

Mitochondrial mechanisms underlying spatial immune phenotypes in non-small cell lung cancer: Implications for ICI response and cold-to-hot tumor conversion (Review)

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Abstract. Non-small cell lung cancer (NSCLC) is characterized by marked spatial immune heterogeneity, commonly manifested as immune-desert, immune-excluded and immune-inflamed phenotypes. These phenotypes are closely

associated with variable responses to immune checkpoint inhibitors (ICIs). Emerging evidence suggests that they are shaped not only by canonical immune pathways but also by mitochondrial processes that regulate immune priming, T-cell trafficking and the maintenance of effector function. The present review summarizes how mitochondrial reactive oxygen species, mitochondrial DNA-cGAS-STING signaling, pathways mediated by tricarboxylic acid cycle metabolites, and non-cell-autonomous mitochondrial transfer may contribute to the establishment and maintenance of distinct spatial immune phenotypes in NSCLC. It is proposed that insufficient activation of innate immunity and impaired dendritic cell priming are particularly relevant to immune-desert tumors, whereas chronic oxidative stress may reinforce barriers mediated by cancer-associated fibroblasts (CAFs), transforming growth factor β , and the extracellular matrix in immune-excluded tumors. By contrast, immune-inflamed tumors may represent a state in which pre-existing immune circuitry remains intact and tumor-infiltrating lymphocytes retain greater mitochondrial fitness and metabolic resilience. The implications of these mechanisms for ICI sensitivity and cold-to-hot tumor conversion strategies were further discussed. Current evidence from human studies remains largely correlative, and prospective, spatially resolved functional studies are needed. Nevertheless, integrating mitochondrial biology with spatial immune architecture within a phenotype-guided framework may generate testable hypotheses for future biomarker development and combination strategies.

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Abbreviations: A2AR, adenosine A2A receptor; ATP, adenosine triphosphate; α -KG, alpha-ketoglutarate; CAFs, cancer-associated fibroblasts; cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; CRT, calreticulin; CTLA-4, cytotoxic t-lymphocyte associated protein 4; CXCL, C-X-C motif chemokine ligands; DC, dendritic cell; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; ETC, electron transport chain; EVs, extracellular vesicles; HIF- α , hypoxia-inducible factor alpha subunit; ICIs, immune checkpoint inhibitors; IFN, interferon; IFN-I, type I IFN; IRF8, IFN regulatory factor 8; KEAP1, Kelch-Like ECH-associated protein 1; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; mtROS, mitochondrial ROS; NSCLC, non-small cell lung cancer; OXPHOS, oxidative phosphorylation; PD-(L)1, programmed cell death protein 1/programmed death-ligand 1; RocA, rocaglamide; STING, stimulator of IFN genes; STK11, serine/threonine kinase 11; TCA, tricarboxylic acid; TBK1, TANK-binding kinase 1; TCR, T-cell receptor; TGF- β , transforming growth factor beta; TILs, tumor-infiltrating lymphocytes; TMB, tumor mutational burden; TME, tumor microenvironment; TNF, tumor necrosis factor; TNTs, tunneling nanotubes; TRAIL, TNF-related apoptosis-inducing ligand; $\Delta\Psi_m$, mitochondrial membrane potential

Key words: NSCLC, spatial immune phenotypes, tumor immune microenvironment, mitochondria, ICIs, cold-to-hot tumor conversion

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1. Introduction

Lung cancer is one of the most common malignancies and a leading cause of cancer-related death worldwide, with non-small cell lung cancer (NSCLC) accounting for ~79% of all lung cancer cases (1). The advent of immune checkpoint inhibitors (ICIs), particularly agents targeting the programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) axis, has markedly transformed the treatment landscape for advanced NSCLC. Nevertheless, the clinical benefits of ICIs remain highly variable. Although some patients achieve durable tumor control, many experience only transient benefit or primary resistance, depending on the treatment setting, biomarker status, combination regimen and tumor microenvironmental (TME) context (2). These variable outcomes suggest that the limited efficacy of ICIs in NSCLC cannot be attributed to a single immunosuppressive pathway. Rather, it likely reflects the interplay among tumor-intrinsic characteristics, immune-cell functional states, and spatial constraints within the tumor immune microenvironment (TIME) (3,4).

