Post-translational modification-governed immune states in cancer immunity: Biomarker implications for checkpoint competence, tumor visibility and immunotherapy resistance (Review)

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Abstract. Immune escape and therapeutic resistance remain major obstacles to durable benefit from cancer immunotherapy, yet transcript-based or abundance-based biomarkers often fail to capture the regulatory states that determine effective immune control. Post-translational modifications (PTMs) form a dynamic protein-state layer that rapidly reshapes protein stability, trafficking, complex assembly, and signaling persistence under tumor-intrinsic and therapy-imposed stress. In the present review, a biomarker-oriented framework is proposed in which PTM biology is interpreted through three recurrent immune constraints: Checkpoint competence, tumor visibility and stress-conditioned immune-state programming. Within this framework, programmed death-ligand 1 is viewed as a protein-state biomarker problem rather than a static expression marker; tumor visibility is defined by durable antigen-presentation competence and interferon-linked reinforcement; and stress-driven immune dysfunction is interpreted through metabolite-sensitive PTM rewiring and chromatin-coupled suppressive stabilization. Rather than cataloguing PTMs comprehensively in cancer immunity, the present review focuses on five core exemplar PTM axes, glycosylation, palmitoylation, ubiquitin editing, phosphorylation and lactylation, because they repeatedly map to rate-limiting immune constraints, are supported by mechanistic evidence, and represent candidate assay-compatible or intervention-relevant state variables at differing levels of translational maturity. It is further outlined how integrated proteogenomic, immuno-peptidomic, and spatial datasets can be used to discover candidate PTM-state biomarkers, validate mechanism-proximal readouts in prespecified pretreatment and on-treatment settings, and prioritize single or co-dominant state constraints for patient stratification, pharmacodynamic monitoring, and rational combination design. By organizing PTM biology around measurable state variables rather than modification class alone, the present review provides a phase-aware translational framework for candidate biomarker discovery, fit-for-purpose validation, constraint-guided stratification, and therapeutic prioritization in cancer immunotherapy.

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1. Introduction

Cancer immunotherapy and multimodal regimens can yield durable benefit in selected patients, yet immune escape and

therapeutic resistance remain major causes of progression (1-3). Across clinical settings, treatment failure often reflects disruption of a limited set of rate-limiting processes required for sustained immune control, particularly checkpoint competence, tumor visibility, and the maintenance of productive effector function (2). Response heterogeneity is frequently discordant with transcript-level immune activation, and resistance can arise early during therapy despite limited genomic change, indicating the involvement of regulatory layers that act on short timescales, encode microenvironmental context, and directly tune protein fate and signaling kinetics (4). Post-translational modifications (PTMs) represent a major such layer because they regulate protein stability, trafficking, subcellular localization, interaction networks and signal duration, thereby converting tumor-intrinsic stress and therapy-imposed selective pressure into adaptive immune phenotypes and resistant states (5).

Although PTM-focused literature is extensive, most syntheses remain organized either by modification class or by individual targets, which can fragment immune regulation into parallel mechanisms and obscure the shared functional constraints that ultimately determine therapeutic response (6). In the present review, PTM biology was instead interpreted through three recurrent immune constraints that limit durable antitumor immunity: Checkpoint competence, tumor visibility and stress-conditioned immune-state programming (7-11). This framework allows mechanistically distinct PTM events to be understood through convergent functional outputs and more directly links molecular regulation to biomarker logic and therapeutic resistance.

A central premise of the present review is that PTMs rarely function as isolated switches. Rather, combinatorial modification states and PTM crosstalk shape coupled changes in protein stability, localization, complex assembly and signaling persistence (5). Focus was therefore addressed on exemplar circuits in which the evidence chain is strongest and the translational implications are clearest. Programmed death-ligand 1 (PD-L1) provides a paradigm for this logic, as glycosylation, palmitoylation, and ubiquitination or deubiquitination converge on a shared functional endpoint: Stabilization of a membrane-resident suppressive state that is not captured by expression alone (7,8,12-16). Therapy-stress-induced PTM rewiring as an early resistance mechanism was also emphasized, because PTM networks can be selected during treatment and subsequently stabilized across treatment-naïve, on-treatment, and relapse phases (2,5).

The present review is therefore not intended to catalogue PTMs comprehensively across cancer immunity. Instead, it addresses a narrower translational question: Which PTM-governed protein states most directly shape checkpoint competence, tumor visibility, and stress-conditioned immune-state programming, and which of these states are most tractable for biomarker development or therapeutic intervention. On this basis, focus was addressed on five core exemplar PTM axes, glycosylation, palmitoylation, ubiquitin editing, phosphorylation and lactylation, because they repeatedly map to rate-limiting immune constraints and provide mechanistically informative state-control examples, although their assay and therapeutic maturity remain uneven across targets and tumor contexts (17,18). Glycosylation, palmitoylation, ubiquitin editing and phosphorylation are emphasized because they recurrently govern checkpoint stability, antigen-presentation competence, receptor trafficking and interferon (IFN)-linked signaling persistence (19-24). Lactylation is considered separately because, within this framework, its main significance lies in linking metabolic stress to chromatin-coupled immunosuppressive fixation (10,25,26). The present review therefore uses these five core axes as the principal organizing examples for interpreting immune-state constraints in cancer immunity. Additional modification classes are discussed selectively where they clarify the same functional state variables, reveal framework boundaries, or provide relevant examples of molecular-state-selective vulnerability. To orient the reader to the historical emergence and conceptual scope of this framework, selected milestones linking PTMs to tumor antigenicity, checkpoint-state control, immune evasion and immunotherapy resistance are summarized in Fig. 1.

2. Framework of PTM control in cancer immune escape and resistance

PTMs influence cancer immune escape and therapeutic resistance through multiple, closely interconnected regulatory routes. To organize these mechanisms into a clinically interpretable framework, this section examines how PTM-governed protein states shape three recurrent immune constraints: Checkpoint competence, tumor visibility and stress-conditioned immune-state programming. It further considers the molecular coordinates, multi-omics resources, and biomarker contexts required to translate these states into measurable and potentially actionable variables.

Mechanistic coordinates of PTM immunobiology. Immune escape and treatment resistance often arise when a limited number of rate-limiting steps fail to convert immune activation into durable tumor control (27-29). PTMs are well suited to this problem because they rapidly tune protein fate and signal propagation in a context-dependent manner, including under therapy-imposed selective pressure (13,30). A constraint-centered synthesis therefore provides a principled way to integrate PTM biology in immuno-oncology while remaining anchored to processes that repeatedly constrain clinical benefit.

A recurring challenge is that numerous immune-relevant phenotypes are determined by protein state rather than transcript abundance (31,32). Across these immune constraints, diverse PTM chemistries converge on a restricted set of state variables that define functional output, including proteostasis, trafficking, complex assembly and signaling persistence (5). This convergence is what makes PTM biology interpretable from a biomarker perspective, because it allows mechanistically distinct PTM events to be read out through a limited number of functionally aligned state variables. Using these variables as mechanistic coordinates enables cross-study comparison while keeping interpretation tied to analyzable control points rather than pathway labels.

PTM-governed protein states should not be interpreted as tissue-independent molecular outputs. Their functional weight is conditioned by the cellular and tissue context in which a modification is installed, maintained, or removed. Pan-cancer

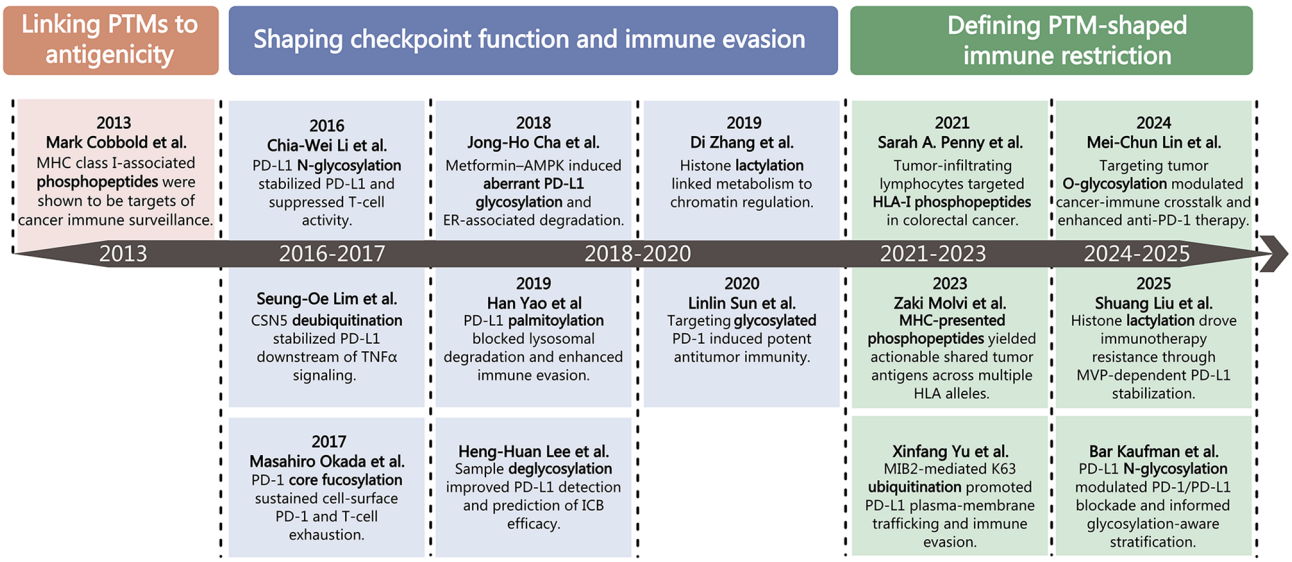

Figure 1. Selected milestones in PTM-centric cancer-immune-resistance research. This timeline highlights representative landmark advances linking PTMs to tumor antigenicity, checkpoint-state control, immune evasion and immunotherapy resistance, with particular emphasis on glycosylation, ubiquitination, palmitoylation, phosphorylation and lactylation. Together, these developments illustrate how PTMs progressively emerged as mechanistically and translationally relevant determinants of cancer immune resistance. PTM, post-translational modification; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

proteogenomic analyses show that oncogenic effects can diverge across RNA, protein and phosphoprotein layers and are accompanied by distinct signaling states (33). Direct *in vivo* nutrient tracing further demonstrates cell-programmed partitioning of glucose and glutamine among myeloid cells, T cells and cancer cells within tumors (34). Nutrient use can also be translated into PTM-dependent transcriptional states. In exhausted $CD8^+$ T cells, altered acetate and citrate utilization redirects acetyl-CoA production and locus-specific histone acetylation, thereby shaping transcriptional differentiation (35). Accordingly, protein abundance or PTM occupancy measured by mass spectrometry should be interpreted together with cellular origin and metabolic context when assigning a dominant immune constraint.

These mechanistic coordinates may also converge within selected therapy-resistant or stem-like tumor cell states rather than being distributed uniformly across malignant populations. Recent studies have shown that immune selection can enrich tumor cell populations in which stemness and immune-evasive programs are coupled, while glioblastoma (GBM) stem cells can sustain PD-L1-associated immunosuppression through a ubiquitin-linked β-catenin signaling circuit (36,37). Within the present framework, stem-like tumor states are therefore not considered a fourth dominant immune constraint. Rather, they represent a resistant cellular context in which checkpoint competence, impaired tumor visibility, and adaptive state persistence may coexist or become selectively reinforced. This distinction allows stemness-associated immune escape to be integrated without replacing the functional constraints that organize PTM-state interpretation.

PTM control of checkpoint gating and blockade sensitivity. Immune checkpoint blockade (ICB) ultimately depends on the persistence and accessibility of the surface-competent checkpoint pool, rather than on total expression alone. PTMs shape functional checkpoint states by biasing proteostasis and trafficking decisions that determine whether ligands or receptors accumulate in suppressive membrane configurations and how long these configurations are maintained (8). For PD-L1, such control is exerted through coordinated regulation of N-glycosylation and ubiquitin editing that stabilizes the ligand and modulates its commitment to proteasomal or lysosomal degradation, together with palmitoylation-dependent stabilization that limits ubiquitination and proteasomal turnover (13,15,20).

This framework also highlights response heterogeneity that can arise when modification state alters antibody engagement independently of bulk abundance. PTM-dependent processing can reshape epitope accessibility and thereby change the effective pharmacology of blockade (38). In line with this, removal of N-linked glycosylation increases anti-PD-L1 antibody binding affinity and signal intensity and improves clinical outcome prediction, while complementary glycoproteomic evidence demonstrates that PD-L1 glycosylation state can measurably affect binding to clinical antibodies (7,39).

PTM regulation of tumor visibility through antigen presentation and IFNγ-STAT1 signaling. Tumor visibility is a dynamic property defined by the capacity to sustain immune recognition pressure over time. Two components dominate this immune constraint: Competence of antigen processing and presentation, and competence and persistence of IFN and signal transducer and activator of transcription (STAT) programs that reinforce visibility under immune pressure (29). PTMs modulate visibility by regulating the stability, assembly and trafficking of antigen presentation machinery and by tuning the kinetics and persistence of IFN-linked signaling circuits (40,41). In antigen-presenting cells, ubiquitin-dependent routing decisions can determine whether peptide-loaded complexes are preferentially recycled or delivered for lysosomal turnover,

thereby controlling the durability of surface display rather than merely its formation (42). In cancer, ubiquitin-dependent control of major histocompatibility complex (MHC) class II trafficking can indirectly reduce MHC I surface expression and antigen presentation to CD8 T cells, indicating that PTM control can directly reparameterize the tumor visibility axis at the level of peptide display (22).

These mechanisms are especially consequential in therapy-exposed tumors, where response failure can arise with limited new genomic change and where transcriptomic inflammation does not necessarily guarantee sustained antigen presentation competence (43). Consistent with this visibility-centered logic, longitudinal clinical analyses of acquired resistance to PD-1 blockade have identified defects converging on antigen presentation and IFN responsiveness, underscoring that visibility can collapse through disruption of either arm even after an initial response. Mechanistically, tumor-intrinsic modulation of IFN pathway signal duration can be achieved through phosphorylation and ubiquitin-controlled negative feedback at the JAK-STAT module, including SOCS1-mediated inhibition of JAK activity and SOCS box-dependent recruitment of ubiquitin machinery that restricts sustained STAT1 signaling and weakens IFN-driven MHC class I programs (44-46). Together, these data support a competence-focused view that separates upstream immune activation from the tumor's capacity to sustain presenting states and IFN-reinforced visibility under therapeutic pressure, thereby linking PTM regulation to impaired priming, insufficient reinforcement and primary non-response under ICB (41,43).

Metabolite-sensitive PTMs and chromatin coupling in immune-state programming. A third recurrent immune constraint concerns whether immune cells retain cytotoxic competence in microenvironments shaped by hypoxia and mitochondrial oxidative stress, which can accelerate exhaustion under sustained antigenic stimulation (47). An important frontier is the increasing mechanistic resolution of metabolite-sensitive PTMs at the chromatin interface, where lactate-linked histone lactylation can couple metabolic constraints to transcriptional programs that tune CD8 T-cell fitness and effector output (3). At present, however, the translational maturity of this axis remains uneven across tumor contexts, and its strongest current value may lie in defining emerging state-transition biomarkers rather than routine deployable assays. Mechanistic interpretation of this axis requires explicit attention to persistence and context because exhausted CD8 T cells can adopt a fixed dysfunctional state with limited reprogramming capacity after PD-1 blockade. Consistent with this, *de novo* epigenetic programs can function as a barrier to checkpoint blockade-mediated rejuvenation, supporting entrenched dysfunction under therapeutic selection (48). Across these immune constraints, functionally relevant regulation often reflects combinatorial PTM states and crosstalk rather than isolated marks, converging on shared state variables such as stability, localization, complex competence and signaling persistence (13).

