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Review

From Molecular Biomarkers to Dynamic Biological States in Precision Immunotherapy

Razieh Dindarlou [1,2,3], Javid Ashtari Mahini [4], Salman Saeidi [5], Hiva Hajijafari [6], Zohreh Jafarinia [7], Leyla Nazarpoorakbari [8], Fatemeh Siahmarzkouhi [1,2,9,*] and Bita Rezaei [10]

[1] College of Medical Sciences, University of Arizona International, Mesa, AZ 85201, USA; r.dindarlou@iau.ac.ir or r_dindarlou@yahoo.com

[2] Pasargad Biochemistry Company, Avicenna Health Technology Development Center, Research Institute for New Technologies of Biological Sciences, Avicenna Research Institute, Shahid Beheshti University, Tehran 1983969411, Iran

[3] Department of Biochemistry, Shahrood Branch, Islamic Azad University, Shahrood 4318936199, Iran

[4] Department of Cellular and Molecular Biology, Islamic Azad University, Tehran 1651153511, Iran; javidashtarimahini@gmail.com or javid.ashtarimahini@iau.ir

[5] Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan 6513613147, Iran; Salman.saeidi@gmail.com

[6] Faculty of Pharmacy, Biruni University, Istanbul 34015, Türkiye; hiva81jafari@gmail.com

[7] Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran 1411713116, Iran; zhrh200@yahoo.com

[8] Department of Comparative Bioscience, Faculty of Veterinary Medicine, University of Tehran, Tehran 1419963111, Iran; Leylanazarpoorakbari@gmail.com

[9] Department of Biochemistry, Sanandaj Branch, Islamic Azad University, Sanandaj 6616935391, Iran

[10] Ministry of Education Iran, Tehran 1416633455, Iran; rezaebita30@gmail.com

[*] Correspondence: f.siahmarzkouhi@iau.ac.ir or f66marzkoohi@gmail.com

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Abstract: Precision immunotherapy has demonstrated that molecular and pathological features can inform therapeutic sensitivity, yet most biomarker frameworks remain anchored to a pretreatment snapshot of a biological system that subsequently changes. Tumour–immune phenotypes emerge from interactions among tumour-intrinsic programmes, antigen presentation, immune-cell states, metabolism, stromal organization and tissue architecture, and these processes are reshaped by therapeutic pressure. We propose that the relevant unit of precision should therefore extend from individual biomarkers to integrated biological states and, ultimately, to the trajectories through which those states evolve during treatment. In this framework, multimodal measurements are used not simply to classify patients, but to reconstruct a context-specific biological state, identify the dominant constraint on therapeutic efficacy and determine whether that constraint remains modifiable. Longitudinal profiling adds information about the direction, rate and persistence of biological change, allowing response and resistance to be interpreted as evolving processes rather than isolated clinical endpoints. We distinguish state estimation from mechanistic inference and intervention, emphasizing that predictive association alone does not establish therapeutic actionability. This framework suggests a transition from biomarker-guided selection toward mechanism-informed precision immunotherapy, in which treatment decisions may eventually incorporate validated informa-

tion about an individual's evolving tumour–immune state. The immediate challenge is not maximal molecular profiling, but identification of the smallest reproducible set of measurements capable of resolving clinically relevant biological uncertainty and demonstrating that acting on this information improves patient outcomes.

Keywords: Precision Immunotherapy; Tumour–Immune Ecosystem; Biomarkers; Longitudinal Profiling; Therapeutic Resistance; Multi-Omics; Spatial Biology; Mechanism-Informed Therapy

1. Introduction

Precision immunotherapy has established a clinically important principle: molecular and pathological features of a tumour can contain information about the probability of therapeutic benefit. PD-L1 expression, mismatch-repair deficiency and microsatellite instability, and tumour mutational burden have each demonstrated clinical utility in defined settings, although their performance depends on tumour type, treatment context, assay characteristics and biological heterogeneity [1–4]. The framework proposed here does not imply that all biomarkers must be reassessed dynamically. For example, MSI-H/dMMR status can be sufficient for particular treatment-selection decisions; serial measurement of that status is not inherently required simply because other determinants of response may evolve. The value of longitudinal assessment must therefore be judged against a specific clinical question and whether new information could change management. These successes have also exposed a deeper limitation. The determinants of immune-mediated tumour control are distributed across interacting biological layers and are not confined to a single molecular alteration, cellular population or anatomical compartment. Tumour-intrinsic programmes, antigen presentation, immune-cell function, metabolism, stromal architecture and tissue organization jointly shape therapeutic response, while each can change under treatment [5–8].

This distinction requires three related but non-equivalent concepts. The tumour–immune state refers to the biological configuration of a tumour and its associated immune microenvironment at a specified time, as characterized by features relevant to a particular research or clinical question. An integrated biological state is a context-specific representation of that tumour–immune state, constructed by combining complementary measurements relevant to a predefined biological or therapeutic question. An inferred state, in turn, is a model-derived estimate or classification based on available measurements and an explicit analytical framework. These distinctions separate the biological system being characterized from its integrated measurement-based representation and from any model-derived interpretation. They also preserve the uncertainty arising from incomplete measurement, analytical assumptions and biological complexity.

A biomarker measures a feature of the system; a biological state represents a configuration of interacting processes. The distinction matters because similar molecular measurements can arise from different biological configurations, whereas similar functional states can emerge through different molecular routes. The cancer-immunity cycle provides a complementary conceptual framework for understanding how successive steps in antitumour immunity depend on coordinated interactions between tumour cells and the immune system [9,10].

Increasing the number of measured variables therefore does not necessarily produce a more useful representation of the patient.

The problem is particularly acute in immunotherapy because treatment perturbs an evolving tumour–immune ecosystem rather than acting on a static biological substrate. Antigen presentation, tumour-cell fitness, immune-cell differentiation, tissue accessibility, stromal interactions and compensatory signalling may change during treatment. Longitudinal studies of immune checkpoint blockade have provided direct evidence that tumour and immune states can evolve under therapeutic pressure, including through changes affecting antigen presentation and interferon signalling [11–13]. A pretreatment biomarker can therefore remain statistically predictive while failing to explain the biological state that subsequently determines response, adaptation or resistance.

The central challenge is consequently shifting from asking which patients carry a biomarker to asking what biological state those measurements represent, how that state changes under therapeutic pressure and which components remain potentially modifiable. Importantly, this perspective does not imply that pretreatment biomarkers are intrinsically inadequate or that all clinically relevant biological features must be monitored longitudinally. Some biomarkers capture relatively stable biological properties that provide sufficient information for a defined

therapeutic decision. MSI-H/dMMR, for example, identifies a tumour phenotype associated with sensitivity to immune checkpoint blockade and can support treatment selection without requiring serial reassessment of mismatch-repair status in every patient. The persistence of such a feature, however, does not imply that all determinants of therapeutic response remain stable. Tumour antigen presentation, immune-cell function, tissue accessibility and compensatory signalling may evolve despite persistence of the original biomarker. The value of longitudinal profiling therefore depends on whether evolving biological processes introduce clinically consequential uncertainty that cannot be adequately resolved by baseline measurements alone. Although the framework may be relevant to multiple forms of immunotherapy, this Review focuses primarily on immune checkpoint blockade in solid tumours. Its extension to other modalities, including adoptive cell therapies, cancer vaccines and bispecific antibodies, requires separate consideration of their mechanisms of action, relevant biological measurements, treatment timescales and clinical endpoints. Its applicability across these settings therefore remains to be established. The objective is not to replace static biomarkers with dynamic measurements, but to identify when information about biological change provides additional value for a specific clinical decision.

This Review develops a framework around that transition. We first consider the biological architecture underlying tumour–immune state and the limits of individual molecular layers. We then examine how complementary measurements can be combined to construct an integrated biological state, how longitudinal profiling converts static measurements into information about trajectories, and how such trajectories may reveal mechanisms of therapeutic failure. Finally, we consider the requirements for translating state and trajectory information into mechanism-informed and potentially adaptive treatment strategies.

The central proposition is deliberately narrower than a call for ever more comprehensive profiling. The objective is to identify the minimum sufficient biological representation required to resolve a clinically relevant uncertainty. In this view, precision immunotherapy progresses from molecular measurement to biological state, from state to trajectory, and from trajectory to mechanism-informed intervention. The critical question is therefore not how much biology can be measured, but how much biological information is required to make a clinically meaningful decision (**Figure 1**).

