Spatiotemporal immune dynamics in lung cancer progression and treatment

Chee Kuan Wong[1,2] and Megat Hafizzuddin Bin Abd Hamid[3]

[1]Division of Respiratory Medicine, Department of Medicine, Universiti Malaya Medical Centre, Kuala Lumpur, Malaysia. [2]Department of Medicine, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia. [3]Department of Molecular Medicine, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia.

Corresponding author: Megat Hafizzuddin Bin Abd Hamid (megat.abdhamid@um.edu.my)

Shareable abstract (@ERSpublications)
Lung tumours continuously shift between immune-hot, -cold and -excluded states. Tracking these spatiotemporal transitions reveals how therapies reshape immune access, guiding stage-specific biomarkers and precision treatment in NSCLC. https://bit.ly/4kq7zxS

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Abstract

The lung tumour microenvironment is a complex and evolving ecosystem in which immune, stromal and malignant cells interact across both space and time. Spatial organisation determines whether immune cells can access and induce immune responses across tumour tissues, while temporal changes driven by disease progression and therapy reshape these interactions. Although advances in high-plex technologies have revealed discrete immune niches in lung cancer, particularly in nonsmall cell lung carcinoma (NSCLC), less is known about how these tissue landscape niches remodel over time. This review integrates spatial and temporal perspectives to provide a unified view of lung cancer immunobiology. We discuss how fibroblast activation, endothelial remodelling and extracellular matrix deposition restrict immune infiltration and how conventional therapies and targeted inhibitors can remodel these barriers. We highlight recent studies that reveal how immune functional states shift with treatment and cancer stages. Finally, we outline how emerging technologies (spatial transcriptomics, multiplex imaging and functional assays) offer new opportunities to link temporal transitions with spatial immune context. Understanding spatiotemporal dynamics will be key to stage-specific biomarker discovery, treatment optimisation and personalised strategies in NSCLC.

Educational aims

- To explain how spatial tissue niches in the lung tumour microenvironment shape immune access and behaviour.
- To show how tumour progression and treatment remodel immune–stromal interactions over time.
- To highlight new technologies that visualise these changes and their clinical implications.

Introduction

Lung cancer outcomes remain highly variable, even among patients with similar stage and histology. Some patients respond well and remain stable for a long time, while others progress quickly or relapse after an initial response. This difference is probably not driven by tumour genetics alone. It also depends on the complex local tissue environment that surrounds the tumour, including the involvement of immune and stromal cells. It relates to how these components change and adapt during disease progression and treatment. These patterns matter because they can influence response, resistance, and how therapies might best be integrated or combined.

This review imposes two simple ideas to organise that complexity: space and time.

- Spatial immune dynamics describes where immune cells are located within the tumour tissue. Immune cells may sit deep within tumour nests, remain mainly in the surrounding stroma or cluster at the

invasive margin. Their location affects what they can interact with and whether they can reach malignant cells efficiently to induce anti-tumour responses.
- Temporal immune dynamics describes how the immune environment changes over time. These changes can occur as the tumour progresses, but they can also be triggered by therapy. In some cases, treatment may briefly increase immune activity, and it can be followed by cancer adaptation and renewed immune suppression.

A practical way to describe these patterns is to classify tumours as immune-hot, immune-cold or immune-excluded. Immune-hot tumours contain many activated lymphocytes within tumour nests and tend to respond better to immune checkpoint inhibitors (ICIs). Immune-cold tumours show few immune cells and often lack effective immune priming. Immune-excluded tumours contain immune cells, but they are mainly residing outside the tumour nests, confined within the stromal or invasive margin compartments.

Importantly, these tumour states are not fixed. Tumours can shift from one state to another in response to treatment or as the tumour progresses. In this review, we will first introduce the main spatial immune states and their tissue features, as well as explain how therapies can shift tumours between states over time. Finally, we will summarise approaches and clinical considerations for using spatiotemporal information in lung cancer.

Mapping the spatial immune niche and landscape in lung cancer

One of the most consistent findings in lung cancer is that not all tumours are immunologically equal. Spatial analysis has revealed at least three archetypes: 1) immune-hot, 2) immune-excluded and 3) immune-cold.

Immune-hot tumours contain abundant lymphocytes that infiltrate the tumour parenchyma, a feature that is often associated with favourable prognosis and responsiveness to immune checkpoint blockade (ICB) [1]. Conversely, immune-cold tumours display sparse immune infiltrates, which may reflect a lack of tumour antigen priming or ineffective recruitment signals in the tumour microenvironment [2]. Immune-excluded tumours represent an intermediate category, where immune cells are present but are confined to the periphery or trapped within stromal bands rather than penetrating the malignant nests [3].

