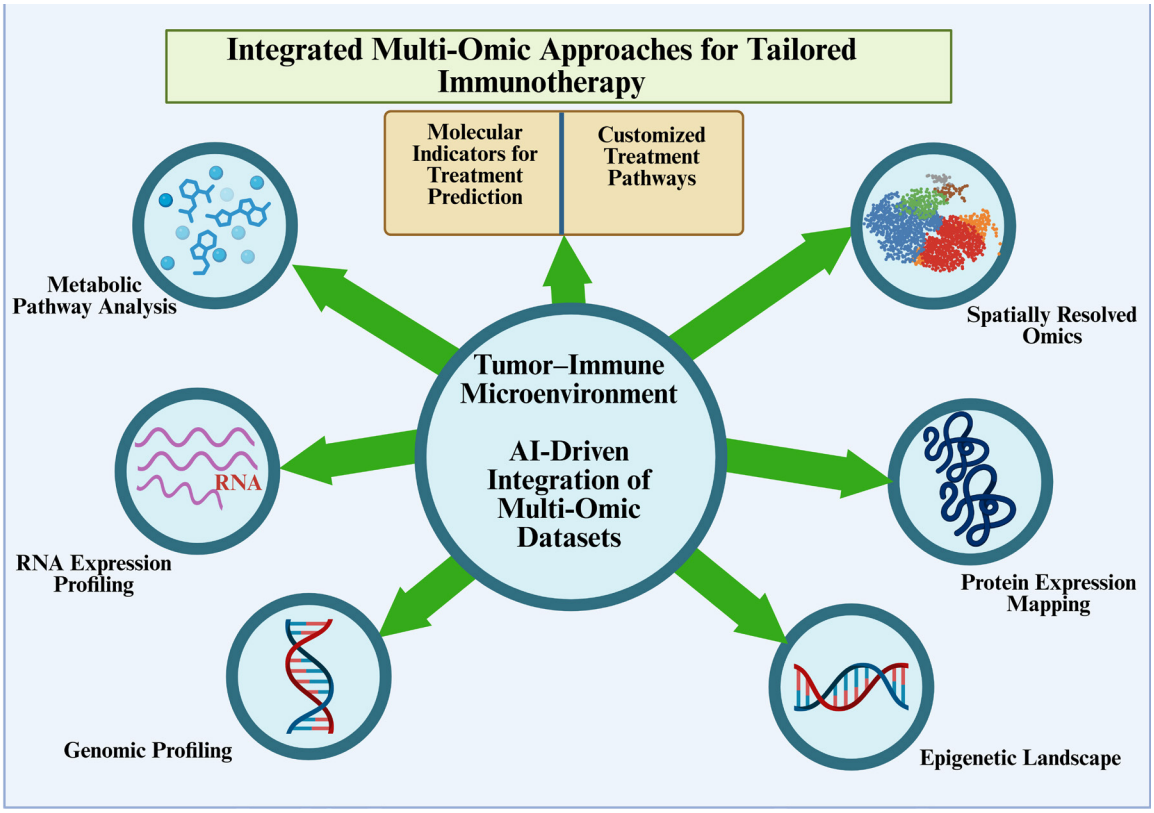

Figure 3. CAI-integrated multi-omic systems framework linking the tumor–immune microenvironment to precision-tailored immunotherapy
This schematic depicts the convergence of six major omic dimensions: genomic, transcriptomic, proteomic, metabolomic, spatial, and epigenomic within an AI-driven analytic core that reconstructs the tumor–immune microenvironment as a dynamic systems network. Genomic profiling defines mutational and neoantigenic architecture; RNA-expression programs reveal immune-checkpoint signaling and interferon responsiveness; proteomic mapping captures cytokine flow and post-translational signaling; metabolic-pathway analysis exposes nutrient-competition and immunosuppressive fluxes (IDO1, arginase, tryptophan–kynurenine axes); spatially resolved omics contextualize cellular neighborhoods and immune-exclusion niches; and epigenetic landscapes delineate methylation and chromatin states governing immune-gene accessibility (134). Artificial-intelligence integration using transformer-based multimodal fusion, graph neural networks, and causal Bayesian modeling merges these heterogeneous layers into unified digital representations of immune phenotypes. The resulting molecular indicators forecast treatment response, toxicity, and resistance trajectories, while customized treatment pathways adapt to evolving tumor–immune feedback (2,95). Conceptually, the figure reframes biomarker discovery as a closed-loop, data-adaptive process in which AI continuously learns cross-omic dependencies to generate patient-specific therapeutic blueprints. This integrated paradigm converts static omic datasets into living, self-updating immunologic atlases, enabling real-time precision immunotherapy in next-generation oncology. This schematic was conceptually designed and illustrated by the author

Table 3. Integrated multi-omic network framework linking genomic, epigenomic, proteomic, spatial, and systems-biology layers to decode NSCLC immunotherapy response and resistance

Omic Axis	Mechanistic Focus	Analytical Tools / Approaches	Clinical / Translational Impact	Advantages	Challenges / Limitations	References
Genomic & Transcriptomic Correlates	Genomic lesions+transcriptomic states co-govern ICI response via antigen presentation, IFN-signaling, and immune-metabolic rewiring	BDVAE deep-learning model; ICBatlas; single-cell+bulk RNA integration; CRISPR-validated EGFR.Sig signature	Predicts responders/non-responders; identifies combinatorial vulnerabilities (e.g., m^{6}A, CAF–immune crosstalk)	Multi-layer precision prediction; mechanistic interpretability	Requires multi-cohort harmonization and large curated datasets	(98)
Epigenomic Reprogramming (DNA Methylation)	Hypermethylation of immune genes (MHC, STING, IFN-pathway) suppresses antigen presentation → immune escape	Single-cell methylomics+HiChIP; DNMT inhibitor+STING agonist synergy modeling	Enables epigenetic-ICI combination strategies; identifies methylation-based resistance biomarkers	Mechanistically reversible immune silencing; therapeutic synergy	Tumor heterogeneity; off-target demethylation; compensatory histone regulation	(135)
Proteomic & Metabolomic Checkpoints	IDO1 / ARG1 axes deplete tryptophan & arginine → T-cell suppression via AHR, mTOR, GCN2 pathways	Spatial proteo-metabolomics; LC–MS/MS Kyn/Trp ratio; AI-based metabolic-network reconstruction	Guides immunometabolic therapy (IDO/TDO/ARG inhibitors, AHR antagonists)	Quantifiable, pathway-specific biomarkers; directly druggable	Dynamic flux adaptation: metabolic compensation across cell types	(136)
Spatial Single-Cell & Multi-Omic Integration	Spatial transcriptomics+scRNA+CITE-seq → map immune-suppressive niches & micro-domain heterogeneity	TopSpace / SPAC toolkits; Bayesian spatial-topic models; multiplex PhenoCycler	Enables niche-level therapeutic targeting & spatial vulnerability mapping	Resolves tumor–immune micro-architecture; links space → function	High computational cost; batch correction & alignment complexity	(137)
Systems-Biology Frameworks	Causal network integration of genome → epigenome → transcriptome → proteome → metabolome → immune phenotype	Multi-omic Bayesian networks; digital-twin immunogenomics; causal inference AI (SCMs, SEMs)	Predicts immunophenotypes & therapy outcomes; supports *in silico* perturbation testing	Mechanistic causality; cross-scale explainability; patient-specific modeling	Data interoperability; experimental validation lag; AI overfitting risk	(138)