Accumulating evidence indicates that structural constraints arising from interactions between tumors and the host immune system within local tissue niches may contribute to the failure of ICI therapy. ICI efficacy is associated not only with established biomarkers, such as PD-L1 expression and tumor mutational burden (TMB), but also with the spatial organization of the TIME (5). The TME comprises a complex network of cellular and structural components, including immune cells, fibroblasts, blood vessels, signaling molecules and the extracellular matrix (ECM) (6,7). Its spatial architecture influences whether immune cells can reach the tumor, penetrate tumor-cell nests, and sustain effector function. It also shapes immune-cell states through nutrient availability, oxygen tension and metabolic by-products (6). In this context, NSCLC exhibits pronounced spatial immune heterogeneity. Some tumors contain abundant intratumoral T cells and exhibit interferon (IFN)-related transcriptional programs, whereas others are nearly devoid of T cells or harbor T cells that remain largely confined to tumor margins or stromal compartments (5).

Based on these spatial patterns, tumor immune states are commonly described in terms of three operational phenotypes: Inflamed, immune-desert and immune-excluded (8). The inflamed phenotype is characterized by immune-cell infiltration into tumor nests; the immune-desert phenotype by globally limited immune infiltration; and the immune-excluded phenotype by the accumulation of immune cells in stromal or marginal regions with limited penetration into tumor-cell nests. However, these phenotypes should be viewed as context-dependent, non-mutually exclusive spatial states rather than categories defined by universal thresholds. They may vary according to the sampled region, disease stage, treatment exposure and analytical platform. Elucidating the mechanisms that give rise to these spatial states may therefore help explain why some NSCLC tumors respond poorly to ICIs despite the presence of targetable immune checkpoints.

Mitochondrial metabolism, which intersects with multiple aspects of cancer biology, offers a mechanistic perspective for understanding these spatial immune constraints. Although the classical Warburg effect emphasizes the role of aerobic glycolysis in tumor-cell energy metabolism, mitochondria are also involved in tumor progression, stress adaptation, therapeutic responses and immune regulation. Mitochondrial reactive oxygen species (mtROS) may function as signaling mediators or amplify cellular stress, depending on their intensity, duration and cellular context. Tricarboxylic acid (TCA) cycle metabolites may link mitochondrial metabolism to epigenetic regulation and hypoxia-responsive programs. The release of mitochondrial DNA (mtDNA) may engage innate immune sensing through the cyclic GMP-AMP synthase-stimulator of IFN genes (cGAS-STING) pathway. In addition, mitochondrial dynamics, mitophagy and intercellular mitochondrial transfer may influence the functional states of tumor cells and tumor-infiltrating lymphocytes (TILs). These mechanisms may therefore connect mitochondrial biology to immune priming, chemokine-driven immune recruitment, stromal barrier formation, and the maintenance of T-cell effector function (9-11).

However, evidence linking mitochondrial processes to spatial immune phenotypes in human NSCLC remains predominantly correlative. Standardized, spatially resolved and cell type-specific measures of mitochondrial function remain limited, complicating causal inference and efforts to determine whether mitochondrial alterations act as upstream drivers, phenotype amplifiers, or concomitant biological states (3,5,12). Accordingly, the present review synthesizes current evidence linking mitochondrial biology to spatial immune phenotypes in NSCLC within an evidence-weighted mechanistic framework. It was examined how mitochondrial processes in tumor, immune, stromal and vascular cells may contribute to immune-desert, immune-excluded and inflamed states. Well-supported clinical associations from preclinical evidence and hypothesis-generating models were also relatively distinguished and the key experimental and translational steps required to validate mitochondria-informed strategies for cold-to-hot tumor conversion were outlined.

2. Review methodology and scope

The present review presents a focused mechanistic narrative review. It aims to synthesize current evidence linking mitochondrial biology to spatial immune phenotypes and responses to ICIs in NSCLC, with particular emphasis on immune-desert, immune-excluded and inflamed tumor states.

Relevant literature published through March 2026 was identified through iterative searches of PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Web of Science (<https://clarivate.com/academia-government/scientific-and-academic-research/research-discovery-and-referencing/web-of-science/>) and Google Scholar (<https://scholar.google.com/>). The searches were structured around predefined conceptual modules, including NSCLC, spatial immune phenotypes, the TIME, ICIs, mitochondrial metabolism, oxidative phosphorylation (OXPHOS), mtROS, mtDNA, cGAS-STING signaling, TCA cycle metabolites, epigenetic regulation, mitochondrial dynamics, mitophagy, mitochondrial transfer,