Clinically, immune-cold and immune-excluded tumours are often less responsive to ICB than immune-hot tumours, but for different reasons. Immune-cold tumours frequently lack sufficient tumour-reactive T-cells for an effective anti-tumour response because antigen presentation and priming are inadequate. In contrast, immune-excluded tumours may contain T-cells, but these remain segregated from the malignant nests by stromal and vascular barriers and suppressive signalling, therefore limiting tumour clearance. This distinction matters because it suggests different treatment strategies: promoting priming and recruitment of immune cells *versus* relieving exclusionary barriers to enable immune infiltration.

These exclusionary patterns are strongly influenced by cancer-associated fibroblasts (CAFs) and the extracellular matrix (ECM). CAFs remodel the tumour stroma by depositing dense layers of collagen and fibronectin, which create physical barriers to lymphocyte entry [4, 5]. In parallel, the ECM impairs lymphocytic function by restricting cell motility within stiff, cross-linked tissue by altering the integrin-mediated adhesion needed for efficient immune migration and contact.

Beyond this structural role, CAFs secrete soluble mediators such as transforming growth factor-β, which suppress effector T-cell activity and recruit immunosuppressive populations [6]. CAFs–ECM programmes may further reinforce exclusion by shaping chemokine gradients that retain immune cells in the stromal compartments rather than directing them into the malignant tumour nests. In tandem, endothelial cells further regulate immune access by altering vessel architecture and adhesion molecule expression. Abnormal vascularisation driven by CAFs and endothelial cells often fails to support lymphocyte trafficking, reducing infiltration even in the presence of strong chemotactic gradients [7].

High-resolution technologies have enabled these processes to be visualised *in situ*. In patients receiving neoadjuvant chemo-immunotherapy, single-cell and spatial transcriptomics revealed reductions in CAF density, redistribution of macrophages towards tumour boundaries and shifts in T-cell subsets, including decreased representation of regulatory T-cells while exhibiting increased representation of the T-helper (Th)17 phenotype. Importantly, these spatial redistributions correlated with treatment responses, showing that therapy can reset the balance between exclusion and infiltration [8].

Spatial transcriptomics has further revealed gene expression programmes linked to CAF-rich niches in lung adenocarcinoma [9], while multiplex imaging has mapped how myeloid cells preferentially occupy

perivascular and stromal regions reinforcing exclusion of effector lymphocytes [1]. These findings emphasise that spatial heterogeneity is not a passive by-product of tumour growth, but an active strategy by which lung tumours sculpt their microenvironment to regulate immune access and function.

Temporal shifts in the immune–cancer landscape

While spatial organisation sets the framework, lung tumours are not static in their immune–cancer landscape. This landscape shifts in response to both natural disease progression and therapeutic intervention.

In early-stage tumours, cytotoxic T-lymphocytes, including $CD103^+$ tissue-resident memory T-cell (T_{rm}) subsets, are often detectable within the tumour microenvironment, exerting effective immune surveillance [10]. CD103 (*ITGAE*) is an integrin associated with tissue retention and epithelial compartment localisation. It is typically used to interpret whether tumour-reactive T-cells are positioned for direct tumour/epithelial engagement, rather than remaining confined to the surrounding stroma. As tumours progress to more advanced stages, inhibitory receptor-expressing T-cell subsets such as $NKG2a^+$ T-cells become enriched, while cytotoxic populations such as T_{rm} cells decline.

This imbalance leads to reduced effector and proliferative immune function, contributing to cancer immune escape [11]. Cancer immune escape refers to the set of tumour-intrinsic and microenvironmental mechanisms that enable malignant cells to evade immune recognition and elimination. Apart from the upregulation of inhibitory receptor-expressing T-cell subsets, other immunosuppressive populations, including regulatory T-cells and myeloid-derived suppressor cells (MDSCs) also expand over time, reinforcing the immunosuppressive cancer milieu [1].