excluded, and immune-desert types, each characterized by quantitative, geographical, and functional limitations on T-cell infiltration, and each with distinct consequences for therapeutic efficacy. In the inflamed phenotype, elevated concentrations of $CD8^+$ T cells, $CD4^+$ T helper subsets, NK cells, dendritic cells, and activated macrophages infiltrate the tumor parenchyma and stroma; vigorous chemokine gradients (e.g., CXCL9/CXCL10–CXCR3 axis), type I/II interferon signaling, and functional antigen presentation mechanisms establish a self-sustaining feedback loop through STAT1/JAK/IRF activation and increased MHC-I expression (139)" "excluded," and "cold" types based on the infiltration patterns of $CD8^+$ T cells, which reflect the underlying immune contexture and therapeutic potential. However, many tumors remain resistant to T-cell infiltration, posing a significant barrier to immunotherapy. This review systematically outlines the seven critical steps of the Cancer-Immunity Cycle that govern $CD8^+$ T-cell infiltration: antigen release, antigen processing and presentation, T-cell priming, trafficking through the vasculature, tumor infiltration, target recognition, and cytolytic activity. At each step, tumor-intrinsic and microenvironmental barriers—including low tumor mutational burden, defective antigen-presenting machinery, immunosuppressive cytokines (e.g., TGF-β, IL-10. Immunoregulatory circuits, Tregs, MDSCs, tumor-associated macrophages, and the overexpression of checkpoint ligands (e.g., PD-L1, TIM-3) induce T cell exhaustion or anergy, hence constraining cytotoxic potency (140). Conversely, the immune-excluded phenotype is characterized by a plethora of T cells localized within the stromal septa or invasive margin, hindered from infiltrating tumor nests due to mechanical, physicochemical, and chemoattractant gradient impediments: dense collagen and fibrotic extracellular matrix induced by TGF-β, aberrant vasculature with irregular endothelial adhesion ligand expression, hypoxic and acidic microenvironments with elevated lactate levels, and diminished chemokine gradients (141). Tumor-intrinsic mechanisms, such as constitutive β-catenin signaling or the regulation of CCL4/CCL5 expression, further inhibit dendritic cell recruitment, hence restricting T cell priming and infiltration (142). The immune-desert phenotype is characterized by a marked scarcity of effector T cells in both the stroma and tumor cores, frequently indicative of inadequate tumor antigenicity, impaired cross-priming (e.g., due to dysfunctional Clec9A+ cDC1 maturation), reduced HLA/MHC machinery, or epigenetic silencing of immunogenic gene sets (142). In deserts, immunosuppressive myeloid populations (e.g., mregDCs, Tregs) and soluble mediators (e.g., PGE_2) prevail, perpetuating immunological ignorance. These traits clinically stratify predictive responses to immune checkpoint blockade. Inflamed tumors routinely exhibit elevated response rates (143). Excluded and desert types typically reject monotherapies and necessitate combination modalities to surmount barrier restrictions (144). Emerging AI-based spatial classification methods promise objective and reproducible phenotyping that correlates with patient outcomes (145). Given the continuous nature of these phenotypes and tumor heterogeneity, mechanistic models (e.g., bistable switching dynamics) may elucidate transitions between states under therapeutic pressure, thereby facilitating phenotype-modulating therapies.

Crosstalk between immune and stromal cells (CAFs, TAMs, Tregs)

In the rapidly changing TME, complex bidirectional interactions involving cancer-associated fibroblasts (CAFs), TAMs, and Tregs constitute a molecular core that perpetuates immunosuppression, facilitates tumor advancement, and fosters treatment resistance. Recent advances in single-cell and spatial transcriptomics have delineated co-localization "neighborhoods" in which $CTHRC1^+$ ECM-producing cancer-associated fibroblasts and $SLPI^+$ profibrotic macrophages establish spatial ecotypes at invasive margins, thereby constructing physical and chemical "barriers" to effector T-cell infiltration (146). At a mechanistic level, CAFs influence tumor-associated macrophage (TAM) polarization by secreting the complement fragments C3a and C5a, which activate the C3aR/C5aR pathway in macrophages, leading to downstream phosphorylation of STAT3. This process enhances PD-L1 and IL-10 expression, promoting a shift toward M2-like phenotypes that inhibit antigen presentation (147). Simultaneously, CAF-derived CXCL12 and CCL2 attract monocytes and facilitate their differentiation into immunoregulatory TAMs, while the secretion of TGF-β, colony-stimulating factor-1 (CSF-1), and IL-6 enhances TAM survival and immunosuppressive metabolism by promoting the expression of arginase-1 and indoleamine 2,3-dioxygenase (IDO)(148). TAMs subsequently provide input on CAFs: IL-1β and oncostatin M released by TAMs stimulate fibroblastic JAK/STAT, NF-κB pathways, and epigenetic modifications (e.g., through EZH2) in CAFs, driving them towards a more contractile, extracellular matrix-remodeling phenotype (147, 148). This CAF–TAM loop promotes perivascular ECM rigidity and FAK/Src mechanotransduction, hence hindering T cell invasion and enhancing immunosuppression (148). Tregs are actively recruited and sustained within this immunosuppressive framework. CAFs generate CCL5 and CCL17 gradients that interact with CCR4/CCR5 on Tregs, while simultaneously secreting latent TGF-β (activated by integrin αvβ8) to differentiate naïve $CD4^+$ T cells into induced Tregs (iTregs)(147, 149). Tregs interact with CAFs through TNFSF14–TNFRSF14 signaling in steatotic HCC models, enhancing Treg residency and inhibiting $CD8^+$ T cell activation; blocking this pathway synergizes with anti-PD-1 to reinstate antitumor immunity (150). Intertumoral Tregs demonstrate remarkable metabolic plasticity by utilizing lactate via MCT1, transforming it into pyruvate for oxidative metabolism. This adaptation allows their survival in the glycolytically adverse tumor microenvironment, while simultaneously depleting glucose for effector T cells and fostering immunometabolic competition (151). Tregs also express CTLA-4, which depletes CD80/86 from dendritic cells and tumor-associated macrophages, thereby further inhibiting antigen presentation in cancer-associated fibroblast/tumor-associated macrophage microenvironments (149). Clinically, elevated expression of CAF–TAM–Treg signature modules is associated with diminished immunotherapy response and reduced survival across several cancer populations (152). The innovative finding presented is that the CAF–TAM–Treg triad operates as a spatially self-reinforcing immune suppressive "stromal consortium," wherein paracrine loops, mechanical remodeling, metabolic partitioning, and ligand–receptor

interactions synergistically contribute to the establishment of substantial immunological exclusion. Therapeutically, one could reprogram this consortium by concurrently targeting complement receptor signaling on tumor-associated macrophages, integrin activation of TGF-β, and Treg–CAF contact interfaces (e.g., TNFSF14) in a combinatorial regimen to dismantle the stromal-immune barrier and enhance tumor sensitivity to immunotherapies.