To consolidate this constraint-centered framework, the dominant state constraints, their corresponding state variables, representative PTM routes, and the supporting experimental contexts are summarized in Table I.

Multi-omics resources for decoding PTM-governed immune states. As the preceding sections indicate, the immune constraints that limit durable antitumor immunity are often not adequately resolved through transcript-level profiling, because they are more directly expressed through changes in protein state, including altered abundance, localization, complex competence and signaling persistence (49). This distinction is especially important in PTM-oriented immuno-oncology, because modification-dependent changes in protein stability, trafficking and signaling architecture are more directly captured by proteomic and phospho-proteomic than by transcriptomic layers alone (49,50). The aforementioned developed framework therefore requires data layers that can resolve these state variables directly. From this perspective, PTM-governed immune states are most effectively decoded through integrated multi-omics rather than through any single modality in isolation. Immuno-peptidomic analyses further show that PTM-associated changes in the tumor proteome can reshape the MHC class I ligandome and generate cancer-specific modified antigens, thereby linking proteomic state to the antigenic information presented at the tumor-immune interface (51). Spatially resolved profiling adds a further dimension by defining how tumor and immune states are organized within tissue and whether they coincide with immune exclusion or immune-evasive niche structure (52).

PTM-governed immune states should not be assumed to be spatially uniform within a single lesion. Single-cell spatial proteomics has revealed extensive region-specific proteomic variation and immune-cell heterogeneity within pancreatic tumors, while spatial profiling of ovarian cancer has identified locally distinct immune phenotypes with differential abundance of PD-L1, B2M, granzyme B and cluster of differentiation (CD)163 (53,54). Spatial multi-omics in GBM further demonstrated that hypoxia and regionally segregated inflammatory or metabolic stimuli are associated with spatially exclusive adaptive programs and enhanced immunosuppressive tumor-myeloid interactions (55). These findings suggest that the protein-state variables used in the present framework to infer checkpoint competence, tumor visibility and stress-conditioned rewiring may be locally discordant within the same lesion. Consistent with this concern, multi-region analysis of treatment-naive esophageal squamous cell carcinoma showed that limited biopsy specimens were insufficient to accurately approximate whole-tumor PD-L1 combined positive scores (56). A single-core biopsy may therefore capture a locally dominant state rather than the lesion-level dominant state constraint. Dominant-state assignment should accordingly be interpreted as a sampling-dependent estimate that requires spatial contextualization rather than as an intrinsically uniform tumor property.

The increasing availability of resource-oriented platforms now makes such analyses feasible across molecular and clinical scales. PTM-focused databases support site-level annotation, functional interpretation and regulator-substrate mapping, including kinase-substrate and E3 ligase-substrate relationships (57). Public cancer atlases provide harmonized genomic, transcriptomic, proteomic, phospho-proteomic and clinical data within a shared patient framework (50). Public immuno-peptidomic resources further extend this landscape by aggregating large-scale MHC-bound peptide data, including

Table I. Constraint-centered framework of PTM-governed immune states in cancer immunotherapy.

First author/s, year	Dominant state constraint	Core functional state variable	Representative PTM route	Immune-proximal consequence	Evidence context	(Refs.)
Li *et al*, 2016	Checkpoint competence	Proteostatic turnover	GSK3β phosphorylation coupled to β-TrCP ubiquitination of non-glycosylated PD-L1	Promotes PD-L1 degradation and relieves PD-L1-mediated T-cell suppression	Tumor cell lines (*in vitro*); mouse tumor models (*in vivo*)	(13)
Chan *et al*, 2019	Checkpoint competence	PD-L1 stability	IL-6/JAK1-mediated PD-L1 Y112 phosphorylation recruiting STT3A-dependent N-glycosylation	Stabilizes PD-L1 and enhances T-cell immune evasion	Tumor cell lines (*in vitro*); mouse tumor models (*in vivo*)	(19)
Cha *et al*, 2018	Checkpoint competence	Membrane localization	AMPK-mediated PD-L1 S195 phosphorylation inducing abnormal glycosylation and ER-associated degradation	Reduces PD-L1 stability and membrane localization and enhances CTL activity	Tumor cell lines (*in vitro*); mouse tumor models (i*n vivo*)	(16)
Wang *et al*, 2019	Checkpoint competence	Lysosomal routing	HIP1R-mediated lysosomal targeting of PD-L1	Decreases PD-L1 abundance and increases T-cell-mediated cytotoxicity	Tumor cell lines (*in vitro*); mouse tumor models (*in vivo*)	(82)
Yao et al, 2019	Checkpoint competence	Membrane residence	ZDHHC3-mediated palmitoylation of PD-L1	Stabilizes PD-L1 by blocking ubiquitination and enhances T-cell suppression	Tumor cell lines (*in vitro*); mouse tumor models (*in vivo*)	(8)
Zhu *et al*, 2021	Checkpoint competence	ERAD resistance	OTUB1-mediated deubiquitination of PD-L1	Prevents ER-associated degradation and sustains cancer cell immunosuppression	Tumor cell lines *(in vitro*); mouse tumor models (*in vivo*)	(21)
Huang *et al*, 2024	Checkpoint competence	PD-1-binding competence	MGAT5-mediated β1,6-branched N-glycosylation of PD-L1	Enhances PD-L1-PD-1 interaction and protects tumor cells from CTL-mediated apoptosis	Tumor cell lines (*in vitro*); human tumor specimens (human cohort)	(104)
Li *et al*, 2018	Checkpoint competence	Ligand inhibitory competence	B3GNT3-dependent glycosylation of PD-L1	Supports PD-L1-PD-1 interaction and suppresses cytotoxic T-cell-mediated antitumor immunity	Tumor cell lines (*in vitro*); mouse tumor models (*in vivo*)	(73)
Hsu *et al*, 2018	Checkpoint competence	PD-L1 stability in stem-like tumor cells	EMT and β-catenin-driven STT3-dependent N-glycosylation of PD-L1	Enriches and stabilizes PD-L1 in cancer stem-like cells and increases resistance to immune-mediated killing	Breast cancer stem-like cell models *in vitro*; syngeneic mouse breast tumor models *in vivo*	(14)

Table I. Continued.

First author/s, year	Dominant state constraint	Core functional state variable	Representative PTM route	Immune-proximal consequence	Evidence context	(Refs.)
Wilson *et al*, 2018	Tumor visibility	Surface MHC-I abundance	MARCH1-mediated ubiquitination of MHC II	Loss of MHC II ubiquitination reduces surface MHC I and impairs peptide and protein antigen presentation to CD8 T cells	Primary immune cells (*ex vivo*)	(22)
Kim *et al*, 2021	Tumor visibility	pMHC-II turnover	MARCH1-dependent ubiquitination of pMHC-II in dendritic cells	Dysregulated pMHC-II turnover yields dendritic cells that poorly stimulate naive CD4 T cells and produce less IL-12	Dendritic cells (*ex vivo*); knockout mouse models (*in vivo*)	(102)
Liu *et al*, 2022	Tumor visibility	Antigen-presenting cell trafficking competence	UBL3-dependent control of MARCH-mediated ubiquitination	Controls MHC II and CD86 trafficking in antigen-presenting cells	Dendritic cells and macrophages (*ex vivo*); knockout mouse models (*in vivo*)	(23)
Apriamashvili *et al*, 2022	Tumor visibility	IFNγ receptor surface abundance	STUB1-mediated ubiquitin-dependent destabilization of the IFNγ-R1/JAK1 complex	Suppresses tumor IFNγ signaling	Tumor cell lines (*in vitro*); anti-PD-1-treated mouse tumor models (*in vivo*)	(24)
Pitter *et al*, 2024	Tumor visibility	IFNγ-STAT1 transcriptional competence	PAD4-mediated STAT1 citrullination	Restrains macrophage MHC II machinery and weakens T-cell activation	Macrophages (*in vitro*/*ex vivo*); mouse tumor models (*in vivo*); human tumor specimens or datasets	(200)
Kacen *et al*, 2023	Tumor visibility	Displayed antigenic substrate	Tumor PTM-driven remodeling of the HLA-I immunopeptidome	Generates cancer-specific modified antigens and expands the tumor antigenic landscape	Human tumor specimens (immunopeptidomic profiling)	(51)
Zhang *et al*, 2024	Stress-conditioned immune-state programming	TIM-3 surface stability	DHHC9-mediated palmitoylation of TIM-3	Stabilizes TIM-3 and promotes immune exhaustion	T cells (*in vitro*); mouse tumor models (*in vivo*); human tumor specimens (human cohort)	(245)

Table I. Continued.

First author/s, year	Dominant state constraint	Core functional state variable	Representative PTM route	Immune-proximal consequence	Evidence context	(Refs.)
De Leo *et al*, 2024	Stress-conditioned immune-state programming	Chromatin-linked suppressive fixation	Glucose-driven histone lactylation in monocyte-derived macrophages	Promotes an immuno suppressive macrophage state in glioblastoma	Macrophages (*in vitro*/*ex vivo*); orthotopic glioblastoma mouse models (*in vivo*); human glioblastoma specimens (human samples)	(25)
Raychaudhuri *et al*, 2024	Stress-conditioned immune-state programming	CD8 effector metabolic competence	Histone lactylation in $CD8^+$ T cells	Modulates $CD8^+$ T-cell metabolism and effector function	T cells (*in vitro*/*ex vivo*); mouse tumor models (*in vivo*)	(3)
Wang *et al*, 2024	Stress-conditioned immune-state programming	Tumor-cell-derived suppressive instruction	H3K9 lactylation in malignant cells	Induces IL-11-JAK2-STAT3 signaling that drives $CD8^+$ T-cell dysfunction	Tumor cell lines (*in vitro*); mouse tumor models (*in vivo*); human tumor specimens (human cohort)	(26)
Kalinichenko *et al*, 2025	Stress-conditioned immune-state programming	Lytic synapse competence	ZDHHC17-mediated palmitoylation of SNAP23	Targets cytotoxic granules to GM1-rich membrane rafts and is required for regulated exocytosis	Primary cytotoxic lymphocytes (*ex vivo*/*in vitro*)	(246)

PTM, post-translational modification; PD-L1, programmed death-ligand 1; GSK3β, glycogen synthase kinase 3 beta; β-TrCP, beta-transducin repeat-containing protein; IL, interleukin; JAK1, Janus kinase 1; STT3A, STT3 oligosaccharyltransferase complex catalytic subunit A; AMPK, AMP-activated protein kinase; ER, endoplasmic reticulum; ERAD, ER-associated degradation; CTL, cytotoxic T lymphocyte; HIP1R, huntingtin-interacting protein 1-related; ZDHHC3, zinc finger DHHC-type palmitoyltransferase 3; OTUB1, OTU deubiquitinase, ubiquitin aldehyde binding 1; PD-1, programmed cell death protein 1; MGAT5, mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetylglucosaminyltransferase 5; B3GNT3, beta-1,3-N-acetylglucosaminyltransferase 3; MHC-I, major histocompatibility complex class I; MHC-II, major histocompatibility complex class II; pMHC-II, peptide-MHC-II; MARCH1, membrane-associated ring-CH-type finger 1; UBL3, ubiquitin-like protein 3; CD, cluster of differentiation; IFNγ, interferon gamma; IFNγ-R1, IFNγ receptor 1; STUB1, STIP1 homology and U-box-containing protein 1; STAT, signal transducer and activator of transcription; PAD4, peptidyl arginine deiminase 4; HLA-I, human leukocyte antigen class I; TIM-3, T-cell immunoglobulin and mucin-domain-containing 3; DHHC9, DHHC-type palmitoyltransferase 9; H3K9, histone H3 lysine 9; JAK2, Janus kinase 2; SNAP23, synaptosome-associated protein 23; GM1, monosialotetrahexosylganglioside 1.

modified antigens, into searchable atlases that support antigen prioritization and cross-cohort comparison (58,59). The value of these resources lies not in cataloguing molecular complexity for its own sake, but in providing the layered resolution needed to define PTM-governed immune states and nominate assay-compatible candidate readouts.

Biomarker classes and clinical use contexts. If PTM-governed immune states are to be captured more directly through integrated proteomic, phospho-proteomic, immuno-peptidomic and spatially resolved approaches, the next issue is not simply how to detect them, but how to use them in clinical decision-making (50,51,60). Within this framework, biomarker classes are best distinguished by the clinical question they are intended to answer rather than by platform alone (61). Importantly, the framework distinguishes direct PTM-state readouts from proximal protein-state and contextual companion readouts. Direct PTM measurements are intended to indicate functional immune constraints, whereas companion readouts provide supporting information on inflammation, immune abundance, spatial organization, or treatment-associated state change. Some baseline readouts are diagnostic, establishing that a biologically relevant state is present in the tumor. Representative examples include checkpoint-competent inhibitory surface states shaped by PD-L1 glycosylation or palmitoylation, attenuated antigenic visibility linked to impaired human leukocyte antigen (HLA)-A expression or broader antigen-presentation deficiency, and metabolically reinforced suppressive states coupled to lactylation-driven macrophage programming (7,8,25,62,63). Others can function prognostically by indicating whether such states are associated with more persistent immune escape or more limited durability of disease control, independent of the specific intervention chosen (61).

A different class is predictive and is intended to guide treatment selection. Here, the most informative features are those that remain mechanistically close to the dominant state constraint and therefore capture a tractable dependency, such as persistent PTM-shaped checkpoint competence, incomplete antigen-presentation programs, or HLA-linked neoantigen-presentation states that enrich for benefit from immunotherapy (7,63,64). By contrast, pharmacodynamic readouts are used to determine whether therapy is actually shifting the relevant state over time (61). Their value lies in showing whether a PTM-regulated immune program is being contracted, reprogrammed, or stabilized during treatment, thereby helping distinguish meaningful state transition from transient pathway perturbation (65,66). These use contexts can overlap, but they should remain analytically distinct, because the same PTM-linked feature may carry one value at baseline, another during on-treatment monitoring, and a third at progression or relapse (65). In this sense, the translational relevance of PTM biology lies not in documenting modification events per se, but in assigning each readout to the decision context in which it can most meaningfully support stratification, monitoring and rational therapeutic design. Operationally, a PTM-state feature should advance beyond mechanistic interest only when it can be assigned preferentially to one dominant state constraint, measured in a prespecified specimen type and treatment window, and interpreted in a way that could alter baseline stratification, on-treatment monitoring, or relapse-state attribution.

To render this framework more operational from a biomarker perspective, candidate PTM-state biomarker modules aligned to the dominant state constraints in cancer immunotherapy are summarized in Table II.

3. Checkpoint competence as a PD-L1 protein-state problem

ICB acts on inhibitory contacts formed at the tumor immune interface (1). For PD-L1, the clinically consequential variable is not total abundance but the fraction that resides at the cell surface in an antibody-accessible, programmed cell death protein 1 (PD-1)-binding configuration (7,67). PTMs tune this configuration by coupling oncogenic and inflammatory cues to PD-L1 folding, trafficking and turnover, with glycosylation, palmitoylation and ubiquitin editing converging to stabilize a synapse-competent pool (8,14,15). Framing PD-L1 as a protein state rather than a static marker not only helps reconcile discordance between staining intensity and therapeutic benefit but also provides a prototype for interpreting PTM-governed immune escape as a biomarker problem centered on functional protein states rather than abundance alone (7).