Treatment introduces another layer of temporal modulation. Chemotherapy, beyond its direct cytotoxic action on cancer cells, can alter immune composition in diverse ways. Some regimens enhance antigen presentation and increase T-cell infiltration, while others promote recruitment of MDSCs [8]. Targeted therapies, such as tyrosine kinase inhibitors against EGFR (epidermal growth factor receptor) or KRAS (Kirsten rat sarcoma virus oncogene homologue), rapidly remodel stromal dynamics. By reducing fibroblast activity and ECM deposition, these drugs may transiently dismantle the physical barriers for lymphocyte infiltration [12, 13]. Resistance mechanisms, however, often restore these exclusionary features. For instance, reactivation of the downstream MAPK (mitogen-activated protein kinase) signalling or bypass activation if alternative receptor tyrosine kinases can drive renewed fibroblast activation and ECM production [14]. These changes rebuild the stromal barriers, reinstating immune exclusion despite continued drug pressure.

Immunotherapy with PD-1 (programmed cell death protein 1) or PD-L1 (programmed cell death ligand 1) inhibitors offers yet another dimension, as reactivation of exhausted T-cells is only effective if these cells can actually reach the tumour nests. In immune-excluded tumours, stromal barriers often limit therapy success unless combination strategies are applied [15]. In preclinical nonsmall cell lung carcinoma (NSCLC) models with immune-excluded phenotypes driven by KRAS-G12C mutations, combining a G12C-selective inhibitor with SHP2 inhibition sensitised tumours to anti-PD-1 therapy, improving T-cell infiltration into tumour cores and increasing tumour rejection [16].

In NSCLC patients treated with nivolumab, high stromal infiltration of both $CD8^+$ and $CD4^+$ T-cells was associated with better durable benefit and overall survival [17]. This is consistent with an immune-excluded pattern, where T-cells are enriched in tumour-adjacent stroma or the invasive margin rather than within tumour nests, yet may still confer benefit through tumour–margin interactions and potential subsequent infiltration if or once the stromal–tumour barrier is relieved. Similarly, a clinical study combining PD-1 blockade with JAK inhibition showed improved therapy responses [18], implying that interrupting suppressive signalling pathways might help overcome the barriers to immune infiltration.

Different treatment modalities impose distinct immune perturbations over time. Some effects are immediate (lung tissue injury and increased/decreased inflammation), while others occur during therapy (modulation of immune activation or suppression). Similarly, other treatment types can appear later when adaptive resistance or relapse occur. To support clinician interpretation, table 1 summarises the typical temporal immune effects of major lung cancer treatments and the clinical significance of these dynamics.

The key lesson is that lung tumours are not permanently fixed as immune-hot, -cold or -excluded. Instead, they transition between these states dynamically in ways that are shaped by both intrinsic disease evolution and extrinsic therapeutic pressure (figure 1). Recognising and anticipating these transitions is critical for tailoring treatment strategies.

TABLE 1 Summary of time-dependent (temporal) immune effects associated with treatment modalities

Treatment modality	Representative timepoint(s)	Typical temporal immune modulation	Clinical significance
Surgery	Immediate peri-operative period up to 6 weeks post-operation	Acute wound inflammation Transient systemic stress response with short-term immunosuppression Shifts in cytokines and myeloid activation	Peri-operative window may influence recurrence risk; the timing of adjuvant therapy may interact with immune recovery
Radiotherapy	Early and late post treatments	Early local inflammation and antigen release Increased chemokines and immune recruitment Late fibrosis/stromal remodelling and suppressive programmes	Potential synergy with immunotherapy (timing-sensitive); late stromal remodelling may contribute to exclusion and resistance build-up
Chemotherapy (systemic)	Early and late post treatments	Promote immunogenic tumour cell death Lymphodepletion/impaired immune function	Regimen and timing can determine whether the net effect supports the anti-tumour response
Targeted therapy	Early and late post treatments	Early tumour debulking Shifts in antigen/chemokine signalling Transient increase in cell infiltration Later resistance build-up remodelling stroma and suppressing immunity	Early adaptation may be exploitable with combination therapy; relapse often associates with an altered tissue state
Immunotherapy	Early and late post treatments	Early reinvigoration of tumour-reactive T-cells in responders Later adaptive resistance including suppressive myeloid programmes, renewed exclusion	Early on-treatment changes may predict response; progression reflects a state transition requiring alternative combination therapy

Linking spatial and temporal contexts

Spatial and temporal perspectives on lung cancer are often studied separately, but their integration provides a more complete framework for understanding immune–stromal dynamics. Temporal transitions can directly alter the spatial distribution of immune cells, leading to changes in exclusion of infiltration [19, 20]. For example, targeted therapies that reduce fibroblast activation can temporarily dismantle stromal barriers, allowing lymphocytes to penetrate tumour nests. However, if resistance emerges, fibroblasts may re-establish these barriers, restoring an immune-excluded phenotype [21].