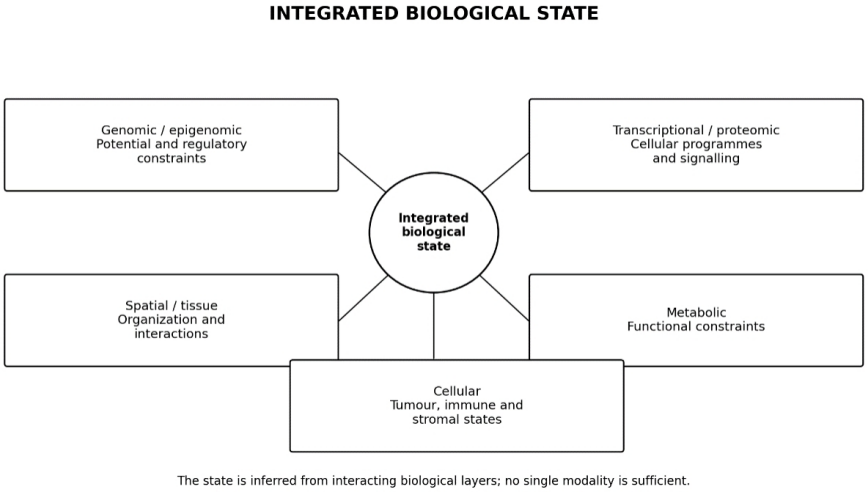

Figure 1. From molecular measurements to an integrated tumour–immune state. Schematic representation of genomic, transcriptional, cellular, metabolic, and spatial data integration to infer a biological or therapeutic state.

2. Literature Search and Conceptual Synthesis

The literature search was an iterative, purposive search designed to support a conceptual synthesis rather than a systematic or scoping review. PubMed, Scopus and Google Scholar were consulted for foundational and recent work on tumour immunology, immune-checkpoint blockade, biomarkers, multi-omics integration, immune-cell states, metabolism, spatial profiling, longitudinal monitoring, therapeutic resistance and computational inference. Representative search terms and combinations included 'immune checkpoint blockade' AND (biomarker OR resistance OR response); 'tumour immune microenvironment' AND (spatial OR single-cell OR metabolism); 'multi-

omics' AND (integration OR machine learning OR latent-variable); and (ctDNA OR circulating tumour DNA) AND (immunotherapy OR longitudinal monitoring OR pseudoprogression). For this revision, targeted reference checks were updated on 16 October 2026. The original search log did not preserve exact search dates or exportable record-level counts.

Publications were retained when they directly supported a biological, methodological or translational claim, provided foundational context, or represented relevant longitudinal or clinical evidence. Priority was given to peer-reviewed primary studies, well-established reviews and guidance documents; a source was not treated as evidence of clinical utility unless its design supported that inference. References were checked for duplicates using title, first author, year and DOI during preparation of the revised list, but a formal record-level deduplication log from the original search was not available. The final evidence set is therefore a purposive selection, not an exhaustive set of eligible studies. The synthesis is organized across four connected levels: molecular measurement, construction of an integrated biological state, characterization of longitudinal biological trajectories, and mechanism-informed intervention. Computational inference is considered an analytical approach that may support the construction and interpretation of integrated biological states and their trajectories, rather than a separate biological level.

3. The Biological Architecture of the Tumour–Immune State

Immune phenotype is often summarized using individual biomarkers or broad categories such as inflamed, excluded or immune-desert tumours. These classifications are useful abstractions, but they compress a system whose determinants operate across multiple levels [5, 14]. Genomic alterations influence antigenicity and evolutionary potential; epigenetic programmes regulate transcriptional competence and cellular plasticity; transcriptional and protein states reflect cellular activity; metabolism constrains function; and tissue architecture determines which cells can interact and under what conditions.

These layers should not be interpreted as a simple hierarchy. Feedback, environmental signalling and evolutionary selection generate extensive cross-talk among them. A transcriptional programme may reflect both tumour-intrinsic signalling and extracellular cues. Metabolic conditions can constrain immune function while selecting for tumour-cell adaptations. Spatial organization can reinforce molecular states through local interactions. The tumour–immune state is therefore better understood as an emergent property of a coupled biological system, of which immune phenotype represents one component, rather than as the output of any individual molecular layer [5,6,14].

3.1. Genomic and Epigenomic Potential

The genomic landscape establishes part of the biological potential from which immune phenotype emerges. Mutations, copy-number alterations, structural variation and mutational processes can influence neoantigen generation, tumour evolution and susceptibility to immune recognition [15–17]. Alterations affecting antigen processing and presentation can reduce tumour visibility, while acquired defects in interferon signalling may blunt tumour-cell responses to immune pressure [11,12,18].

Genomic information, however, does not establish functional state. The presence of a mutation indicates a biological possibility or constraint but does not demonstrate pathway activity in a particular cellular or spatial context. Likewise, predicted antigenicity does not establish antigen expression, processing, presentation or productive immune recognition [19,20].

Epigenetic regulation adds another layer between genetic potential and phenotype. Chromatin accessibility, DNA methylation and histone-associated regulation can constrain or permit transcriptional programmes and influence cellular plasticity under environmental and therapeutic pressure [21,22]. Two tumours with similar genomic features may therefore occupy different functional states.

Genomics and epigenomics are consequently best viewed as descriptions of potential and regulatory constraint rather than complete measurements of immune function.

3.2. Transcriptional and Proteomic States

Transcriptomic measurements move closer to phenotype by capturing coordinated cellular programmes. Inflammatory signalling, interferon responses, antigen-presentation programmes, proliferation, stress responses and

differentiation states can emerge as reproducible transcriptional configurations. Such programmes are often more informative than isolated genes considered independently [22,23].

Yet transcription is not equivalent to molecular execution. RNA abundance does not necessarily predict protein abundance, localization, activation or post-translational modification. Proteomic and phosphoproteomic measurements can therefore reveal signalling states that are poorly represented at the transcript level.

This distinction is particularly important when interpreting immune dysfunction. A transcriptional programme associated with T-cell dysfunction does not, by itself, establish functional capacity or reversibility. Similarly, expression of an inhibitory receptor does not demonstrate that its pathway constitutes the dominant therapeutic constraint [24–27].

Molecular signatures should therefore be interpreted according to their position within the regulatory system. Expression is evidence of a programme; it is not necessarily evidence of its functional consequence.

3.3. Metabolic and Cellular Constraints

The metabolic environment adds another level of biological constraint. Hypoxia, nutrient competition, altered metabolite availability and systemic metabolic conditions can influence immune-cell survival, proliferation and effector function [7,28,29]. A cell may retain the transcriptional machinery required for an immune response while remaining functionally constrained by its environment.

At the cellular level, single-cell approaches have revealed extensive heterogeneity among malignant, immune and stromal populations [30–32]. Broad categories conceal distinct functional states, and bulk measurements can conflate changes in cellular composition with changes within individual cells.

Single-cell resolution nevertheless does not by itself reconstruct function. Cells operate within networks of neighbouring cells, extracellular matrix, vascular structures and local metabolites. The relevant biological unit can therefore shift from the individual cell toward relationships among cellular states.

3.4. Spatial Organization and Tissue Context

Spatial organization determines whether potentially effective immune interactions can occur. Immune abundance alone does not establish immune access to malignant cells. Vascular function, stromal barriers, tumour architecture and local cellular neighbourhoods can determine whether immune populations reach, recognize and engage their targets [33–35].

Spatial technologies have consequently extended the interpretation of immune phenotype from cellular abundance to cellular organization. Two tumours can contain similar proportions of cytotoxic lymphocytes while differing substantially in whether those cells occupy tumour nests, stromal regions or tumour–immune interfaces. Researchers have demonstrated structured spatial organization and its association with clinical outcomes in triple-negative breast cancer; this constitutes evidence of spatial association rather than proof that spatial features predict differential benefit from a specific treatment [29,36].

Spatial information is nevertheless incomplete. Physical proximity does not establish functional interaction, inferred cell–cell communication remains model dependent, and current platforms involve trade-offs among spatial resolution, molecular depth, tissue coverage and throughput [33,34].

The resulting picture is therefore not a sequence in which one molecular technology supersedes another. Each modality observes a different component of the tumour–immune state, and the principal challenge is determining which combination of complementary observations is sufficient to construct an integrated biological state that addresses the biological question at hand. Such a representation should remain context-specific and should not be mistaken for a complete or definitive reconstruction of the underlying biological system.