Functional state variables of PD-L1 beyond expression. Clinical practice often treats PD-L1 as a static biomarker sampled once to infer dependence on the programmed cell death 1 axis, most commonly through immunohistochemistry (IHC) workflows with substantial analytic and interpretive variability across assays and settings (68). Yet PD-L1 is better understood as a proteostasis-governed surface signal whose functional output is continuously shaped by trafficking and turnover (69,70). In numerous tumors, the critical variable is therefore less PD-L1 transcription itself than the ability to sustain an antibody-accessible, PD-1-binding pool at the plasma membrane through signal-coupled control of stability and routing (19,70,71). Bulk PD-L1 readouts are therefore intrinsically ambiguous because they compress synthesis, degradation and epitope accessibility into a single value (71).

A functional-state view reframes PD-L1 around variables proximal to checkpoint output. Stability determines whether transient inflammatory pulses are converted into durable inhibitory tone by maintaining PD-L1 in a protected regime rather than committing it to rapid clearance (19). Routing and membrane residence determine whether PD-L1 is positioned at the tumor-immune interface rather than distributed across internal pools shaped by endocytic sorting and recycling (69). Epitope accessibility governs what IHC detects and what therapeutic antibodies can engage, so that a PD-L1 pool may be biologically present yet analytically underestimated when glycan-dependent features reduce effective recognition (71).

In this framework, PTMs are not ancillary annotations on PD-L1 expression but state specifiers that set residence time, routing, and molecular presentation at the tumor-immune interface, thereby defining the synapse-competent pool that constrains checkpoint blockade depth (19,70-72). The key question is therefore not simply whether PD-L1 is present, but which proteoforms dominate, how they are stabilized, and whether available assays capture the pool that actually mediates inhibition. This also means that assay choice, epitope dependence

Table II. Candidate PTM-state biomarker modules across dominant state constraints in cancer immunotherapy.

First author/s, year	Candidate biomarker module	Dominant state constraint	PTM-governed functional state	Preferred assay platform	Primary use context	Evidence and validation gap	(Refs.)
Lee *et al*, 2019; Kaufman *et al*, 2025	Deglycosylation-sensitive PD-L1 checkpoint state	Checkpoint competence	Glycan-masked but active surface PD-L1	Deglycosylation-assisted PD-L1 IHC on pretreatment FFPE tissue	Predictive baseline stratification for anti-PD-1 or anti-PD-L1 therapy	Improved detection and response association shown; prospective assay harmonization and cutoffs lacking.	(7,92)
Yao *et al*, 2019	Palmitoylated PD-L1 persistence state	Checkpoint competence	Lysosome-evading membrane PD-L1 reservoir	Palmitoylation-sensitive biochemical assay with PD-L1 protein readout	Candidate predictive and pharmacodynamic readout for persistence-dominant checkpoint states	Strong mechanistic support; no standardized tissue assay or response-linked clinical validation.	(8)
Lim *et al*, 2016; Zhu *et al*, 2021; Wang *et al*, 2020	Deubiquitinase-supported PD-L1 rescue-from-turnover state	Checkpoint competence	Deubiquitination-maintained PD-L1 stability	Paired tumor-tissue PD-L1 and DUB-node protein assessment	Candidate predictive stratification of DUB-high checkpoint persistence	Multiple DUB nodes validated; prospective ICB-selection validation remains unavailable.	(15,21,87)
Kacen *et al*, 2023; Fritsche *et al*, 2018	PTM-remodeled HLA-I immunopeptidomic visibility state	Tumor visibility	Modified HLA-I ligand repertoire displayed at the tumor interface	LC-MS/MS immunopeptidomics of tumor HLA-I-bound peptides	Visibility stratification and antigen-target prioritization	PTM-shaped ligandomes are established; prospective PTM-focused response workflows remain limited.	(51,132)
Molvi *et al*, 2023	Tumor-selective phosphopeptide presentation state	Tumor visibility	Recurrent cancer-associated phosphopeptide display	HLA immunoprecipitation followed by phosphopeptide-aware LC-MS/MS	Target prioritization for phosphopeptide-directed immunotherapy	Shared phosphopeptides identified; immunogenicity is allele-dependent and broader validation is needed.	(129)
De Leo *et al*, 2024; Xiong *et al*, 2022	Lactylation-high suppressive myeloid state	Stress-conditioned immune-state programming	Lactate-coupled immunosuppressive myeloid program	Myeloid-resolved lactylation readout paired with immune phenotyping	Baseline identification of metabolically suppressive myeloid niches	Myeloid immunosuppression supported in original studies; assay standardization and pan-cancer validation lacking.	(25,187)
Wang *et al*, 2024	Lactylation-driven CD47 immune-evasion state	Stress-conditioned immunestate programming	Histone-lactylation-linked CD47-high phagocytosis-resistant tumor-cell state	Histone lactylation readout paired with CD47 protein assessment	Candidate selection biomarker for lactate-targeting or anti-CD47 combinations	GBM evidence supports combination rationale; generalizability beyond GBM is unproven.	(143)

PTM, post-translational modification; PD-L1, programmed death-ligand 1; IHC, immunohistochemistry; FFPE, formalin-fixed paraffin-embedded; PD-1, programmed cell death protein 1; DUB, deubiquitinase; HLA-I, human leukocyte antigen class I; LC-MS/MS, liquid chromatography-tandem mass spectrometry; ICB, immune checkpoint blockade; CD47, cluster of differentiation 47; GBM, glioblastoma.

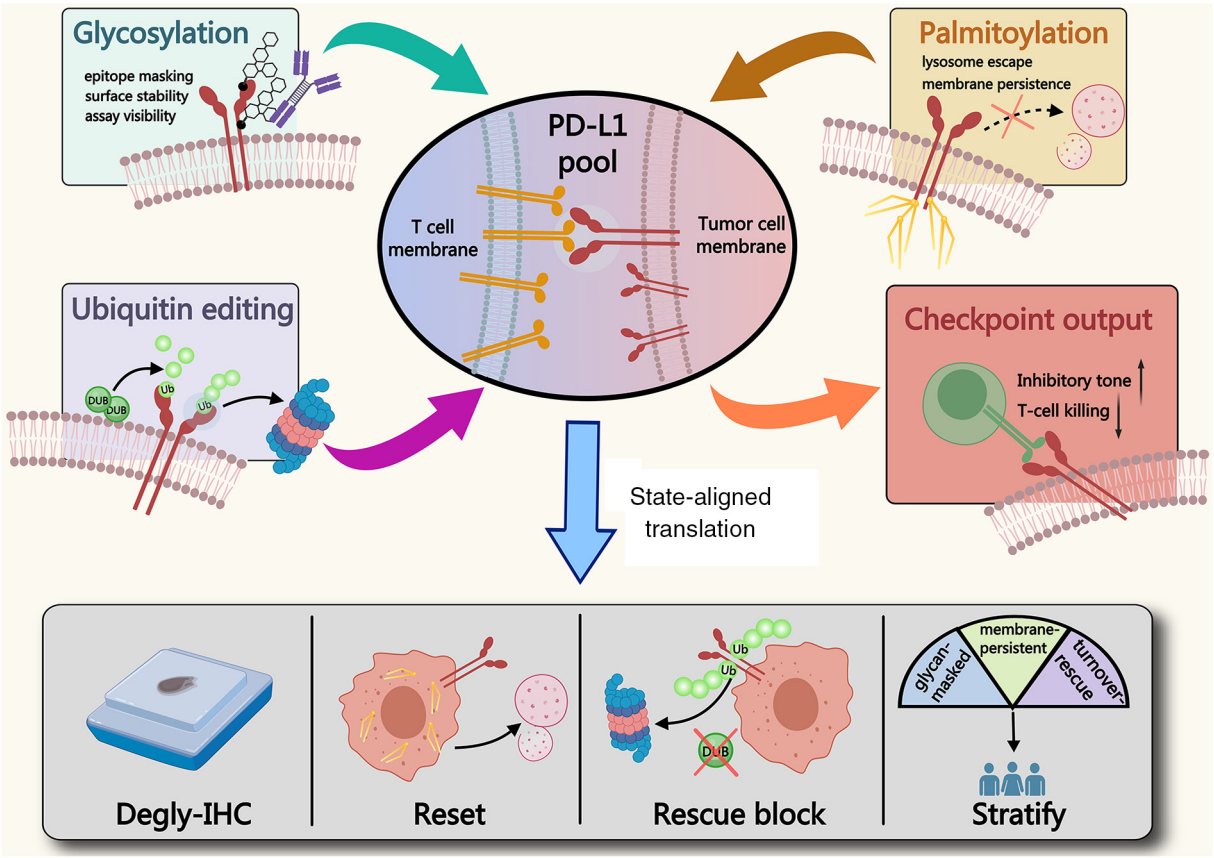

Figure 2. PTM-defined control of the synapse-competent PD-L1 pool. PD-L1 checkpoint output is governed by a functionally relevant surface pool at the tumor-immune interface rather than by total abundance alone. Glycosylation, palmitoylation and ubiquitin editing converge to shape this pool by regulating epitope accessibility and assay alignment, membrane persistence, and rescue from turnover, respectively. These state variables determine sustained PD-1 inhibitory signaling and provide a translational framework for function-matched readouts, intervention design, and patient stratification. PTM, post-translational modification; PD-L1, programmed death-ligand 1; DUB, deubiquitinase; IHC, immunohistochemistry.

and sample processing become part of the biomarker problem rather than secondary technical issues. This view provides a mechanistic explanation for staining-response discordance and supports stratification anchored to functional protein state.

Combinatorial PTM assembly of synapse-competent PD-L1 proteoforms. PD-L1 should be viewed not as a single molecular entity but as a family of proteoforms whose post-translational state dictates stability, trafficking, membrane residence and epitope accessibility. Glycosylation, palmitoylation, ubiquitin editing and vesicular recycling jointly determine whether PD-L1 remains stable, surface-resident, and functionally accessible at the tumor-immune interface (21,73-76).

This protein-state view clarifies why PD-L1 intensity is an inconsistent surrogate for PD-1 axis dependence. Routine staining compresses synthesis and degradation into a single number, and it is additionally confounded by platform- and clone-dependent analytic behavior that can shift classification even when underlying biology is similar (77). Moreover, epitope presentation can be remodeled by glycosylation, creating underestimation under standard IHC that can be partially corrected by deglycosylation workflows. The corrected signal has been reported to align more closely with ICB outcomes in real-world patient material (78). These configurations are not interchangeable because they suggest different state variables to measure and different ways to lower checkpoint tone. Cohort-level evidence adds translational weight when PTM-aware or protein-state measurements map to the same variables and reproducibly track response or immune architecture (78). These convergent PTM axes can therefore be viewed as a common state-control architecture that determines whether PD-L1 is maintained as a synapse-competent pool at the tumor-immune interface, as illustrated in Fig. 2, and how this state may be translated into biomarker and intervention logic.

N-linked glycosylation in PD-L1 stability and epitope accessibility. N-linked glycosylation is not simply a maturation detail for PD-L1. Across experimental systems it acts as a protein-state determinant that influences whether PD-L1 persists in a surface-available configuration capable of engaging PD-1 or is diverted toward turnover. Mechanistically, loss of N-glycan protection can expose PD-L1 to phosphorylation-linked ubiquitination programs, including a GSK-3β and β-TrCP route, thereby accelerating clearance and contracting the persistent surface fraction under inflammatory cycling (79).

Stem-like tumor populations provide a cellular context in which this glycosylation-dependent stability program can be selectively reinforced. In breast cancer (BC) stem-like cells, epithelial-mesenchymal transition activates β-catenin-dependent transcription of STT3 isoforms, with higher STT3 induction in stem-like than non-stem populations. STT3-dependent PD-L1 N-glycosylation subsequently

protects PD-L1 from ubiquitin-proteasome-mediated degradation and enriches the stable PD-L1 protein pool in cancer stem-like cells (14). Genetic depletion of STT3 reduced PD-L1 enrichment and increased the susceptibility of these cells to immune-mediated elimination, directly linking a stemness-associated signaling state to PTM-governed checkpoint competence. This mechanism illustrates how the same PD-L1 proteostasis axis can acquire greater functional weight within a therapy-resistant cellular subpopulation.

The clinical relevance of this layer follows because glycan state can reshape assay observability. Proteomics and antibody-binding analyses indicate that PD-L1 glycosylation can alter recognition by clinically used antibodies, rendering signal intensity a composite of biology and epitope accessibility (39). In tissue workflows, enzymatic deglycosylation has been shown to enhance PD-L1 IHC detection in validation cohorts, with the effect most evident in samples that appear low by standard staining (80). In a triple-negative BC cohort treated with atezolizumab, tumor-cell membrane PD-L1 assessed after enzymatic deglycosylation demonstrated improved correlation with clinical response compared with conventional IHC, consistent with glycan-dependent underestimation in at least a subset of specimens (81).

These observations also constrain therapeutic extrapolation. Because N-glycosylation is ubiquitous and context dependent, broad inhibition is unlikely to be broadly tolerable or informative. A more defensible translational approach is to treat glycosylation as a stability and detectability axis, improving measurement of the relevant PD-L1 state where misclassification is plausible and prioritizing context-restricted interventions that weaken the stabilizing program rather than targeting global glycosylation itself. One proof-of-principle strategy is to target glycosylated PD-L1 directly, which has been shown to drive internalization and degradation and to augment antitumor immunity in preclinical models (73).

ZDHHC3-dependent palmitoylation and PD-L1 surface persistence. Palmitoylation offers a direct entry point into checkpoint competence by defining how PD-L1 is retained as a synapse-competent pool at the tumor immune interface. In colorectal cancer (CRC) models, PD-L1 palmitoylation in the cytosolic domain stabilizes PD-L1 by limiting ubiquitination and suppressing lysosome-dependent degradation, and ZDHHC3 is identified as a principal enzymatic node whose inhibition by 2-bromopalmitate, genetic depletion, or competitive interference reduces PD-L1 persistence and strengthens antitumor T cell immunity *in vitro* and *in vivo* (8).

A complementary pharmacologic example links this modification to endomembrane fate. Benzosceptrin C inhibits DHHC3 activity, prevents PD-L1 palmitoylation, and drives PD-L1 away from membrane retention by blocking return through recycling endosomes and triggering lysosome-mediated degradation, with enhanced tumor-infiltrating T cell immunity and improved tumor control, including in checkpoint blockade combinations (72).

Evidence also supports that palmitoylation-dependent stabilization can extend beyond a single tumor context, and clearance programs can independently set the residence time of PD-L1 at the interface (20,82,83). Translationally, this framework motivates interventions that collapse the persistent membrane fraction rather than only lowering transcripts. A stapled peptide proteolysis-targeting chimera (PROTAC) targeting ZDHHC3 has been reported to downregulate PD-L1 and to increase effector cytokine release in tumor and T-cell coculture systems, illustrating one route to state resetting through the palmitoylation node (84). The operational challenge remains to develop patient material measurements that report palmitoylation-linked persistence and to establish when this state variable is the dominant constraint on checkpoint blockade depth.

Deubiquitination and PD-L1 degradation thresholds. If palmitoylation biases PD-L1 toward membrane persistence, ubiquitination determines whether that persistence can be sustained under degradative pressure. Ubiquitin marks encode a fate decision that commits PD-L1 to regulated disposal, and an E3 ligase axis can drive PD-L1 proteasomal degradation with measurable consequences for antitumor immunity (85). Deubiquitinases (DUBs) can rewrite that commitment by removing ubiquitin chains and stabilizing PD-L1 even when degradation programs are engaged, as shown by OTUB1-dependent protection of PD-L1 from ER-associated degradation with corresponding effects on CD8 infiltration and tumor immunity (21). This rationale becomes especially relevant in inflammation-shaped tumors because inflammatory cytokines can activate immune pressure while also strengthening PD-L1 protein stability through CSN5-dependent deubiquitination (15).