Similarly, ICB can trigger early reinvigoration and expansion of tumour-reactive T-cells [22, 23], but adaptive resistance may subsequently remodel the microenvironment through myeloid suppression or renewal stromal exclusion. This, in turn, could lead to altered spatial positioning of immune cells and loss of effective tumour engagement. In contrast, chemotherapy can induce time-dependent changes. In some settings, it increases antigen release and inflammatory cues that promote immune recruitment and infiltration [24]. Whereas, in others it can cause lymphodepletion or immune functional impairment depending on regimen and timing [25–27]. This consequently shapes whether immune access is gained or lost over the course of treatment.

Functional states of T-cells are also strongly shaped by spatiotemporal context. During tumour progression, cytotoxic T-cells may infiltrate and exert tumour clearance, but chronic exposure to tumour antigens and enhanced inhibitory signals can lead to terminal exhaustion. In parallel, changes in stromal architecture dictate where these exhausted cells accumulate. In our own work, unconventional $CD61^+$ cancer-specific lung-resident T-cells were found to acquire enhanced cytotoxicity during temporal contact with cancer cells, and this phenotype was linked to specific patterns of chemokine-driven infiltration [10, 28]. Likewise, longitudinal profiling of tumours before and after PD-1 therapy has shown that precursor-exhausted T-cells can expand both locally and from peripheral lymphocytic clones, illustrating how temporal reactivation is reflected in spatial positioning within the tumour bed [15].

The integration of time and space thus reveals that lung tumours are not only heterogenous but also a dynamic system in which immune accessibility and functional competence are continuously remodelled. Recognising these interlinked processes is key for understanding why some patients respond durably to immunotherapy, while others relapse or fail to respond at all.