Microbiome–immune–tumor axis in modulating ICI efficacy

Recent findings from 2025 under the developing conceptual framework of the microbiome–immune–tumor axis demonstrate molecular interactions in which commensal and intertumoral bacteria modulate both systemic and local immune responses to influence the efficacy of immune checkpoint inhibitors. Microbial metabolites derived from the gut (e.g., short-chain fatty acids, inosine, secondary bile acids) activate pattern recognition receptors (PRRs) on dendritic cells and macrophages within gut-associated lymphoid tissue, thereby promoting IL-12 and type I IFN production and Th1 polarization. This process extends to tumor-draining lymph nodes and the TME, thereby facilitating the priming of effector CD8^+ T cells and reversing their exhaustion (153). Simultaneously, microbial translocation into the tumor microenvironment (in low-barrier or permeable mucosal contexts) leads to spatially heterogeneous intertumoral microbiota, whose local metabolites create gradients that influence regional immune infiltration and checkpoint ligand expression (154). The concept of the microbiome–immune cell interaction network formalizes this as multi-node signaling webs wherein microbes (bacteria, fungi, viruses) engage directly and indirectly with immune subsets, stimulating antigen-presenting cells, polarizing M1 macrophages, reprogramming regulatory T cells, and myeloid suppressor cells, thereby transforming immunosuppression or "cold" niches into immunogenic "hot" zones (155). In advanced renal cell cancer, antibiotic-induced dysbiosis modified gut microbial composition and resulted in primary resistance to anti–PD-1 therapy (156). In colorectal cancer, the distinct microbiota in mismatch repair–deficient compared to proficient tumors affects immune checkpoint inhibitor response by altering tumor-resident macrophages, CD8^+ T cell infiltration, and MHC I/II expression through SCFA-mediated epigenetic modifications (157). In lung cancer, gut–lung microbial crosstalk via the gut–lung axis shapes systemic inflammation and ICI response heterogeneity (158). At the forefront of translation are precision FMT, engineered probiotics, and spatially targeted phage therapy, which are being developed to reconfigure the microbiome–immune–tumor axis and overcome resistance. This includes employing microbial consortia that enhance beneficial metabolite gradients in "cold" tumor areas while inhibiting suppressive strains in perinecrotic regions. This axis offers a mechanistic and therapeutically applicable framework for future combination immunotherapy strategies.

Targeting TME barriers: Normalization strategies, re-programming macrophages, and vascular modulation

In the advancing field of tumor immunotherapy, a mechanistic, clinically focused approach emphasizes dismantling tumor microenvironment barriers by normalizing vasculature, reprogramming tumor-associated macrophages, and precisely modulating vascular dynamics to enhance synergy with immune and cytotoxic therapies: a convergent "triad" of barrier management. The normalization paradigm aims to eliminate and maintain abnormal, hyperpermeable capillaries to alleviate interstitial pressure, enhance perfusion, and diminish hypoxia, thereby establishing a temporary "normalization window" in which T-cell infiltration and medication delivery are optimized (159). Vascular recalibration requires stringent control; excessive pruning intensifies hypoxia and immunological exclusion, underscoring the importance of dose and timing (160). In conjunction with vascular correction, switching TAMs from pro-tumoral (M2-like) to inflammatory, antigen-presenting (M1-like) phenotypes might alleviate immunosuppression and enhance vessel normalization: Recent 2025 reviews underscore the significance of macrophage plasticity and the necessity for focused therapies due to tumor-associated macrophage heterogeneity (159). Mechanistically, therapies aimed at CSF-1/CSF-1R, PI3Kγ, or epigenetic regulators (e.g., HDAC, BET) alter predominant chemokine and cytokine pathways (e.g., IL-10 to IL-12, CCL22 to CXCL9/10), inhibiting the recruitment of Tregs and myeloid suppressors while promoting CXCR3^+ CD8 T-cell infiltration (152). Vascular modulation may involve the use of controlled nitric oxide (NO) donors, low-dose angiopoietin-2 inhibitors, Tie2 agonists, or pericyte stabilizers, which enhance endothelial-pericyte coupling and restore barrier integrity without compromising flow, thus mitigating leakage and the seeding of metastases (159). The interaction is bidirectional: normalized arteries reduce hypoxia and lactic acid stress, thereby inhibiting hypoxia-induced M2 polarization loops in TAMs. In a groundbreaking advancement, 2025 biomaterial-mediated nanopatterned matrices have been developed to provide spatial gradients of angiogenic inhibitors and macrophage-polarizing signals, facilitating locus-specific microenvironment modulation (e.g., VEGF trap+CSF1R inhibitor in hydrogel scaffolds)(161). Integrative modeling indicates that aligning the peaks of vascular normalization and macrophage reprogramming yields optimal synergy, diminishing tumor burden and improving checkpoint blockade responses in preclinical animals (162). Clinically, biomarker-guided timing (e.g., perfusion MRI, macrophage metabolic imaging) and adaptive dose will be crucial to optimize this trifecta without inducing harmful hypoxia or vascular collapse (Table 4).

Therapeutic personalization strategies

Biomarker-guided checkpoint inhibitor selection (PD-1 vs. CTLA-4 vs. TIGIT, LAG-3, TIM-3)

In a future-oriented framework of precision immuno-oncology, one can anticipate a mechanistic decision tree wherein composite biomarker axes (e.g., spatial PD-L1 density gradient, intratumoral interferon-γ genomics, T-cell exhaustion receptor co-expression maps, and epigenetic chromatin accessibility signatures) inform the optimal selection among PD-1, CTLA-4, TIGIT, LAG-3, or TIM-3 blockade. For instance, when single-cell transcriptomics identifies a predominant progenitor-exhausted $CD8^+$ T subset exhibiting high PD-1 and moderate LAG-3, but negligible TIM-3 co-expression, anti-PD-1 monotherapy may be preferentially used to enhance proliferative self-renewal

Table 4. Multi-dimensional 2025 framework mapping spatial, stromal, microbial, and vascular dynamics of the TME to immunotherapy efficacy and resistance