CSN5 provides a concrete illustration of how inflammatory inputs are translated into a hardened PD-L1 state at the protein level. In CRC models, macrophage-derived CCL5 induces CSN5 via p65 and STAT3, and CSN5 deubiquitinates PD-L1 to prolong ligand availability at the tumor immune interface and suppress T-cell mediated elimination *in vitro* and *in vivo* (86).

USP22 extends this threshold behavior from a single node to a reinforcing circuit. USP22 directly deubiquitinates PD-L1 and also stabilizes CSN5 through deubiquitination, creating a module that sustains PD-L1 protein stability and promotes immune evasion while remaining partly uncoupled from transcript dynamics (87). Complementary *in vivo* evidence in CD274-amplified settings indicates that USP22 depletion increases tumor immunogenicity and tumor-infiltrating lymphocytes and improves the efficacy of CD274-targeted immunotherapy (88).

The development question is therefore best posed in terms of state specificity. Additional DUB nodes have been reported to stabilize PD-L1 in a tumor-context dependent manner, and small-molecule strategies that target DUB dependence directly provide a route to restore ubiquitin-proteasome disposal of PD-L1 and promote antitumor immunity in immune-competent models (89-91).

PD-L1 state regimes in checkpoint biomarkers and combination strategies. Across these three PTM axes, the central question is whether PD-L1 is maintained as a durable surface-exposed fraction capable of repeated PD-1 engagement at the tumor-immune interface. A more actionable view is that these PTMs impose three distinct constraints on the synapse-competent PD-L1 pool, namely engagement and

assay alignment, membrane persistence, and rescue from turnover (21,72,85,86,92). Stratification should therefore focus on the dominant tissue-level constraint rather than on PD-L1 abundance alone.

This convergence helps explain why similar PD-L1 staining intensities can accompany different inhibitory burdens. A single clinical readout compresses production, turnover and detectability into one score, and multicenter harmonization efforts have shown that assay configuration and laboratory workflow can shift PD-L1 status calls (93). Clone- and platform-dependent sensitivity differences that move samples across thresholds have also been documented across tumor types, underscoring assay alignment as a prerequisite for stratification (94-96). Consistent with glycan-dependent detectability as a clinically relevant state variable, deglycosylated PD-L1 assessment has been reported to align more closely with ICB outcome than conventional PD-L1 readouts in real-world BC patient material (78).

A functional PD-L1 state readout shifts stratification from single regulators to state variables closest to checkpoint output, including interface competence, surface persistence and assay alignment. At the translational level, direct PTM-state readouts such as deglycosylation-sensitive PD-L1 or palmitoylation-linked persistence should be distinguished from contextual companion readouts, including spatial immune architecture or pathway activity scores, which support interpretation but do not themselves define the PTM state. The translational question is which constraint dominates in tissue and therefore which lever is most likely to lower checkpoint tone. Glycosylation-weighted states prioritize state-aligned measurement and upstream destabilization rather than global glycosylation inhibition. Palmitoylation-weighted states motivate state-reset combinations that erode the persistent membrane reservoir. Rescue-from-turnover states prioritize protein-state validation in inflammation-shaped settings where persistence can outpace transcription. The remaining gap is whether these state variables converge into reproducible regimes in patient tissue that align with immune architecture and ICB outcomes across cohorts. Spatial receptor-ligand proximity offers one practical validation route, and PD-1 to PD-L1 proximity in mismatch repair-deficient CRCs has been reported to predict benefit from PD-1 blockade (97).

4. Tumor visibility under PTM control in antigen presentation and IFNγ-STAT1 signaling

ICB can amplify antitumor immunity only when antigenic information is effectively displayed and IFNγ signaling is sustained enough to reinforce that display under immune pressure (98-100). The resulting tumor visibility constraint frequently reflects constraints in protein abundance, trafficking and signal duration that diverge from transcript-level immune activation (101,102). At the proximal level, it is governed by whether peptide MHC complexes are generated and maintained at relevant cellular interfaces, and whether IFNγ-STAT1 signaling reaches sufficient amplitude and persistence to install and sustain an immunogenic presentation state in tumor and myeloid compartments (40). PTMs operate across both layers of this visibility architecture: They regulate the routing, stability and surface residence of antigen-presentation machinery, and they tune the amplitude, persistence and compartmental enforcement of IFNγ-STAT1 signaling required to maintain visible tumor states under immune pressure (22,103).

Clinical patterns and proximal determinants of impaired tumor visibility. A recurring clinical paradox is that measurable immune infiltration does not necessarily translate into benefit from checkpoint blockade (4,27). Others remain immunologically cold with low effector density and primary resistance across checkpoint regimens (4,104). Across these phenotypes, a common proximate correlate is reduced antigen presentation capacity together with impaired IFNγ-STAT1 pathway competence, including failure to maintain antigen presentation outputs under immune pressure (101,105). Although upstream causes differ, the operational immune constraint is similar. The immune system either receives too little antigenic information, or it cannot sustain the IFN-driven program needed to keep antigen processing, loading and display active (4).

Two features make this visibility constraint well suited to a PTM-centered analysis. First, visibility is graded rather than binary. It depends on the stability and surface residence of antigen presentation machinery, which can be reset by post-translational control of ubiquitination, endocytosis and degradation (101,106). Second, failure modes can be compartment specific. Tumor cells may downshift MHC class I display through allele-specific HLA loss, whereas macrophage-rich regions can reduce MHC class II abundance and antigen presentation to CD4 T cells under immunosuppressive cues, weakening CD4-dependent support for durable cytotoxic control (107-109). This compartment logic is important mechanistically because it suggests that low visibility can arise from distinct protein state constraints imposed in different cellular contexts even when bulk transcript-level immune activation appears superficially similar.

Therapy-resistant stem-like tumor populations provide one such context in which visibility can be selectively attenuated. In triple-negative BC, ICB was shown to select LCOR-low cancer stem cells with reduced antigen-processing and presentation machinery, thereby promoting immune escape and resistance to checkpoint therapy (110). Restoration of LCOR increased antigen-presentation programs and tumor immunogenicity independently of canonical IFN signaling, establishing that stem-like state selection can directly alter the proximal display layer of tumor visibility. Importantly, this mechanism is transcriptional rather than PTM-driven. Direct evidence that specific PTM networks install or stabilize visibility-deficient states selectively within cancer stem cell populations remains limited. This unresolved interface between stemness, PTM control, and antigen-presentation competence represents an important mechanistic gap in the current tumor-visibility framework.

Within this framework, the tumor visibility axis is best resolved as a coupled two-layer system encompassing antigen-presentation competence and IFNγ-STAT1 reinforcement. The PTM mechanisms emphasized here represent proximal regulatory routes within this architecture rather than exclusive determinants of visibility failure. This distinction is supported by evidence that broader cellular stress states can alter the same visibility layers. Tumor microenvironmental

(TME) peroxynitrite can remodel the MHC-I-bound peptide repertoire and impair recognition of peroxynitrite-sensitive antigens by cytotoxic T cells (111), whereas mitochondrial fission can reduce cancer-cell surface MHC-I through an IRE1α-XBP1s-TPP2 pathway (112). Redox-associated signaling can also modify IFN-linked responses. In melanoma cells, IFNγ-induced neuronal nitric oxide synthase (nNOS) and nitric oxide signaling contributed to STAT1 and STAT3 activation and PD-L1 induction, whereas nNOS inhibition attenuated these responses (113). PTM-controlled protein states should therefore be interpreted as mechanistically proximal and analyzable components of tumor visibility whose functional weight is conditioned by the broader metabolic, organelle-stress and redox environment.

PTM control of IFNγ-STAT1 signaling kinetics along the tumor visibility axis. The second layer of the tumor visibility architecture is IFNγ-STAT1 reinforcement, whose competence is determined by signal amplitude and persistence rather than cytokine availability alone (114,115). The IFNγ-STAT1 module must reach sufficient activation and remain engaged long enough to install and maintain antigen presentation programs (44,116). These STAT1 kinetics function as a maintenance signal for antigen processing and presentation modules and for the turnover of MHC machinery, thereby shaping the replenishment and surface residence of peptide-MHC (pMHC) complexes over time (116). PTMs operate at this control layer. Phosphorylation governs STAT1 activation and nuclear transcriptional function, whereas ubiquitin-dependent processes shape signal persistence by controlling complex stability and turnover of pathway components (117,118).

Operationally, IFNγ pathway failure is often expressed as kinetic patterns that indicate where the pathway is gated. A frequent pattern is premature termination. Negative regulators induced after IFNγ stimulation can recruit ubiquitin machinery to accelerate turnover of signaling intermediates or curtail sustained phosphorylation. The result is a brief STAT1 activation pulse that fails to maintain antigen processing and loading programs (45,46). A corresponding state-aligned readout is the dwell time of phosphorylated STAT1 together with the persistence, rather than transient induction, of antigen presentation modules.

In other contexts, the dominant constraint is amplitude damping. Excessive dephosphorylation of upstream kinases or STAT1 can flatten pathway gain and prevent threshold crossing required for robust antigen presentation and chemokine programs associated with immune recruitment and retention (119). Readouts that map to this constraint include STAT1 phosphorylation intensity and nuclear residency together with IFN response scores anchored to antigen presentation rather than global inflammation.

A distinct but related constraint is spatial or compartmental uncoupling. Pathway components can be diverted from productive signaling locales or retained in non-productive states that limit sustained transcriptional enforcement. In this setting, receptor stability control can regulate complex assembly and residence time, converting IFNγ exposure into ineffective signaling (120). This framing emphasizes that IFNγ non-responsiveness is not always explained by gene loss or absent transcription. In some tumors it can reflect reversible protein state constraints that are best interpreted using state-aligned, compartment-aware measurements. Because these constraints ultimately manifest as altered abundance and composition of pMHC complexes at the immune interface, readouts that directly interrogate displayed peptides provide a complementary perspective on visibility (121,122).

Taken together, tumor visibility is not determined by antigen presentation alone, but by the coordinated coupling of the presentation machinery with IFNγ-STAT1 reinforcement, a two-layer architecture that defines whether antigenic information is durably displayed at the tumor-immune interface, as summarized in Fig. 3. In this sense, antigen-presentation machinery defines the proximal display layer, whereas IFNγ-STAT1 kinetics defines the reinforcement layer that determines whether visible states are transiently induced or durably sustained.

PTM immuno-peptidomics and neoantigens as direct readouts of tumor visibility. A direct way to read out the output of the visibility axis is to measure presented peptides rather than upstream regulators (123,124). This approach quantifies the peptide cargo actually displayed as pMHC at tumor and myeloid interfaces and therefore reports visibility more proximally than transcript-based surrogates (58,125). In this sense, PTM immuno-peptidomics is valuable not simply because it identifies modified antigens, but because it reads out the antigenic state that the immune system is actually being asked to recognize. PTMs can create or alter epitopes that enter antigen processing, are loaded onto MHC molecules, and are displayed at immune interfaces (126). PTMs thus become not only regulators of visibility, but also components of the displayed antigenic substrate itself (127).

Phosphorylated peptides provide a parallel PTM class that can expand the landscape captured by immuno-peptidomics and expose additional layers of state dependent antigen display (128). From a biomarker perspective, PTM immuno-peptidomics provides a readout that is substantially closer to mechanism than transcript-based surrogates, because it interrogates the antigenic surface actually available for immune recognition. It can determine whether tumor and myeloid compartments present modified epitopes, quantify their abundance, and support inference about whether antigenic information is being effectively displayed under a given visibility state (129,130).

In this sense, PTM immuno-peptidomics does more than catalogue displayed epitopes (51,129). By directly resolving naturally presented HLA ligands, including modified tumor-associated peptides, it connects the biochemical state of the tumor proteome with the antigenic surface that is genuinely available for immune surveillance (51,131,132). This feature becomes especially important when transcript-based signatures indicate an immune active or T-cell inflamed microenvironment yet remain insufficient to account for effective recognition or durable clinical benefit, because these outcomes also depend on intact antigen processing, HLA presentation, and sustained tumor cell visibility to cytotoxic T cells (29,62,63,100). Viewed in this way, PTM-defined antigen display serves as a mechanism-proximal readout of the visibility axis and provides a practical bridge from two-layer

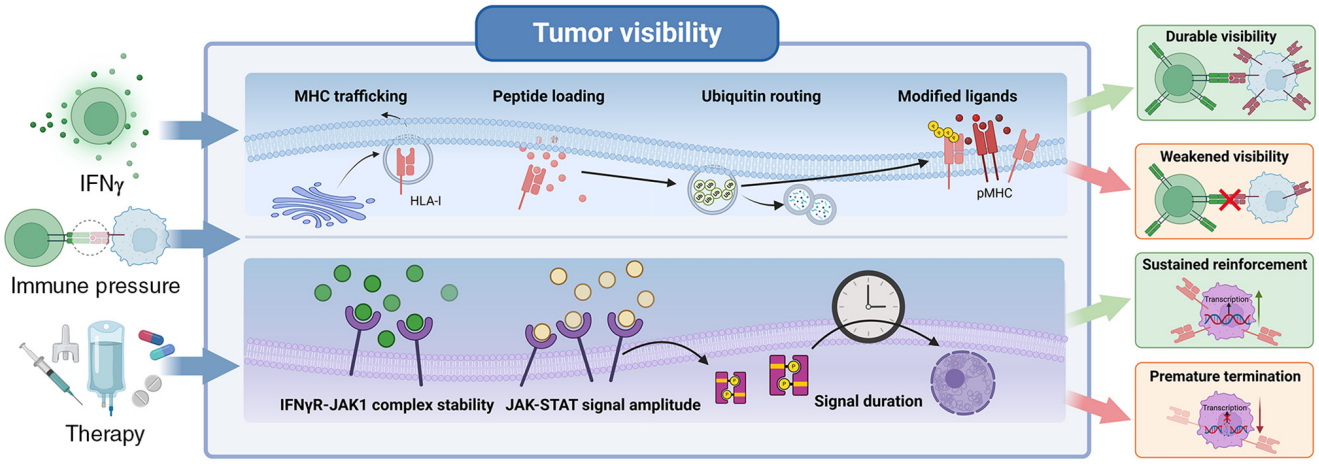

Figure 3. A two-layer PTM-controlled architecture of tumor visibility: Antigen presentation and IFNγ-STAT1 reinforcement. Tumor visibility is determined by two coupled layers: The antigen-presentation machinery that governs peptide loading, trafficking, and surface pMHC display, and the IFNγ-STAT1 reinforcement layer that sustains IFNγR-JAK1 complex stability, signaling amplitude, signal duration, and nuclear maintenance of visibility programs. PTM-dependent disruption at either layer can weaken durable tumor visibility, reduce effective immune priming and reinforcement, and contribute to primary nonresponse. Direct readout opportunities include pMHC display, HLA-I immuno-peptidomic states, modified peptide presentation, and IFNγ-STAT1 persistence, thereby linking the visibility axis to biomarker refinement and visibility-based stratification. PTM, post-translational modification; IFN, interferon; pMHC, peptide-major histocompatibility complex.

visibility biology to biomarker refinement, stratification and target prioritization in immunotherapy-oriented settings.