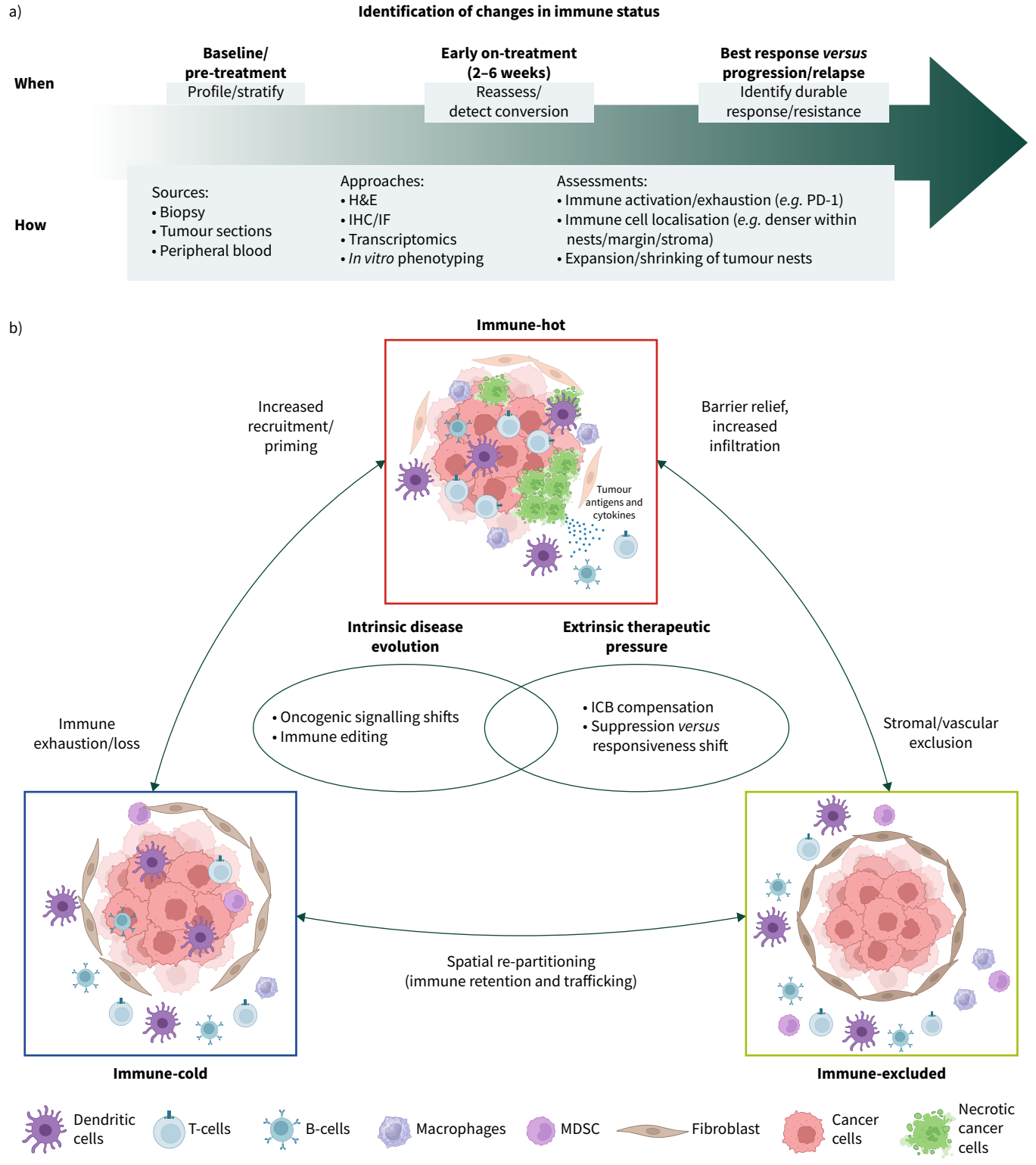

FIGURE 1 Identification of and anticipating transitions between immune-hot, -cold and -excluded tumour states. a) Summary of when immune status may be reassessed (baseline/pre-treatment, early on-treatment within 2–6 weeks, and best response *versus* progression or relapse). The schematic further shows how to evaluate changes using biopsy/tumour sections or peripheral blood with haematoxylin and eosin (H&E) staining, immunohistochemistry (IHC)/immunofluorescence (IF), and transcriptomics profiling. Key readouts include immune exhaustion markers (*e.g.* programmed cell death protein 1 (PD-1)), immune cell localisation (tumour nest *versus* margin/stroma), and changes in tumour architecture. b) Schematic illustrating state transitions: increased recruitment/priming shifting immune-cold to -hot; barrier relief and increased infiltration

shifting immune-excluded to -hot; and immune exhaustion/loss or stromal exclusion driving movement away from immune-hot. Intrinsic tumour evolution and extrinsic therapeutic pressure jointly shape these transitions, including spatial re-partitioning *via* altered immune retention and trafficking. ICB: immune checkpoint blockade; MDSC: myeloid-derived suppressor cell. Figure created using Biorender.com.

Tools that let us see more clearly

The ability to interrogate spatiotemporal dynamics in lung cancer has been transformed by advances in high-resolution technologies. Spatial transcriptomics and multiplex imaging enable researchers to visualise how immune, stromal and malignant cells interact across both axes.

Spatial transcriptomics captures gene expression within intact tissue architecture. By overlaying molecular data on histological context, researchers can resolve ligand–receptor interactions that govern immune–stromal crosstalk at distinct tissue niches. For instance, analysis of acute lung injury revealed immune circuits that are relevant to biological responses, including macrophage–fibroblast signalling that contributes to barrier formation [29]. More recently, combinatory single-cell and spatial transcriptomics profiling identified $SPP1^+$ macrophages co-localised with FAP^+ fibroblasts at lung tumour edges and cores, demonstrating how ligand–receptor pairs create immune-excluding barriers [30].

Multiplex imaging platforms such as CODEX and imaging mass cytometry allow simultaneous visualisation of dozens of proteins *in situ*. These approaches have revealed that regulatory T-cells and cytotoxic T-cells occupy distinct niches in tumour margins, and that the frequency of certain interactions correlates with the response to checkpoint inhibitors [31]. Similarly, epithelial–myeloid crosstalk mediated by MIF–SPP1 pathways has been linked to therapy resistance build-up in NSCLC, showing that interactions at the spatial boundary of tumour and stroma can predict outcomes [32].

Complementing these tissue-based *in situ* technologies, *in vitro* air–liquid interface (ALI) systems of the airway lining offer a tractable platform to validate the molecular drivers of spatiotemporal interactions in lung cancer. By culturing lung epithelial cells together with fibroblasts, endothelial cells and immune populations [33, 34], ALI models can reproduce key structural and signalling features of the tumour microenvironment. This allows investigators to test how specific ligand–receptor pathways, stromal barriers or cytokine networks post-oncogenesis contribute to dynamic shifts in immunoresponsive and immunosuppressives states. Importantly, these models enable temporal tracking of immune infiltration or exclusion under controlled experimental conditions, bridging high-resolution observational studies with mechanistic temporal validation of candidate pathways.