4. From Multimodal Measurements to an Integrated Biological State

The expansion of multi-omics has changed the limiting problem in precision immunotherapy. The challenge is increasingly not whether a biological feature can be measured, but whether heterogeneous measurements can be converted into an interpretable representation of the system [37–39].

We define an integrated biological state as a context-specific representation of the tumour–immune state, constructed by combining complementary measurements relevant to a predefined biological or therapeutic question.

This is distinct from a molecular signature. A signature describes a reproducible pattern of measurements, whereas an integrated biological state provides a structured representation of the biological processes that the combined measurements are intended to characterize.

An integrated biological state is not intended to replace multivariable biomarker panels, immune archetypes, or latent-variable models, but to clarify how their outputs may be interpreted in relation to a specific biological or clinical question. Multivariable biomarker panels combine measured features to estimate a defined outcome or biological property, as illustrated by integrated genomic prediction studies [9], whereas immune archetypes describe recurring patterns of immune organization. Latent-variable models identify lower-dimensional representations of variation across measured features, although their components do not necessarily correspond to distinct biological mechanisms. The proposed framework adds a decision-oriented interpretive layer by relating observed features to a provisional, testable account of the biological constraint that may be limiting therapeutic response, specifying competing mechanistic explanations, and identifying the additional evidence needed to distinguish them. Its added value therefore lies not in integration alone, but in making the interpretation testable and linking it to a clinically relevant question. Whether this approach improves explanatory or decision-making value beyond existing biomarkers and models remains an empirical question requiring validation.

This distinction is important because biological states are only partially observable. No current assay simultaneously captures tumour evolution, immune-cell function, metabolism and spatial organization at complete resolution. State must therefore be inferred from incomplete observations.

An integrated biological state is a context-specific representation of the tumour–immune system constructed by combining available molecular, cellular and tissue measurements in relation to a defined therapeutic question. It is not a directly measured biological object and should not be treated as a complete or definitive reconstruction of the underlying biological system. Its credibility depends on the relevance and quality of the integrated measurements, biological coherence, and robustness across datasets and platforms. When a computational model is used to estimate or classify this representation, the resulting output constitutes an inferred state, whose interpretation must account for model assumptions and measurement uncertainty.

An inferred state is useful only insofar as it provides a parsimonious representation of heterogeneous observations without discarding information necessary to address the clinical question. The objective is therefore not to recover an unknowable complete state, but to estimate the smallest state representation that distinguishes clinically relevant hypotheses [40].

The concept of a minimum sufficient representation should be treated as a testable research objective rather than an established property of an integrated biological state. Sufficiency must be defined relative to a specific clinical decision, such as whether to continue treatment, obtain additional diagnostic information, or consider a mechanism-directed intervention. A candidate representation should be evaluated against an appropriate baseline strategy, including established biomarkers, routine clinical variables, and, where relevant, a simpler model using fewer measurements. Practical criteria should include analytical and classification reproducibility, incremental decision value beyond the baseline, the frequency with which potentially actionable mechanisms are missed, uncertainty in state assignment, and measurement burden in terms of tissue requirements, assay complexity, turnaround time, cost, and accessibility. Acceptable uncertainty should depend on the consequences of the decision: a high-risk treatment change may require stronger evidence than a decision to obtain additional testing. The minimum sufficient representation would therefore be the least burdensome set of measurements that supports the intended decision with prespecified and clinically acceptable reliability, without an unacceptable loss of clinically actionable information. Whether such a representation can be identified and generalized across patients, platforms, and treatment settings requires prospective validation.

To make this framework operational, biological-state inference can be organized into five steps. First, define a clinically relevant question, such as whether a patient is experiencing primary resistance or is likely to develop resistance during treatment. Second, formulate competing biological hypotheses that could explain the observed clinical pattern. Third, select the minimum set of measurements needed to distinguish these hypotheses, considering biological relevance, assay reliability, clinical feasibility, and the availability of suitable samples. Fourth, construct an interpretable state representation and assess whether it generates testable predictions that distinguish between the competing explanations. Finally, evaluate these predictions in independent datasets or prospective studies and determine whether the inferred state provides meaningful explanatory or decision-making value be-

yond established biomarkers or simpler models. The measurements and analytical methods should be selected according to the clinical question; comprehensive multimodal profiling is not inherently preferable to a smaller, hypothesis-driven assessment.

This also clarifies why more dimensions do not necessarily produce better precision. High-dimensional models may discriminate patients while failing to identify a mechanism. Conversely, a relatively simple representation may be clinically more useful if it identifies a dominant constraint that can be tested experimentally or therapeutically [40].

Consider a hypothetical patient with metastatic melanoma receiving PD-1 blockade whose tumour has a high tumour mutational burden (TMB), an interferon-γ (IFN-γ)-associated gene-expression signature, and a spatially excluded $CD8^+$ T-cell infiltrate. These findings capture different aspects of tumour–immune biology. High TMB suggests the potential for immune recognition, while an IFN-γ-associated signature indicates inflammatory signalling. However, neither finding establishes that immune cells can effectively access and eliminate tumour cells. Spatial profiling adds information about whether $CD8^+$ T cells are positioned to interact with malignant cells. The combination of these findings may therefore provide a more informative interpretation than any single biomarker considered in isolation.

In this scenario, the inferred state could be described as an inflammatory tumour microenvironment in which effective antitumour immunity may be constrained by the limited access of $CD8^+$ T cells to tumour cells. The dominant working hypothesis would be that spatial exclusion limits the conversion of inflammatory signalling into effective tumour-cell killing. This hypothesis does not establish that spatial exclusion is the sole cause of resistance. Impaired antigen presentation, dysfunctional T cells, or other tumour-intrinsic and microenvironmental factors could produce a similar clinical outcome. The purpose of defining the state is therefore not to assign a definitive label, but to identify a plausible biological constraint that can be investigated.

Operationally, this state representation should retain the distinction between observed features and inferred mechanisms. In the melanoma example, TMB, the IFN-γ-associated signature, and the spatial distribution of $CD8^+$ T cells are observed features, whereas spatial exclusion as the dominant constraint is a mechanistic interpretation that remains to be tested. The interpretation should be assessed against competing explanations and, where suitable data are available, compared with conclusions drawn from individual biomarkers or simpler models. Its robustness should also be examined across independent samples or datasets, with attention to measurement uncertainty and whether the available tissue adequately represents the tumour. This process makes the inferred state a provisional, evidence-based representation rather than a fixed classification.

The clinical implication is to determine whether this proposed constraint is supported by additional evidence and whether resolving it could change management. Where clinically appropriate and feasible, spatially resolved tissue analysis could assess the distribution of $CD8^+$ T cells relative to tumour cells, together with relevant features of antigen presentation and the surrounding tissue architecture. If persistent spatial exclusion is supported and a treatment targeting the implicated mechanism has an adequate evidential basis, that strategy could be considered in an appropriate clinical context, including a clinical trial where relevant. The state-based interpretation would thus guide the next question to investigate rather than automatically dictate a specific treatment.

The hypothesis must also remain falsifiable. If further analysis shows that $CD8^+$ T cells have penetrated tumour regions and are engaging malignant cells despite continued disease progression, spatial exclusion becomes a less convincing explanation for treatment failure. The interpretation should then be revised, and alternative mechanisms, such as impaired antigen presentation or tumour-cell-intrinsic resistance, should be considered. Similarly, if the IFN-γ-associated signature is not accompanied by evidence of meaningful antitumour immune activity, the assumption that the tumour is functionally inflamed would require reassessment.

This example illustrates how a biological state can connect multiple measurements to a testable mechanistic hypothesis and a clinically relevant next step. The inferred state is neither a direct measurement nor an unquestionable model output; it is a provisional interpretation whose value depends on whether it explains the observed biology, informs a consequential decision, and can be revised when new evidence challenges it [41,42].

The distinction between state and mechanism is therefore essential. Reduced immune infiltration describes a state but does not establish why immune cells are absent. The underlying limitation may involve vascular dysfunction, stromal organization, chemokine distribution, tumour-intrinsic programmes or several interacting mechanisms [33–35]. Likewise, a dysfunctional T-cell state does not establish that additional inhibitory-receptor block-

ade will restore function [25–27].

An actionable state requires a stronger chain of inference: the observed measurements should support a biological configuration; that configuration should identify a plausible functional constraint; and the constraint should suggest a mechanism whose modification could reasonably alter the state.