5. Stress-driven immune-state programming through PTM rewiring and signaling persistence

Beyond checkpoint regulation and tumor visibility, antitumor immunity is also constrained by the ability of immune cells to preserve functional competence under persistent microenvironmental stress. This section examines how metabolic, oxidative, hypoxic and antigenic pressures are translated through PTM-dependent changes in protein state and signaling behavior, and how these changes promote either reversible adaptation or more persistent immune dysfunction.

Definition of the stress-conditioned immune-state programming axis and the state-variable framework. In immunotherapy-relevant settings, this axis concerns whether immune cells retain cytotoxic competence, and whether that competence remains reversible, in microenvironments shaped by hypoxia, lactate accumulation, mitochondrial oxidative stress and sustained antigenic stimulation (133-136). Under these conditions, immune dysfunction is not determined by inflammatory exposure alone, but by whether stress is translated into durable protein-state constraints that preserve effector function or stabilize non-productive states.

To keep mechanistically heterogeneous evidence interpretable, this chapter organizes the discussion around four recurring state variables: stability, localization, complex competence and signaling persistence. Glycosylation, palmitoylation, phosphorylation and ubiquitin editing are emphasized because they repeatedly converge on these variables and thereby provide a practical scale for interpreting how stress is translated into preserved cytotoxicity or fixed dysfunction (137-140).

Pressure landscapes and entry points for state translation. Hypoxia, lactate accumulation, mitochondrial oxidative stress and sustained antigenic stimulation reshape substrate availability, redox balance and signaling thresholds in immune cells. In this framework, nutritional status is therefore interpreted as an integrated metabolic state defined by nutrient access, nutrient utilization and nutrient-coupled signaling rather than by energy availability alone (141).

The metabolic state of the cancer cell is an upstream determinant of this pressure landscape rather than a parallel feature of tumor growth. Pharmacologic lactate dehydrogenase inhibition in solid tumor models reduced glucose uptake by cancer cells and redirected intratumoral glucose availability toward infiltrating T cells, with corresponding gains in T-cell function and antitumor activity (142). Lactate production can also be translated into more persistent PTM-governed regulatory states. In GBM, lactate produced by stem-like tumor cells and microglia or macrophages increased tumor-cell histone lactylation and induced an immunosuppressive transcriptional program that upregulated CD47 and suppressed phagocytosis (143). In pancreatic ductal adenocarcinoma, heightened glycolytic activity and lactate accumulation promoted ENSA K63 lactylation, sustained SRC and STAT3 phosphorylation, increased CCL2 production, and reinforced macrophage-rich immunosuppression and resistance to ICB (144). These findings position cancer-cell metabolic routing as a generator of immune-state pressure and lactylation as one metabolite-sensitive route through which this pressure can be converted into a more persistent regulatory state, as discussed further in Section 6.

Glycolytic reprogramming also generates reactive dicarbonyl pressure beyond lactate accumulation. Methylglyoxal is a reactive glycolytic byproduct whose biological effects are strongly dependent on cellular context and exposure level. In glycolytic GBM and BC models, methylglyoxal challenge induced NRF2 and GLO1 expression and enhanced

detoxification capacity, whereas *in vivo* exposure produced a biphasic growth response in which lower concentrations promoted tumor growth and higher concentrations suppressed growth (145). Methylglyoxal can also impose discrete protein-fate changes. A recent study showed that methylglyoxal triggers BRCA2 proteolysis and transiently compromises BRCA2 tumor-suppressive function, linking glycolytic carbon overflow to altered protein-state control (146). More broadly, non-enzymatic glycation by reactive dicarbonyls can increase proteotoxic burden. Quantitative proteomics identified more than 1,000 protein carboxymethylation sites and showed that glycation induces a proteotoxic response with secondary perturbation of protein-degradation machinery (147).

Carbonyl stress can also suppress immune effectors directly. Methylglyoxal accumulated in myeloid-derived suppressor cells and was transferred to $CD8^+$ T cells, imposing metabolic paralysis, whereas neutralization of dicarbonyl activity relieved T-cell suppression and improved checkpoint inhibition in murine tumors (148). Dicarbonyl stress therefore represents a non-enzymatic route of metabolic state translation that is chemically distinct from the enzyme-regulated PTM axes emphasized in the present review. Accordingly, the PTM effectors considered below should be viewed as proximal state-control routes embedded within a broader metabolic and redox environment rather than as exclusive causes of stress-conditioned immune-state programming.

The key consequence is not simply metabolic stress itself, but the conversion of stress into altered receptor availability, membrane organization, and signaling duration, thereby narrowing the functional margin required to sustain effector programs (149,150). In this setting, inflammatory exposure and immune infiltration may coexist with weak cytotoxic execution because the limiting defect lies in renewable effector competence rather than immune entry alone (151). A central feature of this axis is therefore context-dependent directionality. Similar inflammatory cues may remain compatible with preserved elimination capacity in relatively permissive environments, but can drive progressive dysfunction when embedded in hypoxic, oxidatively stressed and chronically stimulated niches (133,152,153). Under such conditions, protein-state constraints stabilize non-productive configurations, shorten productive signaling windows, and favor exhaustion deepening despite apparent immune activation (154,155). For immunotherapy, this helps explain why early resistance can emerge even in tumors that remain inflamed and infiltrated (156).

PTM routes in immune-state translation. Glycosylation, palmitoylation, phosphorylation and ubiquitin editing are considered in the present review as mechanistic routes that reshape stability, localization, complex competence and signaling persistence under hypoxia, oxidative stress and sustained stimulation, thereby influencing whether cytotoxic programs remain renewable under immunotherapy pressure or drift toward treatment-refractory dysfunction.

Glycosylation most directly affects stability and surface residence by controlling receptor and ligand maturation, trafficking and clearance (73). Across immune contexts, altered glycosylation states are associated with changes in effective receptor abundance and activation threshold (157). Perturbation studies further show that glycan processing and trimming can reweight surface retention and ligand availability, thereby changing the amplitude and duration of downstream signaling (158). Under stress-conditioned conditions, this becomes particularly relevant when surface-competent pools are already limiting, such that even modest reductions in mature receptor availability can shorten productive signaling windows and accelerate functional decline.

Palmitoylation most directly affects localization by controlling membrane partitioning and microdomain residency (138). Human and *ex vivo* data link preserved synapse integrity and membrane organization with maintained effector function (159). Causal studies further show that palmitoylation regulates microdomain residency, receptor clustering and assembly of signaling-competent complexes (160,161). In stressed microenvironments, this spatial control becomes particularly important because activation may proceed either through synapse-aligned signaling or through non-productive membrane configurations that dissipate signals and weaken persistence.

Phosphorylation provides a dominant route for rapid threshold control and for imposing temporal structure on transcriptional output (162). In numerous immune settings, the distinction between transient activation and sustained effector reinforcement maps to differences in phosphorylation kinetics, nuclear routing, and the dwell time of activated transcription factors (163). Perturbation of kinases, phosphatases, or adaptor nodes shows that relatively modest changes in phosphorylation gain can shift cells from sustained signaling to brief pulses that fail to maintain cytotoxic programs (164). Under stressed microenvironments, this vulnerability is amplified because energetic limitation and redox imbalance constrain sustained kinase-driven signaling and favor premature signal damping (165).

Ubiquitination and deubiquitination control turnover and signaling duration by editing degradation, recycling and complex disassembly decisions (166). This role extends beyond antigen processing and presentation. In mammalian protein quality control, the UBR4-KCMF1 ubiquitin ligase complex recognizes mono-ubiquitinated orphan subunits and extends K48-linked polyubiquitin chains, enabling degradation of unassembled or partially assembled protein-complex components (167). Ubiquitin-dependent control also operates at immune-signaling nodes. In $CD8^+$ T cells, a CUL5 E3 ligase complex acting through PCMTD2 restrains TCR and IL-2 signaling, whereas CUL5 loss enhances TCR and cytokine signaling, effector function and tumor control (168). Ubiquitin editing should therefore be interpreted as a protein-fate and signaling-control system that spans selective proteostasis and immune signaling rather than as a mechanism restricted to antigen processing. Human and TME studies associate weakened effector competence with enhanced negative feedback and accelerated turnover of signaling intermediates (169). Causal perturbation studies further indicate that ubiquitin ligases and DUBs determine complex lifetime and whether signaling is sustained or prematurely terminated (150). Under chronic stimulation, this editing function can convert persistent antigen exposure into short-lived signaling pulses, thereby limiting transcriptional reinforcement and stabilizing non-effector states (170).

Taken together, these PTM routes support a common interpretation in which stress-driven immune dysfunction is not explained by stimulation alone, but by progressive restriction of stability, localization, complex competence and signaling persistence.

Locking and reversibility of exhaustion programs. Persistent antigenic stimulation superimposed on hypoxia, lactate accumulation, and mitochondrial oxidative stress can drive immune cells from adaptive dampening into a more fixed dysfunctional state (133,134,154,171). Once this transition occurs, limited recovery after checkpoint release is often better explained by protein-state constraints than by receptor engagement alone (172). The limiting step becomes the ability to sustain productive signaling and the transcriptional reinforcement required for durable effector maintenance under stress. Reversibility therefore depends on whether stressed cells can reestablish signaling persistence and interface competence rather than merely undergo transient activation, a distinction that helps explain why checkpoint release may produce incomplete reinvigoration in stress-hardened tumors (173).

Within the PTM scope considered in the present review, locking is most directly interpreted through changes in signaling persistence, membrane organization and protein turnover (164). Glycosylation can reduce the effective size of surface-competent pools during chronic stimulation (73). Palmitoylation can stabilize or misdirect synapse-relevant membrane partitioning (160). Phosphorylation kinetics can be compressed into weak or short-lived pulses when acidic and metabolic constraints limit pathway gain (150). Ubiquitination and deubiquitination can accelerate turnover and impose negative feedback, thereby shortening complex lifetime and converting persistent inputs into transient signaling (166,174). Together, these processes reinforce long-lived configurations with reduced signaling persistence and weakened complex competence, providing a mechanistic basis for entrenched dysfunction and limited reprogramming capacity (175).

Combinatorial PTM states and crosstalk in functional outcomes. Functional outcomes under stress are rarely determined by a single PTM in isolation. Instead, glycosylation, palmitoylation, phosphorylation and ubiquitin control frequently converge on the same signaling modules or closely coupled nodes, generating combinatorial PTM states that determine whether signaling remains productive or collapses into dysfunction (176,177).

These combinatorial states can be interpreted through the same four variables used throughout this chapter (176). N-glycosylation and ubiquitin-controlled proteostasis shape the effective abundance and turnover of key immune regulators. Palmitoylation governs membrane partitioning and access to productive interfaces at the immunological synapse (13,178). Phosphorylation, together with ubiquitin-dependent braking, constrains signaling duration and reinforcement (138,174,179). Accordingly, similar levels of immune infiltration or inflammatory exposure may yield markedly different cytotoxic output, exhaustion depth, and immunotherapy sensitivity because the underlying PTM configuration differs. This provides a mechanistic rationale for combination strategies aimed at restoring renewable effector competence rather than simply amplifying activation.

Metabolic enzymes further illustrate why PTM interpretation should remain site- and state-variable specific. Recent analyses of cancer glycolysis have emphasized that metabolic enzymes including pyruvate kinase M2 (PKM2), phosphoglycerate kinase 1 (PGK1), hexokinases, ketohexokinase A, and nucleoside diphosphate kinases can acquire non-canonical regulatory functions beyond their canonical metabolic roles (180). Primary studies increasingly resolve this principle at defined modified residues. In cancer cells, hydrogen sulfide-dependent sulfhydration of PKM2 at Cys326 destabilizes the tetrameric enzyme, reduces pyruvate kinase activity, and increases PKM2-mediated transcriptional activation, whereas blockade of this modification stabilizes the tetramer, redirects glucose metabolism toward mitochondrial respiration, and suppresses tumor growth (181). Site-specific glycosylation provides a parallel mechanism. O-GlcNAcylation of PGK1 at Thr255 enhances catalytic activity and promotes mitochondrial translocation, where PGK1 suppresses pyruvate oxidation and reinforces glycolytic metabolism in colon cancer (182). Triosephosphate isomerase (TPI) provides a particularly informative site-resolved example. In non-small-cell lung cancer, PRKACA-mediated TPI Ser58 phosphorylation enhances enzymatic activity and glycolysis and promotes tumor growth and metastasis, while elevated Ser58 phosphorylation is detected in multiple human tumors and correlates with poorer survival (183). Independent biochemical analysis further showed that hemi-phosphorylation of the TPI dimer can enhance catalytic activity, indicating that phosphorylation stoichiometry can influence enzymatic output (184). A distinct phosphorylation state couples TPI1 to nuclear metabolism. Nutrient-responsive mTORC1-CDK2 signaling phosphorylates TPI1 at Ser117 and promotes nuclear translocation, which lowers nuclear dihydroxyacetone phosphate, permits acetate accumulation, and supports nutrient- and cell-cycle-dependent global histone acetylation (185). PTM crosstalk can also regulate TPI1 protein fate. Although demonstrated in idiopathic pulmonary fibrosis rather than cancer, HDAC11-mediated deacetylation of TPI1 at Lys69 reduces K48-linked polyubiquitination and increases protein stability, providing cross-context evidence that acetylation and ubiquitin-dependent turnover can converge on the same metabolic enzyme (186). Together, these findings indicate that metabolic enzymes can occupy PTM-defined states that separately encode catalytic flux, localization, chromatin coupling and proteostatic stability. Their direct consequences for antitumor immune escape remain incompletely resolved, but they identify metabolic enzymes as upstream protein-state variables capable of reshaping the metabolic and transcriptional environment in which immune-regulatory PTM programs operate.

6. Lactylation as a chromatin-coupled node in immunosuppressive state programming

Among the aforementioned state variables, lactylation is particularly informative because it provides a mechanistic route through which metabolic pressure can be coupled to chromatin regulation and translated into durable suppressive

output (10,25,187). This feature distinguishes lactylation from a simple metabolic correlate, because lactate-derived histone lactylation can directly stimulate gene transcription from chromatin and reinforce stable immunosuppressive programs in tumor-associated myeloid compartments (25,187). By placing a recurrent feature of the TME at the level of transcriptional stabilization, lactylation provides a direct framework for linking lactate-rich stress to persistent changes in immune function (3). In this chapter, lactylation is therefore considered a bridge mechanism that extends the aforementioned protein-state logic into a chromatin-coupled layer of immune regulation, with particular relevance to checkpoint sensitivity, myeloid control of the effective presentation environment, and stress-driven rewiring (26).

Lactylation as an emerging biomarker-relevant node. Lactate enrichment is increasingly recognized as a recurrent and spatially heterogeneous feature of solid tumors, arising from uneven metabolic activity and perfusion constraints (188). Lactylation is discussed separately not because it is already the most clinically mature PTM axis in cancer immunity, but because it provides an unusually coherent bridge between metabolic pressure, chromatin-state remodeling, and immunotherapy resistance. In this setting, lactylation is mechanistically informative because it links metabolic pressure to chromatin regulation and thereby provides a route by which similar environmental exposure can yield divergent functional outcomes (10). The value of this framing is not that lactylation replaces transcriptional readouts, but that it adds a more proximal layer for interpreting differences in checkpoint sensitivity, maintenance of effective tumor visibility, and persistence of cytotoxic competence.

Histone lactylation provides a concrete basis for this interpretation. It has been established as a lactate-linked chromatin mark capable of promoting gene transcription and connecting glycolytic metabolism to chromatin regulation (10). Studies in immune contexts further show that lactylation can shape CD8 T-cell metabolic fitness and effector function and can reinforce immunosuppressive programs in tumor-associated myeloid compartments (3,25). Taken together, these observations support a state-based view in which lactylation is most useful when treated as a translator of metabolic pressure into durable transcriptional programs rather than as a passive correlate of lactate exposure.