These technologies are powerful not only because they map where cells are, but because they allow tracking of how those characteristics change in response to therapy or disease progression. The integration of molecular, spatial and temporal data is moving the field from descriptive snapshots to dynamic models of tumour–immune ecology.

Clinical insights: from bench to bedside

The management of NSCLC largely depends on the presence of targetable oncogenic driver alterations [35]. In Asia, the most common targetable alterations are *EGFR* mutations, followed by *ALK*, *ROS-1* rearrangement, *ERBB2* (HER2) mutations, *RET* rearrangements and *MET* exon 14 skipping [36, 37]. However, a substantial proportion of NSCLC patients do not harbour any targetable and actionable mutations. In such cases, the assessment of PD-L1 expression becomes crucial in determining eligibility for ICI therapy.

For these patients, understanding the spatiotemporal dynamics of the tumour microenvironment offers additional yet important insights beyond conventional biomarker testing. For example, regional variation in T-cell activation has been demonstrated using digital spatial profiling, showing that immune activity differs between tumour cores, the stromal boundary and metastatic areas in NSCLC patients [38]. Such stage- and site-specific signatures highlight the potential for spatiotemporal markers to refine prognosis and guide therapy choices.

Although immunotherapy has demonstrated promising outcomes in the management of NSCLC, the proportion of patients achieving durable responses remains limited. In the clinical trials of pembrolizumab monotherapy for advanced NSCLC the median progression-free survival ranged from 7 to 10 months, with an overall survival between 20 and 26 months. Only 30% of these patients achieved a long-term response

or complete remission [39, 40]. The majority eventually experience disease progression due to the development of acquired resistance to ICIs.

Therapeutic resistance can also be better understood through the lens of spatiotemporal tumour dynamics. In nonresponding NSCLC patients with immunotherapy, ligand–receptor interactions near tertiary lymphoid structures have been implicated in sustaining stromal barriers, pointing to novel targets for combination therapy [41]. Large-scale spatial and single-cell analyses of treatment-naïve NSCLC patients have further revealed that tumours can be segregated into immune-hot and immune-cold sections, establishing a comparative landscape against which temporal shifts induced by treatment can be measured [42].

In one study, of surgical resection followed by adjuvant chemoradiation therapy, the 2-year overall survival rate for stage I NSCLC remained only 70–78%, while it dropped markedly to 25–52% for stage III of the disease [43]. These findings indicates that current adjuvant therapies are still insufficient to achieve long-term disease control or cure. Although PD-L1 expression and tumour mutation burden represent the most promising biomarkers for predicting the response to ICIs, a subset of patients with high PD-L1 expression still fail to respond to immunotherapy [44]. This highlights the urgent need for more precise and integrative approaches to guide treatment decisions and refine prognostication.

Beyond conventional biomarkers, spatiotemporal perspectives support the development of stage-specific interventions. For instance, therapies that disrupt fibroblast–immune crosstalk may be particularly effective in early disease stages where stromal exclusion is dominant, whereas later-stage tumours may require strategies that also restore the sensitivity and responsiveness of lymphocytic functions. Similarly, vascular normalisation to enhance effective lymphocyte trafficking may be most impactful in tumours that evolve towards a highly disorganised vasculature during the disease progression.

The central insight is that neither spatial nor temporal data alone can fully capture the complexity of lung cancer responses. Together, they provide a framework for predicting which patients will respond to therapy, for designing rational combinatory therapies and for timing interventions to exploit windows of immune accessibility (figure 2).

This will lead to tailored therapeutic choices, including checkpoint blockade, stromal targeting, vascular normalisation and biological tissue recompensation of therapy effects. This framework highlights a bench-to-bedside translational pathway that covers relevant discovery stages through to precision medicine implementation.

Looking ahead

The study of lung cancer immunology has reached a turning point. It is no longer sufficient to catalogue the cellular composition of the tumour microenvironment at a single moment in time. Instead, the field is moving towards dynamic models that link the evolution of immune and stromal niches with functional outcomes. The integration of spatiotemporal perspectives highlights that tumours are not fixed as immune-hot, -cold or -excluded, but transition between these states in response to both intrinsic progression and extrinsic therapy.

For clinicians, this has important implications. Spatiotemporal signatures could serve as predictive biomarkers that guide stage-specific therapy. For example, early-stage tumours that show fibroblast-driven immune exclusion may benefit from stromal-targeting approaches, while later-stage tumours dominated by exhausted T-cells may benefit from ICB or combination regimens. For researchers, the challenge is to develop longitudinal frameworks that capture not only the transition, but also validate the mechanisms and role of these dynamics and their implication on immunopathological outcomes. This will require not only continued innovation in spatial-omics, but also computational tools for integrating datasets across time and treatment contexts.