The framework must also be evaluated against explicit conditions of failure. An inferred state should generate testable predictions about measurable biological features, treatment response or the effects of a proposed intervention. If these predictions repeatedly fail despite adequate measurement and appropriate validation, the inferred state or its mechanistic interpretation should be revised or rejected rather than retrospectively attributed to an unspecified state transition. At the framework level, if an integrated representation does not improve explanatory or predictive performance, or support more informative treatment decisions, beyond a simpler baseline approach in a defined clinical setting, its added value in that setting remains unestablished. These criteria distinguish a testable approach to state inference from a descriptive lens that can accommodate any outcome.

This hierarchy provides a more stringent criterion for multi-omics than molecular accumulation. The value of integration lies not in the number of assays incorporated, but in whether their combination resolves an uncertainty that is consequential for treatment (**Table 1**). The framework should not assume that a single dominant therapeutic constraint can always be identified. Multiple mechanisms may coexist, interact, or produce similar molecular and cellular measurements, making a unique mechanistic explanation difficult to establish. In such cases, competing explanations should be retained explicitly, and the inferred biological state should reflect the level of uncertainty supported by the available evidence rather than forcing a single causal interpretation. Where the evidence cannot distinguish between plausible mechanisms, the result should be considered unresolved and may justify further testing or monitoring rather than a treatment change. A change in treatment should be considered only when the evidence is sufficiently reliable, the proposed mechanism is clinically relevant, and an alternative intervention has an appropriate evidential basis; otherwise, established clinical management should take precedence.

Table 1. Key terminology used in the biological-state framework.

Term	Definition in This Review	Role in the Framework
Molecular signature	A reproducible pattern of molecular measurements associated with a biological process, phenotype, or clinical outcome.	Describes a measured pattern but does not necessarily explain its underlying mechanism.
Multivariable biomarker panel	A combination of biomarkers used to estimate a defined biological property or clinical outcome.	Supports prediction, classification, or risk assessment.
Immune archetype	A recurring pattern of immune composition, organization, or functional characteristics within a tumour or its microenvironment.	Describes shared features across samples or identifies biologically meaningful groups.
Latent-variable model	A statistical model that infers unobserved variables or lower-dimensional representations from relationships among measured features.	Provides a computational approach to identifying shared patterns in complex data.
Tumour–immune state	The biological configuration of a tumour and its associated immune microenvironment at a specified time, defined by the measured features relevant to a particular research or clinical question.	Defines the biological system being characterized, without implying that all relevant components have been measured or that its underlying mechanisms are fully known.
Integrated biological state	A context-specific representation of the tumour–immune state constructed by integrating complementary measurements relevant to a predefined biological or therapeutic question.	Combines relevant evidence into a structured representation that can support biological interpretation, longitudinal comparison, and clinical hypothesis generation.
Inferred state	A model-derived estimate or classification of the biological state based on available measurements and an explicit analytical framework.	Makes the estimated state operational for analysis or decision support while retaining uncertainty arising from measurement limitations and model assumptions.
Mechanistic inference	A hypothesis about the biological processes that may explain an observed state or treatment outcome.	Generates explanations that require independent biological or experimental evaluation.
Biological trajectory	The pattern of change in an inferred biological state over time or following a therapeutic perturbation.	Helps characterize evolving responses, resistance, or transitions between biological states.
Actionable biological constraint	A hypothesized biological limitation whose modification could plausibly change the relevant biological state or therapeutic outcome.	Identifies a potential intervention target, subject to supporting evidence and clinical evaluation.
Clinical action	A treatment or management decision informed by biological evidence, clinical context, expected benefit, risk, and patient preferences.	Translates validated and clinically relevant evidence into patient management.

Inferred trajectories of the tumour–immune state must also be interpreted in the context of factors that can alter observed measurements independently of a true mechanistic transition. Changes in tumour burden may alter the relative abundance of tumour and immune-derived signals, while shifts in cellular composition may change bulk molecular profiles without corresponding changes in the function of individual cell populations. Sampling location can influence the apparent abundance and spatial organization of immune and stromal populations, and differences between sampled lesions or tumour regions may be mistaken for temporal change. Concomitant treatments, including corticosteroids, chemotherapy and other immunomodulatory agents, may also affect immune-cell states and treatment-associated measurements. Longitudinal interpretation should therefore document relevant treatment exposures, sampling sites, tumour burden and sample composition wherever possible, and assess whether the inferred trajectory remains plausible after accounting for these factors. When such information is unavailable or insufficient, confidence in the inferred mechanism should be reduced and the limitations stated explicitly.

5. Therapeutic Response as a Biological Trajectory

Therapeutic response is commonly represented by discrete clinical categories such as response, stable disease and progression. These categories are indispensable, but they provide only a partial description of the underlying biology. Treatment acts on a pre-existing tumour–immune ecosystem in which malignant cells, immune populations, stromal compartments and vascular structures continuously interact and adapt [5,6].

The clinically observed response is therefore not simply the immediate consequence of drug exposure. It is the observable outcome of a biological trajectory unfolding across multiple temporal and spatial scales. This distinction is particularly important in immunotherapy. Molecular and cellular changes may precede measurable changes in tumour size, whereas an early radiographic response may subsequently be lost as tumour and immune populations adapt to sustained therapeutic pressure. Conversely, limited early tumour shrinkage may coexist with substantial biological activity. Studies of neoadjuvant checkpoint blockade, for example, have shown that early immune and pathological changes can be detected soon after treatment and may provide information about subsequent clinical behavior [43,44].

The relevant question is therefore not only whether the tumour has responded, but what biological transition underlies the observed clinical response and whether that transition is being maintained.

A therapeutic trajectory can be understood as the evolution of the tumour–immune state over time following therapeutic perturbation. Longitudinal measurements provide successive representations of this evolving state, while their integration may help characterize the direction and magnitude of biological change. Treatment alters the selective and signalling environment in which tumour, immune and stromal populations operate. Some changes may represent productive immune engagement, whereas others may reflect compensatory signalling, altered cellular states or tissue remodelling that progressively limit efficacy. This perspective changes the interpretation of longitudinal biomarkers. A dynamic measurement is informative not simply because it changes, but because the direction, magnitude, rate and persistence of that change provide evidence about the evolving tumour–immune state. An early molecular decrease may indicate a biological effect without establishing durable control. Similarly, transient immune activation may represent an intermediate state rather than a stable transition toward effective immunity.

Longitudinal measurements should therefore be interpreted as evidence about state transitions rather than as early replacements for clinical endpoints.

The concept of trajectory also provides a framework for understanding durability. Response magnitude and response duration are related but distinct dimensions of therapeutic benefit. A substantial initial regression may be followed by renewed tumour growth, whereas a more modest reduction may coexist with prolonged control. Durability therefore contains biological information that cannot be inferred from response magnitude alone.

Resistance can be understood as an adverse change in the tumour–immune trajectory, but it should not be reduced to trajectory alone or assumed to be multifactorial in every case. Primary resistance may arise when productive immune engagement fails because of inadequate antigenicity, defective antigen presentation, insufficient immune recruitment, impaired tissue access or constrained cellular fitness. Acquired resistance may follow a discrete molecular alteration, an adaptive change in the tumour–immune ecosystem, or both [11–13,18,45].

These mechanisms can include antigen loss, impaired antigen processing or presentation, altered interferon responsiveness, immune-cell dysfunction, metabolic restriction and remodelling of stromal or vascular environ-

ments [11–13,18,28,45].

They need not occur independently or sequentially. Tumour evolution can alter immune selection; immune dysfunction can permit expansion of less susceptible tumour populations; and tissue remodelling can reinforce both processes. Resistance may arise from a discrete molecular defect or from interacting changes across the tumour–immune ecosystem, and these mechanisms are not mutually exclusive. In some cases, a specific alteration, such as B2M loss or a functionally consequential JAK1/2 mutation, may provide a sufficiently supported explanation for resistance by disrupting antigen presentation or interferon responsiveness, respectively [11,12,18]. In other cases, resistance may reflect coordinated changes across tumour cells, immune populations and the tissue microenvironment that cannot be adequately explained by a single lesion.

Distinguishing between these patterns requires more than detecting a molecular alteration. A single-lesion explanation is strengthened when the alteration has a demonstrated functional consequence, fits the observed resistant phenotype and accounts for the relevant findings without requiring unsupported additional mechanisms. An ecosystem-level explanation becomes more compelling when multiple interacting abnormalities are evident, when no single alteration adequately explains the phenotype, or when longitudinal and spatial measurements reveal changes in cellular interactions or tissue organization that are relevant to treatment failure. Where the evidence supports both, the framework should represent the molecular lesion and its broader biological consequences rather than forcing resistance into either category. The aim is therefore not to assume that resistance is always dynamic, but to determine whether a discrete defect, an evolving ecosystem, or their interaction best explains the observed trajectory (**Table 2**).