From a biomarker perspective, these properties make lactylation particularly relevant as an exploratory stratification and rewiring-state readout in metabolically stressed tumors with suppressive immune wiring and reduced immunotherapy sensitivity (26,189). Because lactate influences immunity through multiple routes, the discussion in the present review is restricted to lactylation at the chromatin interface as the metabolite-coupled path most directly linked to durable suppressive programming.

This focus should not be interpreted as implying that lactylation is the only metabolite-sensitive route to chromatin-state remodeling. Regulated nuclear relocalization and chromatin recruitment of metabolic enzymes can themselves become state-control variables that couple metabolic activity to transcriptional output. Recent native chromatome profiling identified more than 200 metabolic enzymes within the chromatin environment and revealed tissue-specific patterns of chromatin association. Experimental restriction of inosine monophosphate dehydrogenase 2 to nuclear or cytoplasmic compartments further produced distinct transcriptional programs, demonstrating that subcellular localization can add a regulatory layer beyond bulk enzyme abundance (190). A PTM-dependent example is provided by ATP-citrate lyase in endometrial cancer, where AKT-mediated Ser455 phosphorylation promotes nuclear translocation, increases histone acetylation, and upregulates pyrimidine-metabolism genes (191). Chromatin recruitment can provide a complementary route. A nuclear fraction of methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1 (MTHFD1) is recruited to distinct genomic loci through direct interaction with bromodomain-containing protein 4 (BRD4), and perturbation of either MTHFD1 or BRD4 produces related changes in nuclear metabolite composition and gene expression (192). These findings place regulated protein localization and chromatin recruitment alongside histone lactylation as mechanisms through which metabolic state can be translated into transcriptional reprogramming. Lactylation is therefore considered in the present review as one defined chromatin-coupling route rather than the sole metabolite-sensitive mechanism capable of stabilizing transcriptional state.

Lactate pressure, lactylation and immunosuppressive outputs. Lactylation provides a mechanistic route by which lactate-associated pressure can be coupled to durable immune state change (10,25). Two cellular contexts are especially informative in this respect. Tumor-associated macrophages and related myeloid cells shape the suppressive interface through local immune conditioning and support for productive immune recognition, whereas CD8 T cells determine whether cytotoxic programs can be maintained under sustained stimulation. Considered together, these lineages illustrate how lactylation can reinforce suppression on one side of the interface while limiting effector persistence on the other.

Within myeloid compartments, the most informative consequence of lactylation is the stabilization of a suppressive interface. Under lactate-rich pressure, chromatin-coupled consolidation of suppressive programs can reduce the capacity of tumor-infiltrating myeloid cells to sustain productive immune recognition while reinforcing inhibitory mediator programs that suppress T-cell activity (25,187). The central functional outcome is the persistence of an interface in which T cells encounter insufficient support for durable execution. In this way, lactylation becomes relevant not simply because it marks metabolic stress, but because it helps explain how stress is converted into a myeloid state that remains suppressive even when inflammatory activity is still detectable.

In CD8 T cells, the most informative consequence is reduced persistence of cytotoxic competence. Histone lactylation has been shown to shape CD8 T-cell metabolism and effector function, and lactylation-aligned tumor programs are associated with CD8 dysfunction and poor immunotherapy response (3,26). Under metabolic stress, such coupling may bias CD8 T cells away from durable effector maintenance and toward states that are progressively harder to reverse. The resulting picture is not one in which activation is absent, but

one in which activation fails to remain productive over time. Taken together, these two cellular contexts support a unified interpretation in which lactylation couples lactate-rich pressure to durable transcriptional programs that maintain suppressive interfaces and erode sustained antitumor killing.

Lactylation at the intersection of checkpoint control, tumor visibility, and immune rewiring. Taken together, lactylation is best positioned in the present review as an emerging bridge mechanism rather than as a mature stand-alone clinical axis. Its relevance to checkpoint competence is indirect and is mediated through chromatin-coupled suppressive programming rather than through direct control of ligand-state competence (25,26). Its relevance to tumor visibility remains hypothesis-generating and should be interpreted as a plausible link between metabolic stress and weakened antigen-presentation competence rather than as a fully validated visibility biomarker class (189). By contrast, its strongest current support lies in stress-conditioned immune-state programming, where myeloid suppression and CD8 dysfunction converge under lactate-rich pressure (3,10,26). In this sense, lactylation is most appropriately treated at present as an exploratory rewiring-state readout and mechanistic prioritization node, not yet as a routine clinical assay class equivalent in maturity to the aforementioned checkpoint-oriented PTM axes (193,194).

Analytical limitations and tissue validation of lactylation-state readouts. Histone lactylation presents a distinct pre-analytical challenge because the intended readout is a metabolically responsive chromatin state rather than a constitutive protein abundance measure. Isomer-resolved analyses have shown that lysine L-lactylation is the dominant lactylation isomer on cellular histones and responds dynamically to glycolytic perturbation (195). This responsiveness raises uncertainty as to whether signals measured after variable tissue acquisition-to-fixation intervals faithfully preserve the state present at sampling, because lactylation-specific stability across routine pre-analytical and fixation conditions has not been systematically qualified. Evidence from other histone PTMs further shows that pre-analytical stability is modification dependent. Several histone acetylation marks declined within 24 h of post-mortem delay, whereas multiple histone methylation marks remained comparatively stable (196). These observations do not establish equivalent instability for lactylation, but they argue against assuming uniform preservation fidelity across histone modifications.

Analytical identity represents a separate limitation. Lysine L-lactylation, lysine D-lactylation, and Nε-carboxyethyl-lysine are structurally related modifications that require methods capable of resolving their chemical identities (195). A global lactyl-lysine signal obtained without isomer-specific qualification should therefore not automatically be interpreted as chemically resolved histone L-lactylation. Site-specific readouts introduce an additional reagent-validation requirement. Recent characterization of H4K79 and H4K91 lactylation used mass-spectrometry-verified modified peptides and validated site-specific antibodies by dot blot and competitive enzyme-linked immunosorbent assay before mechanistic analysis (197). Formalin-fixed paraffin-embedded (FFPE) processing creates an additional concern for mass-spectrometric interpretation. Comparative analysis of FFPE and fresh-frozen tumor xenografts identified persistent histone chemical modifications with mass shifts corresponding to apparent methylation, dimethylation, acetylation and ubiquitination that were more frequent in FFPE material (198). This finding does not demonstrate artifactual lactylation, but it establishes that fixation-associated histone chemistry can mimic endogenous PTM mass shifts and supports orthogonal confirmation of site-resolved lactylation assignments in FFPE specimens.

Archived FFPE tissue remains technically accessible for histone-state analysis, but analytical feasibility should not be equated with lactylation-specific biomarker equivalence. FACT-seq has enabled sensitive profiling of several histone modifications in archived FFPE tissues and further demonstrated that optimal epitope-retrieval conditions can differ among histone marks (199). These findings support mark-specific FFPE assay development while cautioning against transferring analytical conditions to lactylation without direct qualification. For retrospective lactylation cohorts, incompletely documented acquisition and fixation histories may therefore confound biological variation with unmeasured pre-analytical variation. Initial assay qualification should establish concordance between controlled FFPE and matched fresh or fresh-frozen material, verify antibody isomer and site specificity, and incorporate orthogonal mass-spectrometric confirmation in a representative subset. Because lactylation-associated immune programs can arise in tumor, myeloid, and lymphocyte compartments (3,25,26), compartment-resolved detection should accompany global lactylation measurements. Until preservation fidelity and assay equivalence are established, archived FFPE cohorts are best suited to hypothesis generation and association testing rather than the definition of clinically portable lactylation thresholds.

7. Translational integration of intervention opportunities and biomarker readouts

The preceding sections support a practical conclusion. Under sustained metabolic and inflammatory stress, the limiting determinant of immune control is often not expression level itself, but the state in which key proteins are stabilized, positioned and maintained over time. Distinct PTMs can converge on the same functional constraint by stabilizing inhibitory checkpoints, weakening effective visibility, or reinforcing suppressive chromatin programs, even when abundance alone is an incomplete guide to function (8,13,26,178,189). What becomes clinically actionable is therefore not the modification class in isolation, but the dominant state variable through which inhibitory signaling is maintained, visibility is weakened, or suppressive programs are stabilized.

Intervention priorities across dominant state constraints. From a translational standpoint, intervention priorities are best organized across the three dominant state constraints: Checkpoint competence, tumor visibility and stress-conditioned immune-state programming. One recurrent situation is continued checkpoint dominance in tumors that already show immune infiltration and inflammatory activity. In such settings, the limiting problem is often the persistence of a functionally competent inhibitory surface state rather

than checkpoint abundance alone. As discussed earlier for PD-L1, the clinically consequential variable is the fraction that remains surface resident, interface competent, and available for inhibitory engagement (8,14,178). Once this state is maintained, inhibitory signaling can remain dominant even when transcript-level measures appear similar across tumors. The intervention implication is therefore to contract the persistent inhibitory surface pool and shorten the lifetime of synapse competent checkpoint states, rather than to rely on bulk reduction in expression as a sufficient objective. Different modification layers may influence this state through effects on membrane residence, partitioning, or turnover, but the translational question remains the same, namely whether functional inhibitory availability can be reduced enough to permit durable reinvigoration. Readouts are most informative when they remain close to this biology and capture surface competence, routing and turnover behavior, and interface level engagement in the relevant compartments, together with immune proximal endpoints that reflect persistence of cytotoxic programs rather than transient activation alone.

A second situation is characterized by limited immune visibility, in which immune cells may be present but recognition and amplification remain insufficient for durable control. Here the key constraint lies in the effective presentation interface and in the persistence of IFN-linked reinforcement. Antigen processing and presentation may be induced without being durably maintained, and IFN exposure may occur without sustained transcriptional enforcement of the programs needed for recognition, recruitment and execution (189,200,201). In this setting, intervention is most appropriately directed toward stabilizing presentation competence and prolonging IFN-linked reinforcement rather than merely increasing inflammatory tone. This perspective is important because different modification dependent mechanisms can converge on the same visibility problem by weakening antigen-presenting machinery, damping pathway gain, or accelerating termination of reinforcing signals. The clinically relevant question is therefore whether the tumor becomes durably visible to effectors, not simply whether inflammatory signals can be transiently detected. The most informative readouts in this setting should therefore reflect presentation competence, persistence of IFN responsive programs, and preservation of effector access to tumor nests. When available, measurements closer to the displayed antigenic interface can further strengthen this interpretation.

A third situation emerges when suppressive programming becomes stabilized early and proves increasingly resistant to reversal. In this context, the central problem is not only checkpoint signaling or poor visibility, but the progressive writing of stress into durable transcriptional states that erode cytotoxic persistence despite ongoing stimulation. The discussion of metabolite sensitive chromatin coupling is particularly relevant here because it provides a mechanism by which sustained lactate-rich pressure can be converted into durable suppressive output (10). Lactylation offers a coherent example of this transition. Here, however, lactylation should be interpreted as a mechanistically coherent bridge example rather than as evidence that all rewiring-state biomarkers are already equally mature for routine deployment or therapeutic targeting. Its translational importance lies less in lactate exposure itself than in whether a chromatin-coupled suppressive state has formed in myeloid and CD8 compartments and whether that state is already constraining effector persistence (26,202). Intervention in this setting is therefore most appropriately conceived as preventing early fixation of stress imprinted programs and resetting chromatin-coupled suppressive states before they become clinically entrenched. The most informative readouts are those that capture early switching in myeloid and CD8 compartments and interpret lactylation-aligned chromatin signals together with suppressive and effector modules that distinguish durable rewiring from transient fluctuation.

These dominant constraints do not carry equal weight across treatment phases. Before therapy, the central task is to identify which state is most limiting and to select an initial combination logic accordingly. During treatment, the priority shifts to detecting early state changes that indicate movement toward durable control or toward suppressive rewiring. At relapse, the key question becomes whether the observed program remains reversible or has acquired a more fixed character that will require strategies directed at persistence rather than acute activation.

Analytical barriers to PTM-state biomarker deployment. The deployment of PTM-state biomarkers is constrained not only by biological complexity but also by analytical barriers that directly shape interpretability and clinical use (203-205). Sample compatibility is a first limitation, because some PTM readouts are best captured in fresh or rapidly processed material, whereas others must ultimately be inferred from formalin-fixed tissues under crosslinking-related constraints on molecular resolution (206-208). Assay dependence is equally important, as IHC, mass spectrometry, spatial proteomics, and immuno-peptidomics interrogate different analytical layers of the same biology, making platform choice part of the biomarker definition rather than a secondary technical detail (51,209). PTM lability adds a further layer of difficulty, particularly for states influenced by turnover, metabolic flux, or compartmental redistribution, where pre-analytical handling can alter the signal that is intended to be captured (208,209). Finally, cross-context transferability cannot be assumed, because a PTM state that is informative at one treatment phase or in one sampling context may not retain the same meaning in another (205,210). For this reason, PTM-centered biomarker development requires analytically disciplined readout design in which intended use, sample context and platform constraints are specified in advance rather than addressed retrospectively. These general constraints become especially consequential when candidate readouts introduce selective enrichment or biochemical pretreatment into established analytical workflows. HLA-I immuno-peptidomics and deglycosylation-assisted PD-L1 IHC illustrate distinct standardization problems in the transition from discovery-oriented measurement to multicenter clinical deployment.

For HLA-I immuno-peptidomic readouts, standardization must encompass upstream sample and ligand processing, LC-MS/MS acquisition and computational analysis as an integrated analytical procedure. Different immunoprecipitation formats can recover substantial method-exclusive ligand subsets with distinct physicochemical properties despite broadly comparable overall peptide yields, indicating that

upstream isolation can alter the apparent immunopeptidome rather than only its analytical depth (211). The challenge is greater for PTM-bearing ligands, because modified peptides may occur at low stoichiometric abundance and the modification space permitted during database searching can directly constrain their identification (126). Computational processing is an additional source of non-equivalence. Direct benchmarking of four data-independent acquisition pipelines demonstrated differences in immunopeptidome coverage, replicate reproducibility and experimental false-positive control (212), whereas large-scale aggregation of heterogeneous immuno-peptidomic datasets has required explicit batch-effect control and data-type-specific processing workflows before ligand integration (213). Multicenter translation will therefore require prespecified immunoaffinity workflows, common quality-control criteria, harmonized acquisition and computational procedures, and reporting rules that distinguish exploratory peptide discovery from analytically validated PTM-state readouts. Semi-automated HLA isolation and data-independent acquisition workflows provide a practical route to reduce manual handling and increase throughput (214). Formal cross-platform and interlaboratory bridging will nevertheless be required before such measurements can be treated as clinically interchangeable.