Future therapeutic strategies may focus on modulating the tumour vasculature to improve immune access, disrupting ligand–receptor signalling between stromal and immune compartments or reprogramming macrophage and fibroblast populations to support infiltration rather than exclusion. Importantly, these approaches will need to be tailored to the stages of the disease to maximise benefit.

The promise of spatiotemporal immunology lies in its ability to bridge basic science and clinical practice. By understanding how the “where” and the “when” of immune regulation intersect, the field can move closer to developing truly personalised interventions for lung cancer patients.

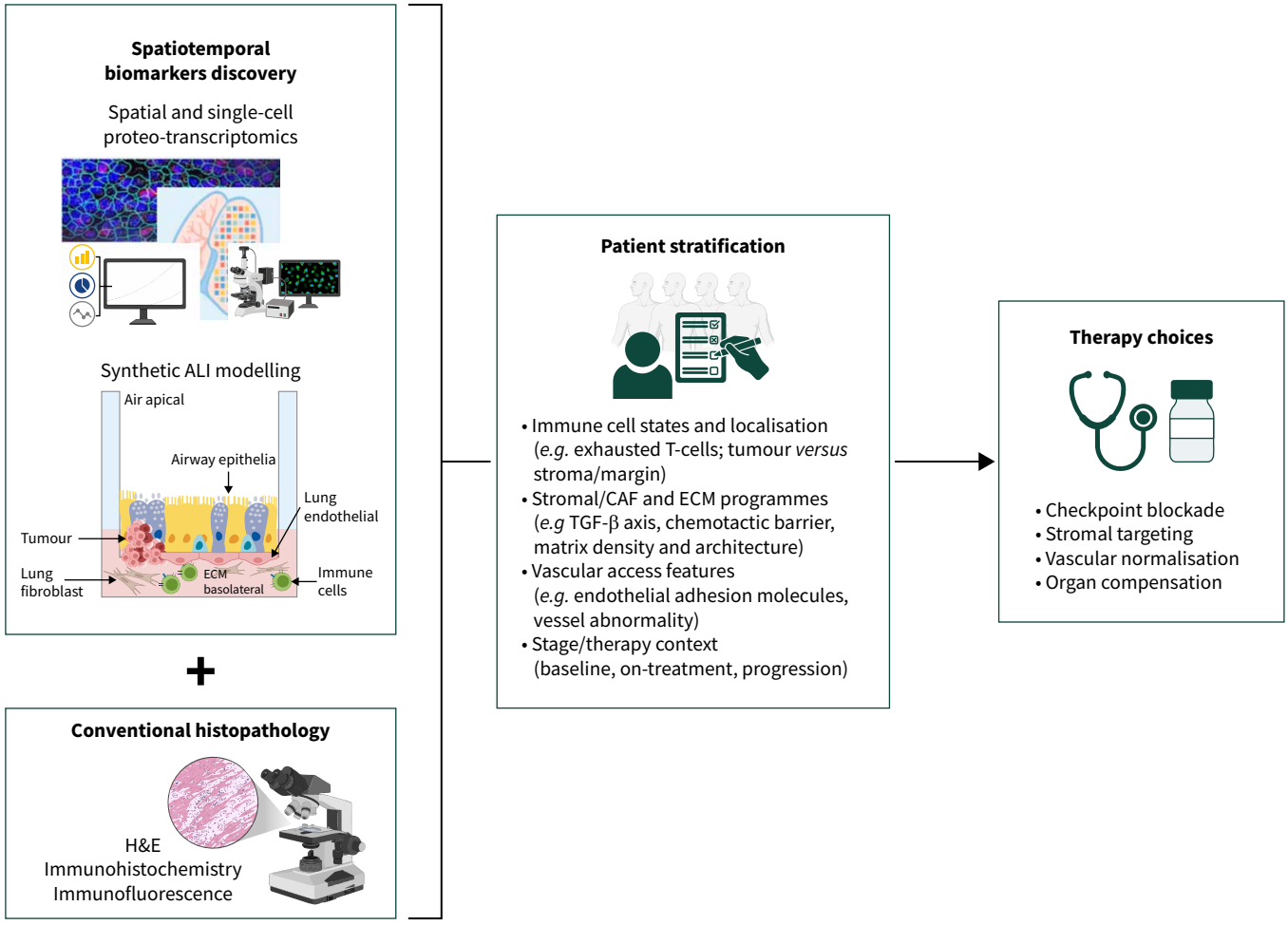

FIGURE 2 Integration of spatiotemporal biomarker discovery with conventional histopathology enables refined patient stratification and therapy choices. This framework integrates three-dimensional synthetic air–liquid interface (ALI) tissue modelling, spatial and single-cell proteo-transcriptomics and routine histopathology to derive clinically interpretable tissue features. Patient stratification is informed by four complementary tissue dimensions: 1) immune features (cell states such as exhausted T-cells, and spatial localisation including in the tumour nests or in the stroma/invasive margin); 2) tumour features (cell states and pathway programmes, such as extracellular matrix (ECM) density and architecture, stromal or cancer-associated fibroblasts (CAFs)–transforming growth factor (TGF)-β axis and dysregulation of chemotactic axes); 3) vascular features (vessel abnormality due to angiogenesis and reprogramming of endothelial adhesion molecule expression); and 4) stage/therapy context (how these features shift across disease progression and treatment). H&E: haematoxylin and eosin. Figure created using Biorender.com.

Conclusion

Spatiotemporal perspectives provide a unifying framework for understanding lung cancer biology. Spatial niches, whether immune-hot, -cold or -excluded, shape whether immune cells gain access to tumours, while temporal changes during progression and therapy remodel these landscapes. Together, they determine whether immune surveillance and anti-tumour functions succeed or fail. Advances in high-resolution technologies now allow these dynamics to be visualised with unprecedented clarity, and early clinical studies are beginning to link these insights to biomarker development and therapeutic strategies.

For the respiratory and oncology community, recognising that the tumour microenvironment is both spatially organised and temporally evolving underscores the need to align treatment with the dynamic nature of the disease. Spatiotemporal signatures hold promise not only as stage-specific predictive biomarkers but also as guides to therapy selection, stromal targeting and immune reactivation. Integrating time and space into both research and clinical decision-making will be critical for the next generation of precision approaches in lung cancer.