TME Axis	Core Mechanism	Key 2025 Tools / Approaches	Therapeutic or Predictive Impact	Challenges / Future Needs	References
TME Phenotypes (Inflamed/Excluded/ Desert)	Spatial–functional gradients of immune infiltration shaped by ECM, vasculature, and cytokine milieu	AI-driven spatial classifiers; multiplex IHC+spatial omics	Stratifies ICI responsiveness; guides phenotype-modulating therapies	Overlapping phenotypes; temporal plasticity under therapy	(163)
CAF–TAM–Treg Crosstalk	Paracrine, metabolic, and mechanical loops enforcing immune exclusion and T-cell exhaustion	Single-cell+spatial transcriptomics; ligand–receptor network inference	Identifies combinatorial targets (e.g., C3aR/C5aR, TGF-β, TNFSF14) for stromal reprogramming	Spatial heterogeneity; off-target immune activation risk	(147)
Microbiome–Immune–Tumor Axis	Microbial metabolites (SCFAs, inosine, bile acids) reshape systemic and local immune tone.	Metagenomics+metabolomics; microbial consortia modeling; precision FMT	Enables microbiome-based sensitization to ICI; predictive of resistance	Inter-patient variability; causal directionality unresolved	(153, 164)
TME Barrier Targeting	Vascular normalization+macrophage reprogramming+matrix remodeling synergize for immune access	Perfusion MRI; macrophage-metabolic imaging; biomaterial gradient scaffolds	Enhances drug delivery and T-cell infiltration; prolongs ICI response	Timing of normalization window; risk of hypoxia rebound	(165)

(by inhibiting SHP-2 recruitment to PD-1 ITSM domains). Conversely, in tumors with terminally exhausted tumor-infiltrating lymphocytes co-expressing PD-1^hi TIM-3^+ and demonstrating upregulation of Gal-9 and CEACAM-1 ligands, a PD-1+TIM-3 dual blockade is anticipated to restore Bat3-Lck mediated TCR/CD28 signaling (as evidenced in models of dual PD-1/TIM-3 synergy)(166) the efficacy of ICIs remains limited, with certain cancers exhibiting resistance to these therapeutic approaches. Consequently, several immune checkpoint proteins are presently being thoroughly screened and assessed in both preclinical and clinical studies. Among these candidates, T cell immunoglobulin and mucin-domain containing-3 (TIM-3; likewise, increased TIGIT expression on Tregs and NK cells, along with higher CD155 expression in tumor and myeloid compartments, indicates the potential selection of a TIGIT inhibitor (e.g., tiragolumab in development) to restore the CD8:regulatory ratio and reprogram dendritic cell IL-12 production (167). Conversely, significant CTLA-4/B7 dysregulation in lymphoid aggregates near tumors, characterized by a high neoantigen load and dense antigen-presenting cell niches, may promote CTLA-4 inhibition to improve T-cell priming before tumor engagement. In recent trials, biomarker stratification, such as quantitative multiplex immunofluorescence of co-inhibitory receptors, is being tested to assign patients to CTLA-4 versus PD-1 combinations. The therapeutic significance is substantial: by reducing ineffective exposure to suboptimal checkpoint medicines, this method can diminish immune-related side events, expedite response time, and enhance lasting benefit rates. This represents a transition from a universal checkpoint immunotherapy approach to an adaptive, receptor-network-guided precision immunotherapy, in which the tumor's inhibitory receptor configuration determines the therapeutic strategy.

Rational immunotherapy combinations: Chemotherapy, radiotherapy, targeted therapy, vaccines, and adoptive cell therapy

In the formulation of rational immunotherapy combinations, a mechanistic framework must incorporate the immunogenic modulation elicited by chemotherapy and radiotherapy alongside the precision of targeted agents, the antigenic priming of vaccines, and the effector efficacy of adoptive cell transfer to surmount immune suppression, antigen heterogeneity, and resistance. For example, low-dose cyclophosphamide or oxaliplatin triggers immunogenic cell death through calreticulin exposure, HMGB1 release, and type I interferon responses, which enhance dendritic cell cross-priming and upregulate MHC I and costimulatory molecules on residual tumor cells, thereby synergizing with checkpoint blockade and vaccine-mediated T cell expansion (as reviewed for radio/chemo-immunotherapy synergy) (168); radiation enhances T cell recruitment through the production of CXCL9/10, activation of the STING pathway, and normalization of vasculature, whereas ablative regimens provide an in situ reservoir of tumor antigens to stimulate vaccination responses (meta-analyses indicate comparable safety for ICI combined with stereotactic radiation)(169). Targeted therapies, such as VEGF or receptor tyrosine kinase inhibitors, restructure tumor vasculature and diminish myeloid-derived suppressor cells or regulatory T cells, thereby enhancing effector T cell infiltration and functional persistence, as seen in immuno-oncology and VEGF-targeted therapy combos in renal cell carcinoma (170). Vaccines, particularly tailored neoantigen mRNA or dendritic-cell vaccines, can activate and expand antigen-specific T-cell clones, which, when administered sequentially before adoptive cell transfer, diminish antigen escape and exhaustion (171). Adoptive cell therapy (ACT), encompassing CAR-T, TIL, or TCR-engineered T cells, is enhanced when integrated with preceding modalities: preconditioning through chemotherapy or radiotherapy creates niches. It diminishes endogenous regulatory cells, while vaccines provide antigenic stimulation following transfer to facilitate clonal expansion and memory formation, thereby alleviating exhaustion and evasion within the tumor microenvironment (172). Neoadjuvant chemoimmunotherapy for limited-stage small cell lung cancer yields a pooled histological complete response rate of approximately 35%, with a tolerable incidence of serious adverse events of 44% (173). Meta-analyses of metastatic prostate cancer have shown a survival benefit for immunotherapy plus other modalities (174). The primary novelty lies not in mere additive therapy but in temporal orchestration: adjusting dose, antigenic scheduling, tumor microenvironment manipulation, and cell transfer timing to convert immunosuppressive habitats into immunogenic ecosystems, thus attaining sustained responses while reducing toxicity.

Temporal sequencing and adaptive treatment algorithms based on dynamic biomarkers

In a time when static biomarkers are inadequate,

an integrated temporal-sequencing framework utilizes dynamic biomarkers in real time to guide adaptive therapy algorithms, thereby bridging the gap between changing pathophysiology and precise intervention. This framework facilitates serial assessment of circulating cell-free DNA (cfDNA) structural variant burdens using CloneSeq-SV, enabling high-resolution monitoring of clonal evolution across therapy cycles in high-grade serous ovarian carcinoma. It uncovers the emergence of resistant clones weeks before radiographic relapse, thereby endorsing algorithmic intervention adjustments during treatment (175). Additionally, high-dimensional single-cell immune phenotyping demonstrates that alterations in peripheral T-cell receptor clonality, exhaustion signature scores, and cytokine modules are associated with emerging resistance to immunotherapy, facilitating predictive indicators for the escalation or de-escalation of checkpoint inhibitors (176). Adaptive algorithms may incorporate mathematically derived biomarkers, such as the ΔAT (delta adaptive therapy) score or eTTP (expected time to progression), derived from initial-cycle longitudinal data, to forecast which patients are more likely to achieve optimal benefit from evolution-aware dosing schedules compared with standard continuous regimens (177). Machine learning-driven integrative models (e.g., ABF-CatBoost) amalgamate temporal omics features such as transcriptomic slope variations, methylation drift, and proteomic flux with pharmacokinetic/pharmacodynamic (PK/PD) models to predict forthcoming resistance windows and suggest therapeutic adjustments *in silico* before clinical failure (178). Clinically, these temporal sequencing methods can reduce the "decision latency window" from months to days, thereby transforming lead biomarkers into actionable modifications to combination therapies, supplementary medications, or medication holidays. This paradigm proposes a conceptual advance: treatment regimens are transformed into closed-loop control systems, in which biomarker sensors provide feedback to optimization engines that dynamically adjust therapy, thereby proactively countering resistance rather than merely responding to it.