Table 2. Representative longitudinal studies of tumour and immune-state changes during cancer immunotherapy.

Study (Year; Reference)	Treatment	Sampling Compartment and Timing	Principal Findings	Evidence and Limitations
Zaretsky et al. (2016) [11]	Pembrolizumab (anti-PD-1)	Paired baseline and relapse biopsies from patients with melanoma who initially responded and subsequently progressed	Acquired resistance was associated with tumour clonal evolution. Loss-of-function mutations in JAK1 or JAK2 were identified in two patients.	Mechanistically informative findings, but based on four patients. The study supports specific resistance mechanisms rather than estimating their prevalence across tumour types.
Lin et al. (2026) [30]	Immune checkpoint inhibitors	Paired tumour biopsies collected at treatment initiation and early during therapy, integrated with longitudinal single-cell datasets	Cross-cancer analyses characterized immune-microenvironment remodelling associated with response to checkpoint inhibition.	Integration of multiple datasets broadened the evidence base. Differences in cohorts, sampling schedules and treatment settings may affect generalizability.
Greenwald et al. (2026) [36]	Nivolumab (anti-PD-1)	Longitudinal tumour samples spanning primary tumours, pretreatment metastases, and on-treatment metastatic biopsies; multiplex imaging and transcriptomic profiling	Temporal and spatial analyses identified tumour-microenvironment features associated with response. Several predictive features were timepoint-specific, with on-treatment samples providing the strongest predictive performance.	Longitudinal clinical cohort from the TONIC trial; findings are based on metastatic triple-negative breast cancer and require validation in independent cohorts with comparable sampling and treatment settings.
Li et al. (2025) [46]	Neoadjuvant anti-PD-1 alone or in combination with anti-CTLA-4 or anti-LAG-3	Longitudinal profiling of tumour-infiltrating $CD8^+$ T cells during neoadjuvant treatment, using transcriptional, proteomic and clonal analyses	Different checkpoint-inhibitor combinations induced distinct $CD8^+$ T-cell trajectories. Anti-PD-1 plus anti-LAG-3 promoted reprogramming towards effector-memory and resident-memory states, whereas anti-PD-1 plus anti-CTLA-4 predominantly expanded pre-existing memory populations.	Clinical-trial-based mechanistic evidence; findings are specific to neoadjuvant head and neck cancer and the treatment regimens examined.
Garralda et al. (2026) [47]	Immune checkpoint inhibitors, alone or in combination with other immunotherapeutic agents	Serial plasma ctDNA profiling; 1,455 longitudinal samples from retrospective and prospective validation cohorts across 24 cancer types	Early ctDNA molecular response and subsequent ctDNA clearance were associated with radiological response and improved survival. ctDNA dynamics also helped distinguish true progression from pseudoprogression.	Multicancer discovery and prospective validation cohorts; heterogeneity in tumour types and treatment regimens limits direct generalization, and clinical benefit from ctDNA-guided treatment decisions was not established.

Table 2. *Cont.*

Study (Year; Reference)	Treatment	Sampling Compartment and Timing	Principal Findings	Evidence and Limitations
Wang et al. (2025) [48]	Immune checkpoint blockade	Serial plasma samples collected during treatment of patients with head and neck squamous cell carcinoma; longitudinal liquid-biopsy analysis	Longitudinal ctDNA assessment identified an early molecular biomarker associated with response to immune checkpoint blockade.	Clinical longitudinal liquid-biopsy evidence; further prospective validation is needed to establish the clinical utility of ctDNA-guided treatment decisions.
Chen et al. (2016) [49]	Anti-CTLA-4 and anti-PD-1	Serial tumour biopsies before treatment, during treatment and, when available, at progression	Early changes in immune-cell infiltration and gene-expression signatures were associated with treatment response. Immune effects differed between checkpoint-blockade settings.	Longitudinal clinical cohort. Biopsy availability and timing varied, and the observed associations did not establish the benefit of state-guided treatment decisions.
Sade-Feldman et al. (2017) [50]	Immune checkpoint blockade	Longitudinal tumour biopsies, including samples obtained at progression	Alterations involving B2M and antigen presentation were associated with resistance in a subset of progressing melanomas.	Findings were evaluated in independent cohorts. The study did not establish whether treatment decisions guided by these alterations improved outcomes.
Quek et al. (2024) [51]	Immunotherapy, including checkpoint blockade	Longitudinal tumour samples; single-cell CITE-seq with matched multiplex spatial imaging	Integrated single-cell and spatial analyses identified changes in tumour-cell populations, immune-cell states and tissue organization associated with response and resistance.	Multimodal analyses provided complementary evidence. Cohort size and observational design limited causal inference and conclusions about clinical utility.
Che et al. (2025) [52]	Anti-PD-1	Serial tumour and draining lymph-node samples collected before, during and after treatment	Treatment-associated B-cell clonal diversity and spatial relationships with T cells were linked to immune activation and clinical response.	The findings support an association between B-cell receptor diversity and treatment response, but do not establish the clinical utility of serial B-cell profiling for treatment adaptation.
Liao et al. (2025) [53]	Combined immunotherapy and targeted therapy	Paired tumour samples before and after treatment; single-cell RNA sequencing and T-cell receptor sequencing	Treatment-associated changes in immune-cell populations and phenotypes differed between responding and non-responding tumours.	Clinical-trial setting. The observed associations did not establish that treatment adaptation based on these changes improves outcomes.
Yost et al. (2019) [54]	Anti-PD-1	Paired tumour biopsies before and after treatment; single-cell RNA sequencing and T-cell receptor sequencing	Expanded T-cell clones following treatment included clonotypes not detected in pretreatment samples, supporting clonal replacement during checkpoint blockade.	High-resolution cellular analyses provided evidence of treatment-associated clonal changes. Sampling and tumour-type limitations restrict generalizability.
Liu et al. (2021) [55]	Immune checkpoint blockade	Thirty-seven tumour samples collected over nine years from one patient	Longitudinal genomic and spatial analyses revealed tumour-lineage evolution, convergent resistance-associated alterations and differences in immune composition.	Deep longitudinal characterization provided detailed patient-level evidence. The single-patient design limits population-level generalizability.
Schiantarelli et al. (2025) [56]	Immune checkpoint inhibitors	Matched pretreatment and post-resistance tumour biopsies from 25 patients	Acquired resistance was associated with alterations involving B2M and JAK1/2. Additional SEC24C/SEC24D alterations were linked to impaired STING-related signalling and antigen presentation.	Patient-matched genomic evidence was complemented by functional experiments, strengthening the mechanistic interpretation.
Leung et al. (2023) [57]	Anti-PD-1	Serial peripheral blood samples and immune profiling during longitudinal follow-up of patients with NSCLC	Dynamic immune-cell and cytokine features were associated with favourable response and provided information about the evolving immune response.	Longitudinal peripheral-blood profiling supports biomarker discovery, but blood measurements do not fully represent the tumour microenvironment. Clinical utility requires prospective evaluation.
Hiltbrunner et al. (2023) [58]	Anti-PD-1	Paired tumour samples before treatment and after acquired resistance; multiplex imaging and molecular profiling	Resistant tumours exhibited immunosuppressive T-cell phenotypes and changes in the tumour immune microenvironment.	Paired samples provide within-patient evidence. The small cohort and observational design limit causal inference and generalization.