Key points

- Spatial tissue niches and temporal evolution jointly determine immune surveillance and response in NSCLC.
- Tumour progression and therapy remodel stromal barriers, altering immune infiltration patterns.
- High-resolution technologies reveal ligand–receptor networks with direct clinical relevance.
- Spatiotemporal signatures hold promise to uncover stage-specific predictive biomarkers and therapeutic targets.

Self-evaluation questions

1. What are the main spatial features of the lung tumour microenvironment that restrict immune access?
2. How does tumour progression from the early to the late stage affect immune–stromal interactions?
3. What changes occur in the tumour microenvironment after immunotherapy or targeted therapy?
4. Which high-resolution technologies allow simultaneous spatial and temporal analysis of the lung cancer immune landscape?
5. How could spatiotemporal signatures serve as predictive biomarkers for patient outcomes?

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Suggested answers

1. Dense CAFs and cancer-associated endothelial cells, abberrant tumour vasculature with downregulated adhesion molecules, compartmentalised expression of immune checkpoint ligands, and upregulation of regulatory cytokines can both physically and chemically exclude T-cells from tumour nests, creating an "immune-excluded" state.
2. Early-stage tumours often display immune-hot features with T-cell infiltration and intact antigen presentation, but as tumours progress, immunoediting selects cancer clones that have reduced MHC expression and neoantigen loss. Additionally, there is a shift in permissive fibroblastic phenotype to immunosuppressive CAFs states, as well as upregulation of regulatory T-cells that further suppress anti-tumour activities.
3. Successful therapy involves reinvigoration of exhausted T-cells, improving local antigen presentation, reducing tumour-derived immunosuppressive cytokines, and preventing upregulation of compensatory checkpoints.
4. Spatial transcriptomics enable unbiased mapping of gene expression across tissue sections, while multiplexed imaging, such as immunofluorescence, provides high-throughput spatial protein profiling. In parallel, single-cell transcriptomics provides temporal resolution of clonal dynamics transition state changes, while live-cell imaging and microscopy allows real-time tracking of T-cell motility, synaptic contact formation and killing dynamics observation.
5. Spatial proximity of T-cells to tumour cells at diagnosis can predict efficacy of checkpoint blockade and cytotoxic acitivities. Secondly, providing temporal trajectories of immune infiltration from baseline level, with spatially resolved co-expression patterns of immune checkpoints, may rationally guide combination therapy strategies. Composite spatiotemporal biomarkers could provide a multimodal predictive score that outperforms a single-analyte approach.