Case-based personalization frameworks from clinical trials (KEYNOTE, CheckMate, IMpower)

In the advancing field of precision immuno-oncology, case-based personalization frameworks grounded in pivotal trials such as KEYNOTE, CheckMate, and IMpower require mechanistic foundations to move beyond population averages and achieve true N=1 customization. For instance, retrospective biomarker sub-analyses of KEYNOTE-426 revealed that elevated T-cell-inflamed gene expression profiles, rather than PD-L1 IHC alone, significantly correlate with enhanced progression-free survival (PFS) and overall survival (OS) in the pembrolizumab/axitinib cohort (HR≈0.69) by modulating interferon-γ–STAT1–CXCL9/10 chemokine pathways that attract CXCR3+ CD8+ T cells (179). AI-driven predictive biomarker models, such as PBMF contrastive frameworks, effectively categorize responders in early-phase KEYNOTE trial arms by using multi-omic latent representations, hence facilitating case-level probability scores for individual enrollment assignment (178). The CheckMate family, encompassing CheckMate 214 in renal cell carcinoma and melanoma cohorts, endorses classification based on tumor mutational burden and gut microbiota composition: Mechanistic investigations have demonstrated that polysaccharide A produced from Bacteroides activates STING-IFN signaling and enhances cross-presentation, hence augmenting the efficacy of CTLA-4 and PD-1 dual blocking in responsive subjects (180). Concurrently, IMpower (e.g., IMpower150/IMpower130) cohorts provide modular customization by integrating VEGF/Angiogenesis signatures: elevated angiogenic gene sets (VEGFA, ANGPT2) predict enhanced efficacy of atezolizumab combined with bevacizumab, facilitated by vascular normalization and augmented T-cell infiltration (181)yet challenges remain. In ovarian cancer, the evolution of poly (ADP-ribose. A case-based personalization workflow comprises: 1) multi-omic patient preprofiling (e.g., transcriptome, tumor mutational burden, microbiome), 2) trial-specific probabilistic scoring utilizing AI classifiers, 3) dynamic modification of the immunotherapy backbone (checkpoint monotherapy, IO+VEGF, IO+TKI) informed by mechanistic feedback loops, and 4) real-time feedback through circulating tumor DNA and spatial immune imaging to facilitate treatment modulation. These frameworks enhance trial enrollment to a precision level, providing personalized switch rules and adaptive escalation at the case level, representing the next advancement in immunotherapy trial design (Table 5).

Table 5. AI-driven multi-omic integration transforms non-small cell lung cancer (NSCLC) immunotherapy into dynamic, continuously learning precision medicine

Subsection	Core Mechanistic Concept	Analytical /Computational Approaches	Clinical / Translational Significance	Advantages	Challenges / Limitations	References
Biomarker-Guided Checkpoint Selection	Composite biomarker axes (PD-L1 gradient, IFN-γ genomics, exhaustion-receptor maps, chromatin accessibility) determine PD-1, CTLA-4, TIGIT, LAG-3, and TIM-3 blockade choice	Single-cell transcriptomics, multiplex immunofluorescence, receptor co-expression profiling	Matches checkpoint to inhibitory topology; reduces toxicity; enhances durable benefit	Adaptive receptor-network logic; precision patient stratification	Real-time biomarker measurement; incomplete inhibitory-axis coverage	(182, 183)
Rational Immunotherapy Combinations	Integration of chemo-/radio-/targeted therapy, vaccines, and ACT to exploit immunogenic cell death and vascular remodeling	Radiomic immunomic fusion, STING-pathway modeling, temporal dosing synchronization	Converts immune-excluded tumors to an inflamed state; achieves durable synergistic responses	Spatiotemporal synergy; improved infiltration and antigen presentation	Scheduling complexity, toxicity, and pharmacokinetic variability	(168)
Temporal Sequencing & Adaptive Algorithms	Dynamic biomarkers (cfDNA, TCR clonality, cytokine shifts) steer treatment adaptation in real time	CloneSeq-SV, ABF-CatBoost, ΔAT & eTTP indices, PK/PD fusion models	Enables closed-loop control; anticipates resistance; optimizes timing	Evolution-aware precision; rapid feedback	Costly longitudinal data; regulatory approval lag	(184)
Case-Based Personalization Frameworks	Multi-omic patient profiling combined with trial-specific AI models for individualized therapy design	PBMF contrastive AI, federated learning on KEYNOTE/CheckMate /IMpower datasets	N=1 trial allocation; dynamic switching of IO+VEGF/TKI regimens	Trial-level adaptivity; mechanistic enrichment of enrollment	Data-privacy barriers; assay standardization needs	(178, 185)