Deglycosylation-assisted PD-L1 IHC presents a different standardization problem because enzymatic pretreatment becomes part of the analytical procedure used to define the readout. A dedicated patient-sample protocol has incorporated enzymatic removal of N-glycans from FFPE tissue sections into the conventional IHC workflow before PD-L1 assessment (215). The analytical consequence of deglycosylation is also dependent on antibody recognition characteristics. Pre-analytical stress and deglycosylation can differentially affect PD-L1 antibodies according to the location and conformational properties of their binding epitopes (216). Consistent with this principle, deglycosylation increased PD-L1 detection with 28-8, CAL10 and SP142 but slightly reduced detection with 73-10 in lung cancer tissues, and the same study identified protocol standardization, clinically applicable cutoffs, and alignment between detection and therapeutic antibodies as unresolved requirements (71). More broadly, a comparison of 22C3, 28-8 and SP142 across 418 routine clinical specimens found concordant positive or negative tumor-cell classification with all three assays in only 60% of cases (95). Multicenter validation of 22C3 laboratory-developed tests further showed that centrally disseminated protocols did not by themselves ensure diagnostic accuracy and that iterative protocol modification was required to improve laboratory performance (217). Thus, deglycosylation conditions, antibody clone, staining workflow and scoring definition should be locked as a single assay configuration, with interlaboratory reproducibility and prospectively defined thresholds established before routine deployment. Increased signal after deglycosylation should not by itself be equated with clinical assay interchangeability. A defined workflow must first demonstrate reproducible patient classification across laboratories and clinically relevant cohorts.

Biomarker readouts and a minimal validation pathway. An intervention framework centered on state constraints is only useful if the accompanying measurements track the biology that actually governs response. The goal is not to accumulate a large number of loosely related markers, but to define a compact set of readouts that remain proximal to functional output across platforms, cohorts and treatment phases. At minimum, a candidate PTM-state biomarker should satisfy four conditions before being prioritized for validation: It should map preferentially to one dominant state constraint, remain close to functional output rather than distal correlation, be measurable in a prespecified specimen type and treatment window, and carry a plausible consequence for baseline stratification, pharmacodynamic interpretation, or relapse-state attribution.

Within this framework, checkpoint competence is more informatively represented by readouts that remain close to the functional inhibitory interface, including surface-accessible PD-L1 state, membrane persistence and effective receptor-ligand engagement, rather than by bulk ligand abundance measures. By contrast, the visibility axis is best captured by durable antigen-presentation competence, sustained IFN-responsive reinforcement, and evidence that effectors can productively engage tumor tissue, because benefit tracks with preservation of these programs whereas resistant lesions recurrently lose them (29,43,66,98,218).

The rewiring axis is best represented by chromatin-linked suppressive remodeling, including lactylation-aligned signals in the relevant myeloid and CD8 compartments, because histone lactylation has been directly implicated in immunosuppressive macrophage activity and in transcriptional control of CD8 T-cell function (3,25).

Spatial heterogeneity should be treated as a validation variable rather than an optional contextual layer when PTM-state biomarkers are used to assign a dominant state constraint. Multiregional single-cell profiling has shown that cellular composition and T-cell states can vary among regions within the same lesion, whereas selected TME communication networks remain conserved across regions, indicating that regional concordance can help distinguish recurrent tumor-level features from local variation (219). Spatial analysis under immune-checkpoint therapy further demonstrated that resistant malignant subclones can occupy distinct niches characterized by different immunosuppressive microenvironments (220). Accordingly, where tissue access permits, validation should incorporate spatially separated tumor regions and spatially resolved profiling to determine whether a candidate PTM-state readout is regionally restricted or recurrent across the lesion. Spatial discordance should be retained as biologically informative rather than collapsed into a single binary assignment, whereas recurrent patterns across regions provide stronger support for lesion-level dominant-state classification. Temporal heterogeneity requires a complementary design. Longitudinal single-cell and spatial multi-omics in melanoma have identified treatment-associated emergence of resistant tumor subclones and remodeling of spatial immune structures (221), while paired pretreatment and early on-treatment profiling has linked expansion of a TCF4-governed mesenchymal-like tumor state to resistance to ICB (222). Multiregional assessment and serial sampling should therefore be considered complementary components of PTM-state biomarker validation.

A practical validation path follows naturally from these considerations. A minimal validation path should prespecify the intended use context, one fit-for-purpose and analytically

valid PTM readout, the companion variables required for biological interpretation, and the sampling timepoint most relevant to the intended clinical decision (205,210,223). Pretreatment material is needed to test whether prespecified state variables stratify subsequent outcome. Paired on-treatment samples are needed to determine whether early changes in visibility, checkpoint-relevant immune state, or suppressive rewiring accompany benefit, as longitudinal analyses have shown dynamic tumor and microenvironment remodeling during nivolumab therapy and stronger predictive performance for on-treatment signatures than for pretreatment signatures (43,66).

Relapse-associated samples are needed to define whether treatment failure is accompanied by stabilized defects in IFN responsiveness, antigen presentation, or immune contexture, as acquired resistance has repeatedly been linked to lesions in IFN pathway signaling, β2-microglobulin, HLA class I antigen processing and presentation, and broader genomic or immunophenotypic rewiring at progression (29,98,218).

Clinical endpoints are therefore most informative when interpreted together with immune proximal endpoints, particularly response durability and resistance timing, because the value of this framework lies in linking measurable state change to sustained immune control.

To translate the state-centered framework outlined above into a practical validation strategy, PTM-state-aligned and companion readouts, assay platforms, sampling windows, immune-proximal endpoints and validation uses across treatment phases are summarized in Table III.

Druggability, combination strategies and toxicity-aware translation. As the preceding sections indicate, the translational value of PTM biology depends not only on mechanistic relevance but also on whether a pathway can be modulated with sufficient selectivity and an acceptable therapeutic window (224,225). Because PTM-directed interventions often act close to core proteostasis and signaling nodes, translational design must weigh biomarker proximity against systemic liability, rather than treating efficacy logic and toxicity logic as separable questions. Kinase-directed therapy provides the most mature example of clinically tractable PTM-linked intervention, yet long-term clinical experience with tyrosine kinase inhibitors also demonstrates that effective target engagement does not preclude substantial treatment-related toxicity (224-226). Epigenetic regulators pose a related but distinct challenge. Chromatin-directed therapies can reshape tumor and immune states, and clinical studies combining epigenetic agents with ICB have underscored the practical importance of tolerability, schedule and dose optimization (227,228). More recently, targeted protein degradation has expanded the translational scope of PTM biology by harnessing the ubiquitin-proteasome system to eliminate disease-relevant proteins rather than merely inhibit catalytic activity (229,230). The therapeutic window is likely to narrow as intervention moves from a substrate-selective PTM dependency toward broadly shared proteostasis or phosphorylation machinery.

Modification-defined proteoforms may provide a more selective therapeutic entry point when a disease-enriched protein state creates a pharmacologically distinct vulnerability. In Jurkat T-cell acute lymphoblastic leukemia cells, deamidated TPI accumulated under the experimental conditions and was not detected in the normal T lymphocytes examined. Disulfiram and curcumin preferentially bound and inhibited recombinant deamidated TPI, while cellular exposure reduced endogenous TPI activity and viability in Jurkat cells without significant corresponding effects in normal T lymphocytes. Sodium dichloroacetate pretreatment further enhanced the cytotoxic effects of both compounds and reduced the concentrations required to achieve comparable cytotoxic activity (231). Complementary evidence from BC models showed accumulation of deamidated human TPI in MDA-MB-231 cells but not primary mammary epithelial cells. Rabeprazole and auranofin markedly impaired TPI activity and viability in the cancer cells while exerting substantially smaller effects in the normal-cell comparator, and rabeprazole suppressed tumor growth in a xenograft model (232). These findings support the concept that disease-enriched deamidated proteoforms can create molecular-state vulnerabilities that may be more selective than global inhibition of TPI or broadly shared PTM machinery. Modification-state-directed intervention is also becoming experimentally tractable through induced-proximity approaches. Epidermal growth factor receptor (EGFR)-targeting phosphorylation targeting chimeras promoted targeted receptor dephosphorylation, and a covalent construct selective for mutant EGFR inhibited dysregulated EGFR signaling and reduced cancer-cell viability (233). Collectively, these studies identify molecular-state selectivity as a plausible route to therapeutic-window engineering, while emphasizing that proteoform enrichment, on-target dependence, and selectivity in relevant normal tissues require direct validation before clinical extrapolation. However, these examples primarily establish the feasibility of molecular-state-selective intervention in cancer cells; their direct relevance to antitumor immunity, immunotherapy response, or clinically improved immunotherapeutic windows remains to be demonstrated.

Clinical proteasome inhibition provides a cautionary boundary case for the systemic liability of broad proteostasis intervention. In the phase III APEX trial, bortezomib improved outcomes in relapsed multiple myeloma, but grade 3 or 4 adverse events occurred in 75% of treated patients (234). Phosphorylation-directed therapy presents a distinct selectivity problem because clinically evaluated kinase drugs frequently engage broader target spectra than their nominal targets. Chemical proteomic profiling of 243 clinically evaluated kinase drugs identified previously unrecognized targets and both kinase and non-kinase off-target interactions (235). At the pathway level, the phase III BELLE-3 trial provides a separate example of the therapeutic-window challenge. The pan-class I PI3K inhibitor buparlisib improved progression-free survival, yet grade 3 or 4 transaminase elevations and hyperglycaemia were frequent, and its safety profile did not support further development in that setting (236). These observations argue against inferring clinical tractability from pathway-level target engagement alone. Targeted degradation may improve selectivity, but this advantage is conditional rather than intrinsic. Mutant-selective BRAF PROTACs preferentially degraded mutant BRAF while largely sparing wild-type RAF proteins through differential ternary-complex

Table III. PTM-state readouts, assay platforms, and validation uses across treatment phases.

First author/s, year	Dominant state constraint	PTM-state readout	Recommended assay platform	Preferred sampling window	Key immune-proximal endpoint	Intended validation use	(Refs.)
Mei *et al*, 2021	Persistent checkpoint-competent inhibitory surface state	Deglycosylated PD-L1 signal better aligned with the functionally relevant PD-L1 pool	Deglycosylation-assisted PD-L1 immunohisto-chemistry	Pretreatment	Improved concordance between measured PD-L1 and functional checkpoint competence	Refine baseline stratification for PD-1/PD-L1 blockade	(71)
Ayers *et al*, 2017	Limited durable tumor visibility	IFNγ-related T-cell-inflamed gene expression with antigen-presentation-associated transcripts	Tumor RNA expression profiling	Pretreatment	Preserved interferon-responsive reinforcement and presentation competence	Identify visibility-competent tumors more likely to benefit from PD-1 blockade	(100)
Saberzadeh-Ardestani *et al*, 2023	Insufficient receptor-ligand engagement at the tumor-immune interface	Spatial PD-1 to PD-L1 proximity rather than bulk marker abundance alone	Multiplex immunofluores-cence with spatial proximity analysis	Pretreatment	Higher effective receptor-ligand engagement at relevant cellular interfaces	Identify tumors more likely to be truly PD-1/PD-L1-axis dependent	(97)
Wang *et al*, 2024	Early suppressive chromatin-coupled rewiring	H3K9 lactylation with linked IL-11-JAK2-STAT3 signaling and CD8 dysfunction markers	PTM-oriented histone assays with paired immune phenotyping	Pretreatment	Reduced suppressive signaling and attenuated CD8 dysfunction	Flag lactylation-high tumors with increased risk of poor immuno-therapy response	(26)
Ma *et al*, 2022	Restricted effector access to tumor nests	Baseline $CD8^+$ T-cell density at the invasive margin and early intratumoral redistribution	Quantitative immunohisto-chemistry or multiplex immuno-fluorescence with spatial analysis	Pretreatment and early on-treatment	Increased intra-tumoral $CD8^+$ T-cell access and expansion	Identify spatially excluded tumors less likely to respond to PD-1 monotherapy	(219)
Du *et al*, 2021	Treatment-emergent pathway-state remodeling	On-treatment pathway signatures derived from tumor specimens	On-treatment biopsy with pathway-level transcriptomic analysis	Early on-treatment	Emergence of response-associated pathway repro-gramming	Improve response prediction beyond pretreatment-only profiling	(66)

Table III. Continued.

First author/s, year	Dominant state constraint	PTM-state readout	Recommended assay platform	Preferred sampling window	Key immune-proximal endpoint	Intended validation use	(Refs.)
Riaz *et al*, 2017	Dynamic evolution of dominant state constraints during therapy	Baseline-to-on-treatment genomic, transcriptomic, and TCR clonal changes	Longitudinal paired biopsies with integrated exome, transcriptome, and TCR sequencing	Pretreatment and on-treatment	Intratumoral T-cell clonal expansion with immune transcriptional activation	Distinguish baseline predictive biomarkers from dynamic treatment-response markers	(43)
Gettinger *et al*, 2017	Acquired loss of antigen-presentation competence	HLA-I antigen-processing and presentation defects, including B2M loss	Progression biopsy with sequencing and HLA-I/APM profiling	Progression or relapse	Loss of tumor recognition by $CD8^+$ T cells	Distinguish acquired immune escape from absent baseline immune engagement	(98)

PTM, post-translational modification; PD-L1, programmed death-ligand 1; PD-1, programmed cell death protein 1; IFNγ, interferon gamma; IL-11, interleukin 11; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; H3K9, histone H3 lysine 9; CD, cluster of differentiation; TCR, T-cell receptor; HLA-I, human leukocyte antigen class I; B2M, beta-2 microglobulin; APM, antigen-processing machinery.

formation in cells (237), whereas a trastuzumab-PROTAC conjugate restricted BRD4 degradation to HER2-positive BC cells while sparing HER2-negative cells (238). Conversely, MD-224, originally developed as an MDM2 degrader, was subsequently shown to degrade PXR and additional nuclear receptors through a non-canonical binding surface (239). Proteome-wide screening has further demonstrated that subtle chemical changes can markedly alter neo-substrate specificity and that some degraders induce substantially broader protein regulation than others (240). Therapeutic-window engineering should therefore prioritize substrate or molecular-state selectivity, tumor-directed delivery, and proteome-wide degradation profiling before targeted degradation is assumed to reduce systemic toxicity. To make this framework more operational from a biomarker perspective, Fig. 4 maps dominant state constraints to the corresponding readout layer, assay layer, and treatment-phase context in which each state is most informatively captured.

Within the framework proposed in the present review, rational combination design should therefore be guided by the prioritized state constraint or combination of co-dominant constraints rather than by PTM class alone. Checkpoint-dominant states are most plausibly addressed by strategies that reduce the persistence or effective engagement of the inhibitory surface pool (8). Visibility-deficient states are more likely to benefit from interventions that restore antigen presentation and preserve IFN-linked reinforcement (24,200). Rewiring-dominant states, by contrast, are more likely to require early interruption of suppressive stabilization before dysfunction becomes fixed (26). Toxicity should accordingly be treated as part of the design logic rather than as a downstream complication, because clinically useful translation will depend on demonstrating that the intended PTM-state dependency can be perturbed at exposures that remain tolerable in relevant normal tissues and in combination regimens that avoid unnecessary overlap of immune-mediated or organ-specific adverse effects. A toxicity-aware framework is therefore essential if PTM-directed strategies are to move from mechanistic plausibility toward clinically usable combination design.