Table 2. *Cont.*

Study (Year; Reference)	Treatment	Sampling Compartment and Timing	Principal Findings	Evidence and Limitations
Shiao et al. (2024) [59]	Pembrolizumab with radiotherapy	Tumour biopsies at baseline, after one pembrolizumab cycle and after a subsequent cycle combined with radiotherapy	Responding tumours displayed distinct immune trajectories, including pre-existing antitumour immunity or treatment-associated immune activation. Non-responding tumours showed limited immune changes.	Serial single-cell and spatial profiling captured treatment-associated transitions. The combined regimen makes the independent contribution of each treatment difficult to isolate.
Wei et al. (2026) [60]	Immune checkpoint blockade	Forty-five tumour samples from ten patients; longitudinal genomic, single-cell and spatial analyses	Resistant tumours showed convergent evolution towards a spatially organized immunosuppressive ecosystem, with distinct cellular niches and candidate signalling mechanisms.	Independent-cohort validation strengthened the biological associations. The small number of patients and observational nature of the study limit conclusions about treatment-guided clinical benefit.
Lee et al. (2018) [61]	Anti-PD-1 antibodies	Serial plasma ctDNA measurements during treatment of metastatic melanoma	Longitudinal ctDNA profiles helped distinguish pseudoprogression from true progression and were associated with subsequent clinical outcomes.	Clinically relevant monitoring cohort. ctDNA is an indirect measure of tumour dynamics and does not directly identify the immune mechanism underlying progression.
Lee et al. (2020) [62]	Immune checkpoint inhibitors	Serial plasma ctDNA measurements in patients with melanoma and brain metastases	Longitudinal ctDNA monitoring was evaluated as a marker of treatment response and disease progression in patients with brain metastases.	Disease-specific cohort. Extracranial ctDNA shedding and the biology of intracranial disease may limit interpretation in individual patients.
Takai et al. (2021) [63]	Immune checkpoint blockade	Serial plasma samples with longitudinal mutation-specific ctDNA analysis	ctDNA trajectories reflected heterogeneous tumour-clone dynamics. Changes in mutation profiles and ctDNA kinetics provided information about response, progression and possible pseudoprogression.	Longitudinal molecular observations provide mechanistic clues, but patient-level patterns require validation in larger prospective cohorts.
Murray et al. (2024) [64]	Immunotherapy-based regimens	Serial plasma ctDNA, peripheral T-cell repertoire and protein profiling, with matched tumour and white-blood-cell sequencing where available	Combined longitudinal monitoring captured variation in molecular response and immune-cell dynamics in patients with NSCLC.	Multimodal monitoring broadened assessment beyond tumour burden alone. Prospective studies are needed to determine whether using these measurements to change treatment improves outcomes.

Note: Studies were selected to illustrate complementary approaches to longitudinal profiling of tumour and immune-state changes during cancer immunotherapy, including genomic alterations, immune-cell dynamics, clonal evolution, spatial remodelling and circulating tumour DNA monitoring. The table is intended to provide representative examples rather than a comprehensive or systematic inventory of the available literature. The reported findings should be interpreted in the context of each study's design, population, sampling strategy and level of validation. Abbreviations: B2M, β2-microglobulin; CITE-seq, Cellular Indexing of Transcriptomes and Epitopes by Sequencing; ctDNA, circulating tumour DNA; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HNSCC, head and neck squamous cell carcinoma; JAK, Janus kinase; LAG-3, lymphocyte-activation gene 3; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein 1; STING, stimulator of interferon genes; TNBC, triple-negative breast cancer.

The distinction between state and trajectory also has therapeutic implications. A resistant phenotype may reflect a relatively stable genetic alteration, but it may also arise through transcriptional, epigenetic, metabolic or environmental adaptation. Some configurations may retain substantial plasticity, whereas others may represent more persistent evolutionary change. Determining which constraint is dominant and how plastic it remains is therefore more informative than assigning resistance to a generic category (**Figure 2**).

6. From Biological State to Mechanism-Informed Intervention

The translational value of an integrated biological state ultimately depends on whether it can change a therapeutic decision. This requirement distinguishes a clinically useful framework from a descriptive taxonomy of tumour biology. Biomarker-guided selection asks whether a pretreatment feature is associated with benefit from a treatment, whereas mechanism-informed treatment asks which biological constraint is limiting therapeutic efficacy at a given point in the treatment trajectory and whether that constraint can be modified. Baseline biomarkers and longitudinal state estimation are complementary rather than competing approaches. Baseline measurements establish the initial biological context and can identify patients for whom particular therapeutic strategies are plausible [1–4]. However, when relevant biological processes evolve during treatment, longitudinal assessment may

resolve uncertainty that baseline measurements cannot. Reassessment is most valuable when its findings could change management, particularly when additional measurements can distinguish plausible resistance mechanisms and inform subsequent treatment selection.

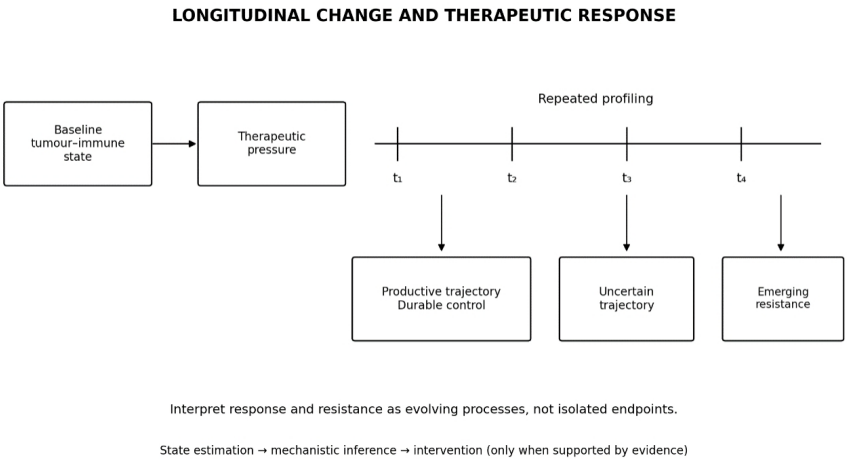

Figure 2. Schematic biological trajectories following therapeutic perturbation, illustrating possible patterns of response, persistence, adaptation, and resistance. These trajectories are conceptual and not quantitative predictions.

The choice of longitudinal measurement should be hypothesis driven. Tumour-derived measurements may be informative when tumour evolution is suspected; immune profiling when cellular dysfunction is central; tissue or spatial measurements when exclusion or remodelling is suspected; and blood-based measurements when frequent surveillance is required [46, 47, 65, 66]. The selected modality should therefore be determined by the biological question and the clinical decision it is intended to inform, recognizing that no single approach captures the entire tumour–immune system.

Circulating tumour DNA (ctDNA) illustrates both the potential and limitations of longitudinal monitoring. Serial ctDNA measurements can provide relatively frequent information about tumour-derived molecular changes and may detect treatment-associated changes before conventional imaging in some settings. However, ctDNA does not directly capture local immune function, tissue architecture or all aspects of tumour biology [47,65,66]. Pseudoprogression illustrates the importance of contextual interpretation: radiographic worsening may precede a subsequent response, and small clinical studies in metastatic melanoma have suggested that favourable ctDNA trajectories may help distinguish pseudoprogression from true progression. Such findings remain context dependent and must be interpreted alongside clinical status, follow-up imaging and other relevant evidence; ctDNA should not be considered a definitive standalone discriminator [47,67].

Clinical utility of ctDNA-guided decision-making has also been demonstrated in a distinct therapeutic setting. In the randomized DYNAMIC trial, ctDNA-guided adjuvant management in stage II colon cancer reduced chemotherapy use from 28% to 15% while meeting the prespecified non-inferiority criterion for 2-year recurrence-free survival compared with standard management [67,68]. Although these findings support the use of ctDNA for a defined postoperative treatment decision, they do not establish its utility for response monitoring or treatment adaptation during immune checkpoint blockade. Such applications require separate prospective validation.

Serial tissue profiling can provide greater mechanistic resolution than circulating measurements, particularly when local immune activity, tissue architecture or spatial heterogeneity is central to the biological question. However, its interpretation remains constrained by invasiveness, sampling bias and the possibility that a biopsy may not represent the broader tumour microenvironment [33,46]. The timing of assessment is also critical: measurements obtained too late may identify resistance after the opportunity for effective intervention has diminished, whereas excessively frequent sampling may capture transient fluctuations rather than meaningful biological transitions. Accordingly, the timing of reassessment should be guided by the expected timescale of the mechanism under investigation.

Monitoring, however, should not be equated with treatment adaptation. An unfavourable molecular change

does not, by itself, justify modifying therapy. Biological systems fluctuate, measurements are subject to technical and sampling error, and transient signals may not indicate durable state transitions. Before longitudinal findings can inform treatment decisions, their persistence, biological plausibility and potential clinical consequences must be considered. Thus, the central question is not simply whether a biological state has changed, but whether the observed change provides sufficiently reliable evidence to support a clinically meaningful decision.

In practice, longitudinal evidence may support three distinct clinical responses. First, continued monitoring may be appropriate when observed changes are consistent with the expected treatment course or when an isolated signal is insufficient to establish a meaningful biological transition. Second, additional testing may be warranted when findings suggest a plausible but unresolved resistance mechanism, provided that further information could influence management. Third, treatment modification may be considered when the implicated mechanism is sufficiently supported by validated evidence and an intervention with a credible clinical basis is available. Neither model-generated predictions nor isolated molecular changes should independently determine treatment decisions. All responses must remain consistent with the patient's clinical condition, treatment-related toxicity, preferences and established standards of care. This proposed decision pathway requires prospective evaluation and should not be interpreted as a validated treatment algorithm.