Future perspectives: AI-driven precision immuno-oncology

AI-powered biomarker discovery and data fusion

In NSCLC, the traditional dependence on PD-L1 immunohistochemical scores for patient stratification in anti-PD-1-PD-1/PD-L1 therapy is increasingly regarded as mechanistically inadequate. This has prompted a new paradigm where AI-driven biomarker discovery integrates spatial, genomic, transcriptomic, proteomic, and metabolomic data to produce mechanistically interpretable predictors of therapeutic response. Recent advancements in computational pathology, exemplified by the HistoTME deep learning model trained on multiplexed immunofluorescence and digital histology, can already predict TME cell-type deconvolution from standard slides and anticipate immune checkpoint inhibitor (ICI) responses beyond PD-L1 alone (10). In conjunction with multi-omic profiling, the Frontiers study on unresectable NSCLC employed integrative proteomic and metabolomic assays alongside machine learning techniques (random forest and logistic regression) to identify 5-sulfooxymethylfurfural, anthranilic acid, IGHV1-45, and MFAP4 as a composite set of liquid biomarkers indicative of immunotherapy responsiveness (186), or the BCAA-metabolism signature (including HMGCS1) identified in plasma and transcriptomes as prognostic in immunotherapy cohorts (187). These modalities enable AI models to deduce functional axes of resistance and sensitivity, such as immunometabolism, antigen presentation, and interferon responses. In addition to single-modal predictors, techniques like cross-modality masked learning have integrated CT imaging and clinical tabular data to enhance survival predictions in ICI-treated NSCLC patients, resulting in enhanced c-index and intermodality resilience (188). Diffusion-based generative models, such as ImmunoDiff, simulate post-treatment CT phenotypes based on baseline imaging, clinical variables, and PD-L1, facilitating the prediction of response trajectories instead of static labels (2). Spatial multi-omic frameworks currently extract cell-state phenotypes (e.g., immune-exhausted T cells, TLS B cell niches) from spatial proteogenomic maps, then consolidate them into transcriptome-derived gene modules whose local density corresponds with checkpoint responsiveness across tumors (2). Mechanistically unraveling latent features is now achievable through semi-supervised architectures such as the Biologically Disentangled Variational Autoencoder (BDVAE), which encodes pathway-specific latent variables (immune, metabolic, genomic) that correlate with resistance gradients rather than binary response states, providing interpretable axes to inform therapeutic modulation across pan-cancer cohorts (15). A prognostic multi-omic signature for NSCLC was developed by integrating multi-omic phenotypes associated with ICI benefit via Bayesian fusion and survival modeling (189). An integrated investigation of LUAD utilizing scRNA-seq, bulk RNA, and GWAS deconvolution revealed different immune-rich subtypes (CS2) exhibiting increased susceptibility to immune checkpoint inhibitors and specific important biomarkers (e.g., RRM1) within the multiomic signature (190). At the metabolic immunoregulation level, multi-omics identified G0S2, a lipid metabolism regulator in Tregs, as a prognostic target in NSCLC through Treg-specific metabolic gene models (191). In treatment allocation, machine learning models have included clinicogenomic data to enhance the decision-making process between ICI monotherapy and ICI-chemotherapy combinations by simulating treatment effect heterogeneity (e.g., recognizing APC, FBXW7) beyond PD-L1 thresholds (12). In conclusion, the future of NSCLC immunotherapy is not dependent on a singular PD-L1 threshold but rather on an AI-driven integration of multi-omic and imaging modalities, producing biomarker frameworks that are predictive, interpretable, and clinically actionable through the modulation of resistance circuits (metabolic, antigenic, microenvironmental), thereby facilitating personalized immunotherapy regimens customized for each tumor's inherent vulnerabilities.

Real-time immunomonitoring and next-gen targets

In the evolving landscape of NSCLC immunotherapy, dynamic immunomonitoring offers greater clinical granularity than static PD-L1 assessment by incorporating serial multi-omic, spatial, and radiomic readouts to support adaptive treatment strategies. This includes the use of longitudinal plasma metabolome–proteome correlation patterns, such as 5-sulfooxymethylfurfural–IgHV1-45 signaling axes, to track PD-1/PD-L1 pathway activity and emerging resistance mechanisms in unresectable NSCLC (10). Complementary approaches leverage single-cell profiling to map temporal transitions in tumor-infiltrating lymphocyte states, capturing exhaustion-to-activation dynamics through markers such as TCF1, TOX, TIM-3, LAG-3, and $CD8^+$ TCR clonotype remodeling (192). Additionally, radiomic–tumor immune microenvironment inference frameworks show promise as noninvasive tools to estimate immune infiltration and spatial heterogeneity under treatment pressure (computational and emerging translational evidence)(10, 192, 193). This data is situated within a multi-omic tumor microenvironment map that elucidates the plasticity of immunosuppressive cancer-associated fibroblasts, tumor-associated macrophages, and activated cancer-associated fibroblasts, as well as hypoxic niches that are resistant to immune checkpoint blockade (194). The convergence of these modalities facilitates real-time feedback loops, in which an early proteo-metabolomic "escape signature" triggers a transition to co-stimulatory agonists (e.g., anti-4-1BB) or targeted myeloid reprogramming (e.g., CSF1R blockade), thereby transforming non-responders into responders *in silico*. This paradigm aims to optimize adaptive biomarker-guided salvage treatments, enhancing patient classification, reducing overtreatment, and optimizing sustained responses in NSCLC, moving beyond dependence on baseline PD-L1 alone.

Toward immune digital twins and ethical integration

Innovative frameworks for immune digital twins are now designed to incorporate mechanistic tumor-immune co-dynamics tailored to individual patients via multi-omic, spatial, and clinical time-series data, surpassing static PD-L1 thresholds. These dynamic digital twins perpetually integrate evolving mutation, transcriptomic, proteomic, metabolomic, and imaging signals to model virtual immunotherapy trials and forecast T cell-tumor interactions, neoantigen drift, spatial niche development, and resistance trajectories (195). In NSCLC, integration of spatial proteogenomic signatures (2), BCAA-metabolism plasma signatures (186), peripheral metabolite–protein predictors (10), and single-cell stem cell

signatures (196) facilitates multiscale anchoring of virtual twin submodules. The twin models mechanistically address coupled differential equations about cytokine diffusion, immune checkpoint receptor-ligand binding kinetics, clonal TCR expansion, and metabolic competition within the microenvironment, all limited by Bayesian priors derived from BDVAE-style disentangled latent modeling (15). These *in silico* twins can recommend personalized immunotherapy protocols (monotherapy, chemotherapy combined with immune checkpoint inhibitors, or viral adjuvants) and predict potential side effects. Incorporating interpretable limitations, audit trails, and consented patient updates into dual architectures enhances accountability and equity in decision support (197). These immunological twins may clinically stratify NSCLC patients who, despite low PD-L1 levels, possess latent, responsive multi-omic profiles, thereby informing off-label ICI utilization or escalation while maintaining human oversight and ethical transparency in practical applications.

Future directions and conceptual frameworks

Digital twins and adaptive immunotherapy

In the evolving framework of NSCLC precision immunotherapy, a mechanistically informed integration of dynamic digital twin models and adaptive immunotherapy guidance aims to surpass static PD-L1 stratification by incorporating multi-omic, spatial, and temporal dimensions into a patient-specific virtual representation: Digital twin approaches in oncology are being explored conceptually to enable real-time calibration of tumor–immune dynamics through multimodal data sources; however, their use in simulating treatment adjustments remains experimental and requires clinical validation (195). Building on the conceptual foundations of virtual patient and QSP-IO modeling in immuno-oncology (198). Mechanistic submodels of antigen presentation, T cell fatigue, cytokine feedback loops, spatial immunological exclusion, and neoantigen drift can be encoded and dynamically interconnected via differential equations and probabilistic switches that regulate the simulation of clonal evolution. In NSCLC, multi-omic biomarker investigations have identified metabolites (e.g., 5-sulfooxymethylfurfural, anthranilic acid) and proteins (e.g., IGHV1-45, MFAP4) that, when combined, produced an area under the curve of approximately 0.955 for predicting sensitivity to immune checkpoint inhibitors (10); spatial multi-omics signatures (proteomic compartments+transcriptomic neighborhoods) further stratified responders in three NSCLC cohorts (2). A 23-gene prognostic profile encompassing various programmed cell death pathways (apoptosis, ferroptosis, necroptosis, pyroptosis) and organelle dysfunction is also associated with immune infiltration and immunotherapy response in NSCLC (9). Digital twin models have been proposed to mechanistically simulate how perturbations in PD-1/PD-L1 blockade may affect metabolic and immune dynamics, although such applications remain theoretical (15). Hybrid digital twin–adaptive immunotherapy frameworks have been suggested as a potential model for '*in silico* test dosing,' but this concept remains exploratory. As prognostic models using cross-modality masked learning (187) and generative immunotherapy response models (188), as they mature, they may be integrated into a perpetually updated twin, with the long-term possibility of patient-specific virtual simulations contributing to adaptive immunotherapy decisions, pending prospective validation and real-world feasibility.