Public-data analysis and structural bioinformatics as supporting triage layers for PTM-state validation. With these resource layers in place, a supporting practical task is to determine which PTM-linked signals merit downstream validation, and which should remain at the level of mechanistic interest. Once a dominant state constraint and candidate PTM readout have been defined, PTM-aware public-data analysis can serve as a compact and reproducible framework for prioritizing features for deeper functional and clinical validation. In practice, candidate PTM enzymes, readers, or modification-sensitive substrates can first be anchored to curated PTM resources that provide site-level annotation and regulator-substrate relationships (57). These candidates can then be evaluated in proteogenomic cohorts that integrate proteomic, phospho-proteomic, transcriptomic and clinical layers, allowing alignment with immune phenotypes, pathway activity and clinical state (49,50). Proteogenomic concordance alone does not establish whether a candidate PTM directly alters molecular recognition or complex stability. Structural bioinformatics can therefore provide a complementary triage

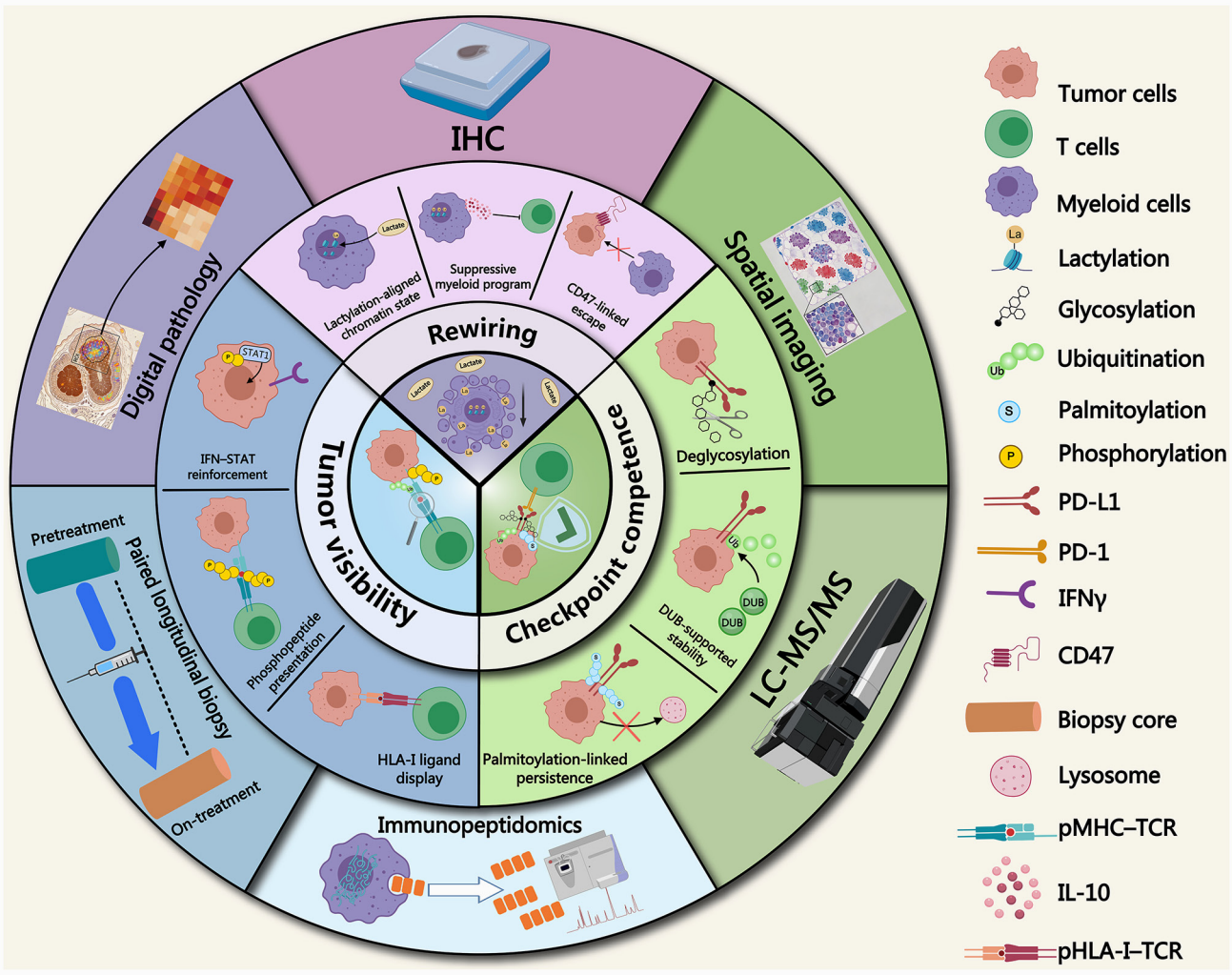

Figure 4. State constraints, PTM-state and companion readout modules, assay platforms and treatment-phase deployment in cancer immunotherapy. This figure summarizes a constraint-guided framework for biomarker deployment in cancer immunotherapy. The innermost layer defines three recurrent state constraints that limit durable antitumor immunity: Checkpoint competence, tumor visibility, and stress-conditioned immune-state programming. The intermediate readout layer highlights representative direct PTM-state, proximal protein-state, and contextual companion readouts aligned with each constraint. Not all readouts in this layer directly measure a PTM; transcriptomic, spatial, cellular and longitudinal features are included as companion measurements that contextualize functional state. The outer assay layer organizes the principal analytical platforms through which these states can be captured in tissue or longitudinal samples. The figure is intended to guide discovery-to-validation translation by aligning each constraint with the readout class, assay layer, and clinical timepoint most suitable for stratification or monitoring. PTM, post-translational modification; IHC, immunohistochemistry; IFN, interferon; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; pMHC, peptide-major histocompatibility complex; pHLA, peptide-human leucocyte antigen; TCR, T cell receptor; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

layer between molecular association and empirical validation. For glycosylated proteins, PTM-aware structural reconstruction and glycan conformational modeling can restore missing three-dimensional glycan information and estimate modification-dependent changes in protein surface accessibility (241,242). Molecular modeling of glycosylated TIM-3 further showed that N-glycan conformation and composition can alter ligand stabilization at an immune-checkpoint binding site, illustrating the value of docking-based analysis for prioritizing modification-sensitive binding sites (243). For palmitoylated membrane proteins, membrane-aware molecular dynamics can resolve structural effects that depend on bilayer context. In PD-L1, microsecond-scale all-atom simulations supported by FRET and immune-eliminating experiments linked palmitoylation-dependent raft partitioning to altered membrane orientation and PD-1 association (244). Structural predictions should therefore be used to prioritize PTM-state mechanisms with plausible effects on epitope accessibility, ligand recognition, or complex assembly. They do not establish functional causality and require biochemical, biophysical and cell-based confirmation before translational prioritization. Where available, immuno-peptidomic atlases can determine whether PTM-linked variation is accompanied by altered presentation of modified peptides or broader shifts in displayed antigenic content (51,58). Spatially resolved datasets can further test whether the inferred states localize to tumor cells, myeloid compartments, or effector lymphocytes and whether interface-level organization is linked to immune outcome (52,97).

The analytical value of this strategy lies in moving beyond descriptive association toward constraint-resolved inference. A practical validation framework should establish whether a candidate feature maps preferentially to one dominant state constraint, remains reproducible across more

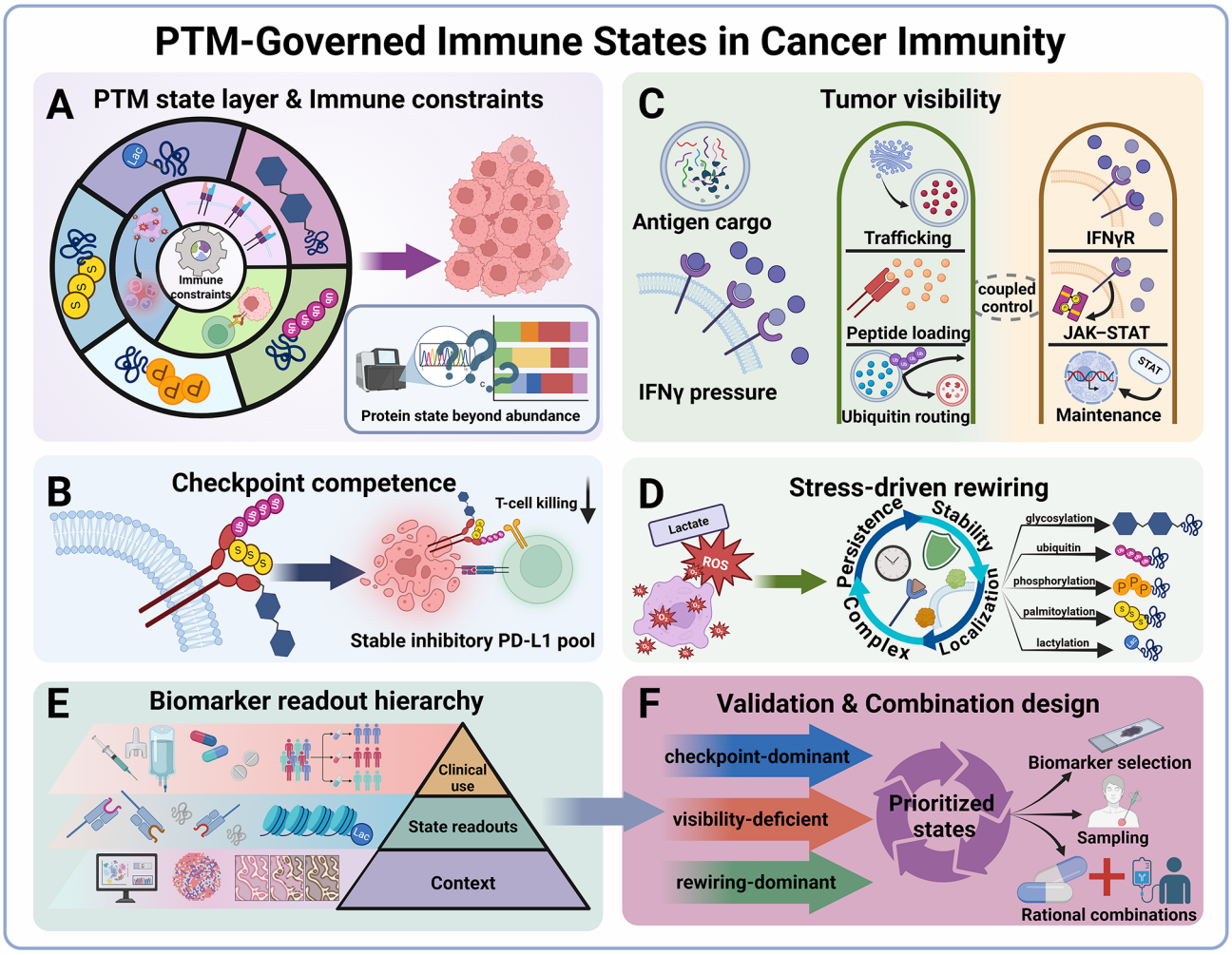

Figure 5. Integrated framework of PTM-governed immune-state regulation in cancer immunity. (A) PTM-state layer linking protein stability, localization, trafficking, complex assembly and signaling competence to immune constraints, with emphasis on protein state beyond abundance. (B) Checkpoint competence shaped by PTM-dependent stabilization and membrane persistence of inhibitory checkpoint proteins, particularly PD-L1, with consequent suppression of T-cell elimination capacity. (C) Tumor visibility regulated by antigen processing, peptide loading, antigen-presentation machinery and IFNγ-JAK-STAT signaling. (D) Stress-driven immune rewiring induced by lactate, reactive oxygen species, and other tumor-microenvironmental pressures through glycosylation, ubiquitination, phosphorylation, palmitoylation and lactylation. (E) Biomarker readout hierarchy integrating contextual companion information, state-proximal PTM readouts, and clinical utility. (F) Validation and combination design based on prioritized or co-dominant immune-state constraints, including checkpoint-dominant, visibility-deficient, and rewiring-dominant states. PTM, post-translational modification; PD-L1, programmed death-ligand 1; IFN, interferon; ROS, reactive oxygen species.

than one molecular layer, and tracks with treatment phase in pretreatment, on-treatment, or progression-associated settings (43,49,66). Features that satisfy these criteria are better positioned to support biomarker refinement, patient stratification, and rational combination design. Framed in this way, public-data and structural analyses provide complementary triage layers between PTM mechanism and clinically oriented validation, sharpening candidate prioritization without displacing fit-for-purpose experimental confirmation.

Framework boundaries and translational limitations. Several boundaries of the proposed framework should be acknowledged. First, the five core exemplar PTM axes emphasized in the present review represent a selective set of mechanistically supported state-control routes rather than an exhaustive account of PTM regulation in cancer immunity. Additional modification classes, non-enzymatic protein-state changes, and higher-order PTM crosstalk are discussed selectively when they illuminate the same functional state variables or define the boundaries of the framework; they are not intended to constitute equally developed core axes of the synthesis. Second, checkpoint competence, tumor visibility and stress-conditioned immune-state programming are intended as translational coordinates rather than a complete taxonomy of antitumor immunity. Evidence remains uneven across immune compartments, tumor lineages, and tissue contexts and is currently weighted toward selected checkpoint, antigen-presentation, T-cell and myeloid programs. Third, prioritizing a dominant state constraint necessarily simplifies a biologically coupled and dynamic system. Multiple constraints may coexist within the same lesion, differ across spatial compartments, or shift between pretreatment, on-treatment and resistant states (56,219-222). Accordingly, state assignment should not force mutually exclusive classification: Mixed, co-dominant and indeterminate states should be retained when the available spatial, molecular, or longitudinal

evidence does not support a single predominant constraint. Finally, much of the mechanistic evidence discussed in the present review derives from defined experimental models or selected cancer cohorts. Prospective clinical validation will therefore require prespecified intended use, analytically valid PTM-state readouts, and longitudinal sampling strategies aligned with the relevant clinical decision point (205,210). These boundaries constrain immediate clinical extrapolation and should be considered when selecting candidate readouts or intervention targets. The proposed framework should therefore be regarded as a prioritization strategy for organizing mechanistically proximal and measurable protein states rather than as a comprehensive inventory of immune effectors or PTM-dependent mechanisms. Its translational value will ultimately depend on whether the proposed state variables remain reproducible, contextually interpretable, and clinically informative across independent prospective cohorts (Fig. 5).

8. Conclusions and future perspectives

The present review argues that the biological and translational significance of PTMs is best understood through the immune constraints they regulate, not through an isolated catalogue of modification chemistries. From this perspective, diverse PTM programs converge on three interconnected processes: Checkpoint competence, tumor visibility and stress-conditioned immune-state programming. Across these processes, distinct PTM classes regulate a limited set of functional state variables, including protein stability, subcellular localization, complex assembly and signaling persistence (245,246). This framework helps explain why PD-L1 should be interpreted as a protein-state problem rather than a simple abundance marker, why tumor visibility depends on sustained antigen-presentation competence and IFN-linked reinforcement rather than inflammatory tone alone, and why lactylation represents a mechanistically coherent route by which metabolic stress is translated into durable immunosuppressive programs. The central implication is that the most actionable unit for biomarker discovery, validation and therapeutic intervention is not the PTM class itself, but the prioritized state constraint, or combination of co-dominant constraints, that sustains immune evasion and informs patient stratification across treatment phases.

Future studies should therefore move beyond static abundance-based measurements and establish phase-aware biomarker workflows anchored to functional state across pretreatment, on-treatment and progression settings. Key priorities include the discovery of compact PTM-state candidates in spatially and proteogenomically resolved clinical samples, validation of fit-for-purpose readouts linked to prioritized or co-dominant state constraints, and clarification of which PTM-regulated immune states remain reversible, and which become fixed during resistance. Equally important is the development of clinically interpretable biomarker panels that support patient stratification, response monitoring and rational combination selection rather than descriptive PTM cataloguing alone. The next decisive step is not further expansion of PTM inventories, but prospective validation of compact PTM-state biomarker panels that can guide stratified immunotherapy in real clinical use contexts.

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Authors' contributions

JP, BL, ZH and YC conceived the review topic and designed the overall conceptual framework. JP and BL drafted the manuscript. RL, CF and LYL contributed to literature collection, organization and critical revision of the manuscript. ZH and YC supervised the work and revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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