Causal inference is essential to mechanism-informed treatment adaptation. A longitudinal association between a molecular transition and subsequent disease progression does not establish that the measured process caused treatment failure. The observed signal may be downstream of another resistance mechanism or may simply reflect increasing tumour burden. Establishing a causal role therefore requires evidence beyond temporal association, ideally including perturbational studies demonstrating that modifying the proposed biological constraint produces changes consistent with the hypothesized mechanism. Such evidence strengthens the rationale for targeting the implicated process but must still be distinguished from proof of clinical benefit.

The framework consequently requires a clear hierarchy of evidence. Analytical validity concerns the reliability and reproducibility of a measurement, whereas biological validity addresses whether the inferred state corresponds to a reproducible biological configuration. Prognostic value refers to an association between a marker and the likely course of disease, while outcome prediction concerns the estimation of a clinical outcome from available data. Treatment-effect prediction requires evidence that the relative benefit of a specific treatment, compared with an alternative, differs according to the marker or inferred state; an association with outcome among patients receiving a single treatment is not sufficient. Pharmacodynamic response describes a measurable biological change following treatment that may indicate target engagement or a downstream effect, but does not necessarily establish clinical benefit. Finally, clinical utility requires evidence that using the information to guide decisions improves patient-relevant outcomes compared with the relevant standard of care. These distinctions determine whether a molecular measurement is informative, predictive or sufficiently validated to guide clinical decisions [69].

7. Adaptive Precision Immunotherapy: Requirements for Clinical Translation

Translating the proposed framework into clinical practice requires more than identifying biologically meaningful changes over time. It requires establishing whether longitudinal state assessments can be performed reproducibly and whether their interpretation provides reliable, clinically actionable information within defined care pathways.

The analogy to a closed-loop system is useful but should not be overstated. Clinical decisions must remain grounded in patient-specific factors, treatment feasibility, competing therapeutic options and the strength of available evidence. Accordingly, the proposed framework should be regarded as a testable strategy for supporting clinical research and decision-making, rather than as an autonomous or self-directing control system.

Prospective evaluation is essential to determine whether tumour–immune states can be characterized reproducibly and whether their longitudinal changes provide information that improves clinical decision-making and patient outcomes. Retrospective analyses may identify candidate trajectories and generate mechanistic hypotheses, but they cannot establish that clinical decisions based on these trajectories improve patient outcomes [37,40].

A trial designed to evaluate this framework should distinguish the clinical value of state-guided decision-making from the prognostic value of the biological state itself. One possible approach would compare standard-of-care management with a strategy in which treatment decisions are informed by predefined longitudinal biological-state assessments at specified clinical decision points. The study protocol should define eligibility criteria, the modalities

used for state characterization, the reassessment schedule, and the conditions under which standard clinical management takes precedence. The primary endpoint should reflect the intended clinical benefit, such as progression-free survival or another disease-appropriate outcome. Secondary endpoints could include overall survival, treatment-related toxicity, quality of life, and the frequency and consequences of treatment modifications. Analytical reproducibility, stability of state classification and the impact of state-guided assessments on clinical decisions should also be evaluated.

Importantly, changes in treatment selection, monitoring intensity or the volume of information collected should not be interpreted as clinical benefit unless they translate into improved patient-relevant outcomes.

Decision thresholds should be prespecified within the study protocol and justified by the intended clinical application, rather than selected retrospectively to maximize apparent model performance. Their development should account for the relative consequences of false-positive and false-negative classifications, including unnecessary treatment modification and delayed intervention. Where thresholds are derived empirically, they should be established in a development cohort and evaluated in an independent validation cohort before being used to inform clinical decisions. The protocol should also allow an indeterminate classification when the available evidence is insufficient for a reliable assignment, rather than forcing uncertain cases into definitive categories. Prospective assessment should determine whether the selected thresholds provide clinically meaningful discrimination without exposing patients to avoidable harm.

The same principle applies to computational models. Machine learning may identify nonlinear relationships across high-dimensional datasets, but predictive performance alone does not establish biological validity or clinical utility [37,40].

The choice of computational strategy should be guided by the clinical question, the structure and quality of the available data, and the intended use of the model. Early integration, in which features from different modalities are combined before modelling, provides a straightforward analytical framework but may be sensitive to differences in scale, dimensionality and measurement reliability. Late-integration approaches, which combine predictions or representations derived from separate modalities, can accommodate modality-specific models but may fail to capture biologically meaningful interactions. Latent-variable methods can identify shared patterns across correlated measurements and reduce dimensionality, yet their factors may be difficult to interpret biologically and may reflect technical variation or dominant sources of heterogeneity rather than clinically relevant mechanisms. Supervised multi-omics methods can prioritize features associated with a defined outcome, but their performance may be unstable when the number of candidate features is large relative to the number of patients. Predictive associations also do not establish causal mechanisms. These trade-offs are consistent with broader assessments of clinical multi-omics profiling [39].

Data quality and uncertainty require explicit attention. Missing measurements should be characterized by modality and by their likely causes, because data may be absent for technical reasons, limited sample availability or clinical factors associated with disease severity. Imputation or other missing-data strategies should be selected according to these mechanisms, with sensitivity analyses used to assess whether conclusions depend on particular assumptions. Batch effects and platform differences should be evaluated separately from biological variation and addressed using appropriate normalization or harmonization procedures, while ensuring that independent test data do not influence preprocessing or model development. Validation should assess not only discrimination but also calibration, uncertainty in individual predictions, robustness across relevant populations and platforms, and sensitivity to changes in the input data. Interpretability should be evaluated in relation to the intended decision: identifying influential features is insufficient unless their contribution is stable, biologically plausible and consistent with the evidence.

Model complexity should therefore be justified by demonstrated incremental value in the intended clinical setting. Integration strategies should be selected according to their ability to distinguish clinically relevant mechanisms, provide reproducible estimates, and support predefined therapeutic decisions. Adaptive precision immunotherapy should consequently be understood as targeted information acquisition rather than maximal molecular surveillance [33,46,65,66].

The clinical feasibility of this framework depends on whether the required measurements can be obtained safely, consistently and within a clinically relevant timeframe. Although tissue-based profiling may provide detailed biological information, its repeated use may be constrained by limited tissue availability and the practical burden of

invasive procedures. Circulating biomarkers offer a less invasive alternative for longitudinal assessment, but their suitability depends on the biological processes being investigated. Clinical protocols should therefore specify the minimum requirements for reliable state characterization, the circumstances in which alternative measurements may be accepted, and how uncertainty should be handled when the available data are insufficient to support a confident biological interpretation.

Clinical decisions must also incorporate treatment-related toxicity, performance status, comorbidities, patient preferences, financial burden and competing therapeutic options. A biologically plausible intervention should not be recommended solely because a model identifies a potentially modifiable constraint; its expected benefit, risks, feasibility and supporting clinical evidence must be considered alongside established standards of care. The cost of profiling, turnaround time, access to specialized assays and availability of confirmatory testing may determine whether a strategy can be implemented in routine practice. Requiring advanced multi-omics profiling at every decision point could restrict access to well-resourced centres and exacerbate existing disparities. Prospective evaluation should therefore report the resources required for implementation and examine whether access to profiling, confirmatory testing and resulting clinical benefits is equitable across different care settings.

Establishing the clinical value of this framework will require a stepwise accumulation of evidence, beginning with reproducible state characterization and prospective validation of longitudinal trajectories, followed by demonstration of mechanistic relevance and evidence that modifying the implicated biological constraint alters the trajectory. Ultimately, randomized studies must determine whether decisions guided by this framework improve patient-relevant outcomes compared with standard care (**Figure 3**).