Explainable and federated intelligence

In an advanced framework of precision immuno-oncology for NSCLC, explainable federated intelligence can systematically integrate classical single-biomarker stratification (e.g., PD-L1 immunohistochemistry) with dynamic multi-omic predictors, thereby facilitating interpretability and privacy-preserving learning across institutions. Federated learning architectures have been explored as approaches to decentralized model training, although their routine implementation remains in development. This is illustrated in NSCLC prognostic risk modeling, where robust federated learning (RFed) achieves cross-center AUCs exceeding 0.90 for postoperative progression prediction (199). This federated framework enables the integration of explainable AI modules (e.g., SHAP, counterfactual attribution, pathway-guided attention) to elucidate how multi-omic features (e.g., neoantigen load, T cell receptor clonality, cytokine gene-expression signatures, methylation of immune checkpoints) influence the anticipated efficacy of immunotherapy. Integrated explainable machine learning, in conjunction with multi-omics data in immunotherapy cohorts, has revealed gene modules that interact with the PD-1/PD-L1 axis and improved survival predictions beyond those based solely on PD-L1 (200). In NSCLC, machine-learning methodologies such as A-STEP (attention-based scoring for treatment effect prediction) explicitly characterize heterogeneous treatment effects and incorporate variables such as FBXW7, APC, and PD-L1 to inform selection between ICI monotherapy and chemoimmunotherapy, achieving a hazard ratio of approximately 0.60 for progression-free survival (12). Furthermore, explainable machine learning applied to NSCLC cohorts has now begun to outperform PD-L1 as a standalone predictive marker for treatment benefit, demonstrating stronger discrimination for both progression-free and overall survival and reinforcing its emerging relevance in guiding therapeutic decision-making (201). Moreover, multimodal deep learning frameworks that integrate histopathological, genomic, and clinical information in NSCLC have demonstrated enhanced predictive capacity and generate interpretable attention maps that highlight biologically meaningful regions and molecular drivers linked to therapeutic response (202). To advance further, one might incorporate biologically disentangled multi-omic latent models (e.g., BDVAE) that delineate pathway-specific modules (immune, metabolic, genomic) with interpretable latent axes that predict the continuum of resistance (e.g., metabolic exhaustion, immune suppression) across various cancer types, including lung cancer (15). In a federated, explainable system, clinicians might examine per-patient contributing features (e.g., methylation at the PD-L1 promoter, interferon gamma signature, TCR clonality, imaging texture) and compare alternative treatment pathways. Such architectures have been proposed as a pathway toward future patient-specific computational representations, though this remains conceptual rather than clinically established. This analytical approach has demonstrated potential for predicting treatment-response patterns in preliminary

modeling studies, but clinical deployment will require further validation. This model might clinically stratify borderline PD-L1 expressors, suggest combination tactics, and evolve over time through federated constant learning, while maintaining patient privacy and interpretability. Overall, these concepts should be considered exploratory and remain at a theoretical or early development stage. Their translation into routine NSCLC clinical practice will require extensive validation, multi-institutional evidence, regulatory approval, and demonstration of clinical utility beyond existing biomarker frameworks.

Challenges, limitations, and ethical considerations

Despite the rapidly growing interest in AI-driven biomarker discovery and digital twin frameworks in NSCLC immunotherapy, several constraints must be acknowledged before these technologies can be meaningfully translated into clinical practice (197). Current AI models are highly dependent on training data structure and availability, and many datasets exhibit demographic, molecular, or institutional imbalance, which may reinforce inequities in treatment response prediction (203). For example, underrepresentation of early-stage disease, ethnic minorities, immunotherapy nonresponders, or rare actionable subgroups may introduce latent bias that remains invisible during development but emerges during clinical deployment. Moreover, multimodal deep architectures frequently operate as "black boxes," creating interpretability gaps that limit clinician trust, hinder mechanistic understanding, and restrict the ability to justify treatment recommendations in evidence-based medicine (34). From a regulatory perspective, most existing AI systems and digital twin proposals have not undergone the level of analytical validation, external reproducibility testing, or prospective trial evaluation required for clinical approval under frameworks such as FDA SaMD, EMA AI-Act, or emerging ISO standards for trustworthy machine learning (204). Even if performance accuracy appears favorable in retrospective cohorts, the absence of standardized validation pipelines, cross-platform reproducibility benchmarks, and harmonized reporting criteria remains a major barrier to adoption (205). Additionally, longitudinal digital twin deployment introduces computational governance challenges, including model drift monitoring, real-time recalibration, and clearly defined responsibilities when algorithm-guided decisions diverge from physician judgment. Ethical considerations further complicate implementation (138). Generating, updating, and federating patient-specific digital avatars requires handling deeply sensitive genomic, immunologic, and behavioral data. Questions remain unresolved regarding digital identity ownership, consent for model re-use beyond the originating institution, long-term storage of patient-specific computational profiles, and the ethical implications of predictive labeling for disease progression or therapeutic resistance (206). Privacy-preserving strategies such as homomorphic encryption, secure multiparty computation, and federated learning have shown promise, yet practical deployment at scale remains limited and untested in heterogeneous healthcare systems (206).

While AI-driven immunotherapy modeling and digital twin systems represent an innovative direction for precision oncology, their clinical implementation remains preliminary. Key challenges, including data heterogeneity, interpretability limitations, regulatory uncertainty, and lack of prospective multi-center validation, must be addressed before routine deployment. In addition, publication bias and overrepresentation of positive modeling outcomes may inflate perceptions of readiness, as negative or inconclusive findings are rarely reported. Current evidence, therefore, reflects an evolving research stage rather than a clinically established framework. Lessons Learned: AI-based models and digital immune simulations show promise for predicting treatment response and guiding adaptive therapy. However, most remain exploratory due to data imbalance, limited interpretability, and insufficient clinical validation. With future regulatory alignment and federated learning infrastructure, these tools may progress toward meaningful integration in clinical immuno-oncology.

Translational outlook and implementation pathway

While the frameworks discussed in this review illustrate the long-term potential of digital immune twins and AI-integrated biomarker systems, their transition from conceptual innovation to clinical practice will require phased and evidence-based implementation (207). Initial deployment is expected within controlled research and clinical trial settings, where digital simulations can function as decision-support layers rather than replacements for established diagnostic criteria or clinician judgment (207). Practical integration will depend on alignment with existing infrastructures such as liquid biopsy workflows, spatial profiling platforms, radiomics pipelines, and electronic health record systems to enable real-time data ingestion, longitudinal immune tracking, and adaptive therapy modeling (208). In parallel, regulatory readiness remains essential. Harmonized interoperability standards, model explainability requirements, data harmonization protocols, and federated learning frameworks will be necessary to ensure safety, reproducibility, transparency, and alignment with ethical governance policies (208). As multi-center prospective trials, real-world evidence studies, and cross-platform benchmarking mature, digital immune-twin systems may gradually evolve from investigational research prototypes into clinically supported tools that complement established biomarker-driven decision pathways in precision immuno-oncology (209). Lessons Learned: AI-driven models and digital immune simulations demonstrate strong promise for enhancing treatment prediction, identifying resistance patterns, and supporting adaptive immunotherapy strategies. However, these capabilities currently remain exploratory and require prospective validation, regulatory alignment, and standardized deployment frameworks before they can be considered for routine clinical use.

Discussion

This review highlights the rapidly evolving landscape of biomarker-driven and AI-supported immunotherapy stratification in NSCLC. While several molecular and liquid biopsy biomarkers demonstrate strong mechanistic and translational potential, the level of supporting evidence varies widely. Clinically validated biomarkers such as tumor mutational burden, ctDNA kinetics, and TCR repertoire diversity currently have the strongest real-world and trial-based support and are most closely aligned with patient

outcome prediction. In contrast, emerging biomarker candidates, including exosomal PD-L1, spatial omics–based signatures, and metabolic immune checkpoints, remain largely at a preclinical or early translational stage. AI and computational modeling frameworks, including digital immune twins, predictive machine-learning classifiers, and multimodal fusion models, represent a novel dimension in NSCLC precision medicine (computational/model-based evidence). These tools show promise for forecasting treatment response, resistance trajectories, and adaptive dosing strategies; however, most of these models remain hypothesis-level or simulation-based and require rigorous clinical validation. The integration of computational predictions with biological and clinical evidence will be essential to avoid premature implementation and ensure patient safety. Overall, the strength of evidence across the reviewed domains remains uneven, and findings should be interpreted with caution. Computational predictions and preclinical observations provide valuable mechanistic insight but cannot yet replace clinically validated biomarkers in decision-making. Harmonized clinical trial frameworks and prospective validation studies will be necessary to transition emerging biomarkers and AI-driven platforms into routine clinical use for NSCLC immunotherapy personalization. To further clarify the relative maturity and validation status of the findings discussed in this review, a structured evidence-level summary is provided in Table 6.

Conclusion

The landscape of immunotherapy in NSCLC is transitioning from dependence on single static biomarkers, such as PD-L1, toward an integrated framework informed by multi-omic profiling, spatial biology, and increasingly sophisticated computational modeling. Rather than functioning as a binary determinant of eligibility, PD-L1 is now understood as a dynamic and context-dependent signal shaped by tumor microenvironmental pressures, inflammatory cues, metabolic rewiring, and treatment exposure. This evolution reflects a broader shift in precision oncology from descriptive association toward mechanistic interpretation supported by spatial technologies, liquid biopsy platforms, and emerging machine-learning frameworks. AI–enabled tools, including multimodal fusion models, explainable machine learning, and digital immune twin proposals, represent promising avenues for simulating immune–tumor interactions, forecasting therapeutic response, and informing adaptive immunotherapy strategies. While early modeling studies and retrospective analyses suggest the potential value of these approaches, their clinical translation remains preliminary. Current evidence is limited by variability across datasets, the lack of prospective validation, and the absence of harmonized standards for reproducibility, regulatory compliance, and integration into clinical workflows. Accordingly, these computational approaches should be viewed as emerging complements rather than replacements to validated clinical biomarkers such as tumor mutational burden, T-cell receptor repertoire metrics, and circulating tumor DNA dynamics. Moving forward, efforts to operationalize biomarker-guided immunotherapy in NSCLC will require rigorous multi-institutional validation, prospective real-world evidence, and carefully designed

Table 6. Evidence levels supporting PD-L1-associated biomarkers and computational models in non-small cell lung cancer (NSCLC) immunotherapy

Biomarker / Approach	Strengths (Clinical Value)	Limitations	Current Evidence Type & Validation Status	Translational Readiness	References
PD-L1 Expression (tissue-based IHC)	Widely available; approved companion diagnostic; correlates with ICI response in subsets	High assay variability (22C3 vs SP142); temporal and spatial heterogeneity; threshold uncertainty	Clinical evidence; validated in multiple trials	High (established)	(210)
Tumor Mutational Burden (TMB)	Reflects neoantigen potential; improves predictive ability when combined with PD-L1	Not predictive alone; inconsistent cutoffs across platforms; assay cost	Clinical + computational evidence; partial validation	Moderate–High (conditional)	(189)
Exosomal / Soluble PD-L1	Non-invasive; reflects systemic immunosuppression and dynamic immune response	No standardized isolation or quantification method; high variability	Emerging translational evidence; early cohorts only	Moderate (pending validation)	(211)
ctDNA Dynamics	Enables real-time monitoring of treatment response and resistance evolution	Dependent on tumor shedding, may miss low-volume disease	Clinical evidence (increasing trial validation)	High (trial-ready)	(211)
TCR Repertoire Diversity / Clonality	Captures immune activation and ICI-associated clonal expansion	Lack of universal scoring metrics; complex interpretation	Translational + computational evidence	Moderate	(12)
Spatial Multi-omics / Multiplex Profiling	Captures immune micro-architecture; identifies immunosuppressive niches	Expensive; limited standardization; restricted clinical availability	Hybrid evidence (preclinical + emerging clinical)	Moderate (research-phase)	(2)
Multi-omic Composite Signatures	Higher accuracy than single biomarkers; integrates immune, genomic, spatial, and metabolic layers	Requires harmonized pipelines and large datasets; reproducibility challenges	Emerging clinical + computational evidence	Moderate–High (pending large validation)	(189)
AI-Driven Predictive Modeling (radiomics, digital twins, deep learning)	Enables adaptive prediction, response modeling, and personalization	Requires large datasets, explainability, regulatory approval, and dataset bias	Computational / simulation evidence; limited prospective validation	Low → Future-High	(12)

regulatory pathways to ensure interpretability, safety, and equitable implementation. As biomarker science, systems immunology, and AI converge, the field is poised to evolve toward increasingly adaptive, patient-specific treatment paradigms, but realizing this potential will depend on bridging the gap between conceptual frameworks and demonstrated clinical utility. These findings should be interpreted in the context of evidence tiering, as preclinical and computational observations remain exploratory until validated in clinical settings.

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Availability of Data and Materials

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Authors' Contributions

HJA A conceived the study; Q A and HJA A developed the methodology; Z B and MM R conducted formal analysis; M B, S S, and V A performed data curation; A S and A K conducted investigation; HJA A provided resources; Z B and HJA A supervised; Q A and HJA A prepared the original draft; Q A illustrate the figures; all authors reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no known competing financial or personal interests that could have influenced the work reported in this paper.

Declaration

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