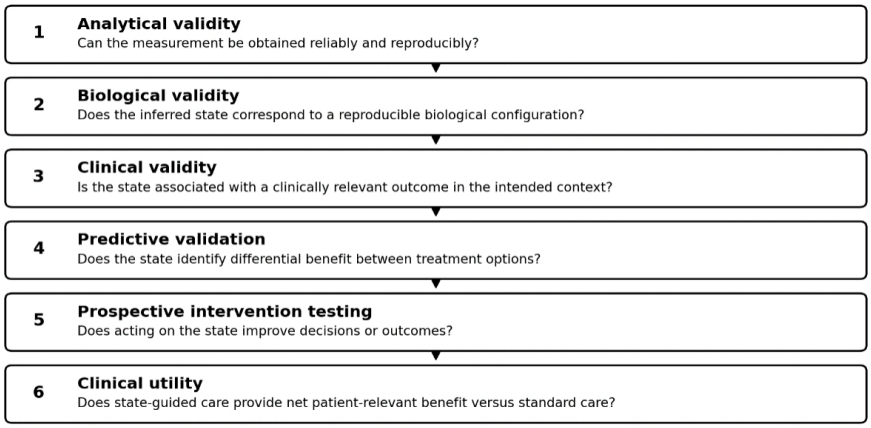

Figure 3. Evidence hierarchy for mechanism-informed precision immunotherapy. Proposed framework for adaptive treatment evaluation, integrating state estimation, longitudinal reassessment, hypothesis testing, and prospective evaluation.

8. Future Perspectives: From Molecular Snapshots to Individual Trajectories

Future progress in precision immunotherapy will depend on determining how complementary molecular and spatial technologies can be combined to characterize clinically relevant changes in the tumour–immune ecosystem. Genomic, epigenomic, transcriptomic, proteomic, metabolic, cellular and spatial approaches provide different forms of biological information. Future studies should establish which combinations offer distinct advantages in specific clinical contexts and whether the resulting information improves the interpretation of treatment response or resistance [37,38].

Future longitudinal studies should establish the temporal resolution required to capture clinically meaningful changes in the tumour–immune ecosystem. This will involve determining the optimal intervals for reassessment in relation to treatment schedules, expected biological responses and the emergence of resistance. Such studies should also clarify whether different biological compartments require distinct sampling schedules to provide complementary information about disease evolution.

An important direction for future research is to determine how spatial organization contributes to the interpretation of tumour–immune interactions. Spatial profiling may reveal cellular relationships, immune-exclusion

patterns and localized signalling processes that are not readily captured by measurements of molecular abundance alone. However, its added value should be assessed in relation to specific biological hypotheses and clinical questions, particularly whether spatial information changes the interpretation of a therapeutic target or resistance mechanism [33–36].

Beyond conventional prognostic modelling, future research should investigate approaches that estimate how alternative therapeutic interventions might influence disease trajectories. Unlike conventional prognostic models, which estimate outcomes based on observed patient characteristics, intervention-aware models seek to compare potential outcomes under different treatment strategies. Their development requires appropriate causal assumptions, reliable longitudinal data and methods for addressing confounding and treatment-selection effects. The validity of these models must ultimately be assessed through rigorous prospective evaluation rather than predictive performance alone.

Patient-specific computational models may offer a means of integrating longitudinal molecular, spatial, imaging and clinical data into a unified representation of an individual's disease. Within this context, the concept of a digital twin has attracted growing interest in precision oncology. Such models could potentially support the estimation of a patient's current biological state, quantify uncertainty and simulate plausible disease trajectories under alternative therapeutic strategies. However, their clinical application remains contingent on the availability of sufficiently comprehensive longitudinal data, model transparency, rigorous external validation and evidence of improved patient outcomes [48,70–72]. For the foreseeable future, however, their most realistic role is likely to be as decision-support tools that make uncertainty explicit, rather than as autonomous computational replicas of individual patients.

An emerging direction in precision immunotherapy is the evaluation of dynamic, patient-specific representations as potential complements to conventional biomarkers. Rather than relying exclusively on isolated measurements, these approaches may incorporate the direction and rate of biological change, the suspected mechanisms underlying treatment response or resistance, and the uncertainty associated with these estimates. However, their clinical relevance cannot be assumed from conceptual or computational advantages alone. Future comparative studies should determine whether such representations provide reproducible and clinically meaningful information beyond established biomarkers and simpler predictive models, and whether their use improves treatment selection or patient outcomes.

9. Limitations and Future Directions

A fundamental limitation of the proposed framework is that the tumour–immune state cannot be directly observed and must instead be inferred from indirect biological measurements. Consequently, the resulting representation is inherently dependent on the selected features, sampling strategy, analytical methods and assumptions used to integrate and interpret the available data. Different combinations of measurements or analytical approaches may therefore produce different representations of the same underlying biological system. In the absence of an independently established reference for the inferred state, its accuracy and reproducibility cannot be assumed and must be evaluated through convergent biological evidence and independent validation.

A further limitation concerns the difficulty of distinguishing genuine biological evolution from variability introduced by measurement and sampling procedures. Differences between serial or spatially distinct samples may arise from changes in cellular composition, tissue representation, assay performance or analytical processing, rather than from a meaningful transition in the underlying tumour–immune system. Conversely, biologically important changes may remain undetected when the sampled material does not adequately represent the relevant tumour regions or immune compartments. These uncertainties complicate the interpretation of longitudinal trajectories and require standardized sampling procedures, analytical reproducibility and independent confirmation of changes considered biologically meaningful.

Another important limitation is the uncertain correspondence between computationally inferred patterns and the biological mechanisms they are intended to represent. Even when an integrated model demonstrates reproducible associations with treatment outcomes, its outputs may not fully capture the underlying functional processes or distinguish between coexisting mechanisms that produce similar molecular patterns. Furthermore, model performance may vary across tumour types, treatment settings and patient populations, limiting the generalizability of findings derived from a particular cohort. Accordingly, the biological interpretation and external applicability

of inferred states require evaluation beyond internal model performance, using independent datasets and, where appropriate, experimental evidence.

An additional limitation concerns the context in which evidence for this framework is likely to be generated. Studies conducted in specialized academic centres may rely on patient populations, diagnostic pathways, sample-processing capacity and computational resources that are not representative of broader clinical practice. Consequently, even technically reproducible state definitions may not transfer directly across institutions with different patient populations, treatment pathways and laboratory platforms. The external validity of the framework will therefore require evaluation across geographically and institutionally diverse cohorts, including settings with differing levels of diagnostic and computational capacity.

Until these methodological, biological and implementation-related challenges are adequately addressed, the proposed framework should be regarded as a hypothesis-generating and testable approach for precision immunotherapy research, rather than an established clinical decision-making tool. Its eventual clinical relevance will depend on demonstrating that inferred biological states are reproducible, biologically interpretable and capable of supporting clinically meaningful decisions.

10. Conclusion

Precision immunotherapy has shown that molecular characteristics of tumours and their immune microenvironments can inform therapeutic sensitivity, but it has also revealed the limitations of treating any individual biomarker as a complete representation of therapeutic state. Tumour–immune ecosystems are heterogeneous, spatially organized and continuously reshaped by treatment. The state measured before therapy is therefore not necessarily the state that determines response months later.

This Review proposes a framework in which precision immunotherapy progresses from molecular measurement to the construction of an integrated biological state, from characterization of the tumour–immune trajectory to mechanistic interpretation, and ultimately to mechanism-informed intervention. The central premise is that molecular measurements become more clinically informative when interpreted as observations of an evolving system rather than as isolated predictors of outcome.

Within this framework, multi-omics integration provides the basis for characterizing the biological features that may shape therapeutic response and resistance. Its contribution lies in connecting molecular measurements with the functional and spatial context in which they arise, thereby supporting the interpretation of tumour–immune dynamics. Longitudinal assessment can then determine whether these features remain stable, evolve during treatment or indicate the emergence of potentially actionable biological constraints.

The clinical value of this framework ultimately depends on whether the biological changes identified through longitudinal assessment can be translated into meaningful therapeutic decisions. This requires distinguishing changes that are merely measurable from those that reflect relevant biological transitions and potentially modifiable mechanisms. The framework therefore offers a basis for investigating how evolving tumour–immune information may support treatment decisions, while recognizing that its clinical benefit remains to be established through prospective evaluation.

Ultimately, the framework will be valuable only if it helps clinicians make better decisions. Its success should be judged not by the breadth of molecular profiling or the sophistication of computational models, but by reproducible evidence that mechanism-informed decisions improve outcomes that matter to patients.

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Informed Consent Statement

Not applicable. This study is a review article and did not involve human participants.

Data Availability Statement

No new data were generated or analyzed in this study, as this article is a review article. Therefore, data sharing is not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

AI Use Statement

During the preparation of this manuscript, the authors used ChatGPT solely for grammatical corrections and sentence-level language refinement. No AI tools were used for data analysis, interpretation, or generation of scientific content. No AI tools were used in figure generation. All AI-assisted language edits were reviewed by the authors. The authors take full responsibility for the integrity and accuracy of the work.

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