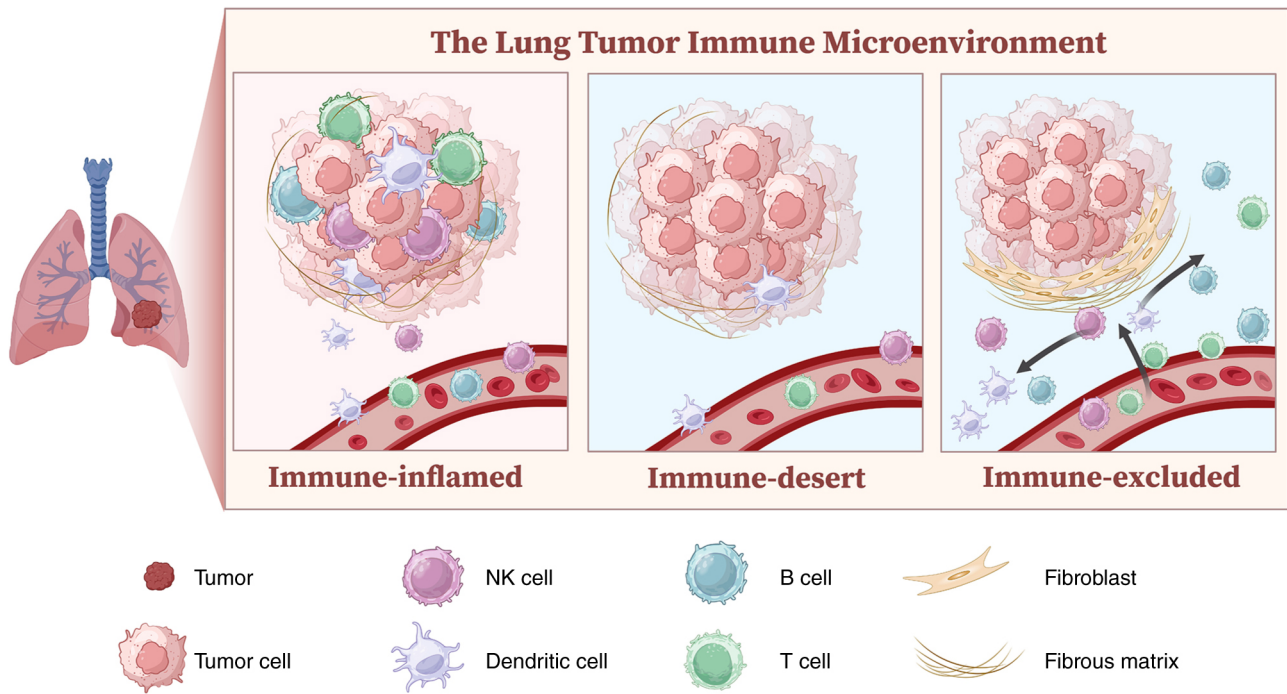


Figure 1. Spatial immune phenotypes of the NSCLC TME. Schematic illustration of the three major spatial immune phenotypes in the TME of NSCLC, conceptually categorized according to the spatial distribution of immune cells relative to tumor nests. Immune-inflamed tumors are characterized by abundant immune-cell infiltration within tumor parenchyma, with immune cells frequently located in close proximity to tumor cells. Immune-desert tumors exhibit sparse immune-cell infiltration within both tumor nests and surrounding stroma, suggesting limited immune recruitment or impaired immune priming within the TME. Immune-excluded tumors display immune cells enriched in the peritumoral stroma or invasive margin but with limited penetration into tumor nests, often associated with stromal remodeling or physical barriers restricting immune-cell infiltration. The schematic also depicts representative cellular components involved in NSCLC immune architecture, including tumor cells, T cells, B cells, NK cells, dendritic cells, fibroblasts, and fibrous ECM. NSCLC, non-small cell lung cancer; TME, tumor microenvironment; NK, natural killer.

cancer-associated fibroblasts (CAFs), hypoxia, and cold-to-hot tumor conversion. Additional studies were identified by screening the reference lists of relevant original articles and reviews.

Because the available literature encompasses heterogeneous evidence from human NSCLC cohorts, spatial profiling studies, animal models, *in vitro* systems, and other tumor types, evidence was prioritized in the following order: Human NSCLC studies with clinical or spatial information; NSCLC studies involving ICI response or resistance; mechanistic studies using NSCLC models; and, when direct evidence from NSCLC was unavailable, mechanistic evidence from other tumor types or general cancer biology. Evidence derived from non-NSCLC models or non-spatial experimental systems was interpreted as supportive or hypothesis-generating rather than definitive evidence for spatial immune phenotypes in human NSCLC.

Accordingly, the present review does not provide a quantitative evidence synthesis. Instead, it adopts an evidence-weighted narrative approach to distinguish relatively well-established clinical associations from preclinical mechanistic findings and context-dependent hypotheses requiring further validation in spatially resolved human NSCLC samples.

3. Operational and clinical definitions of spatial immune phenotypes in NSCLC

The spatial immune architecture of NSCLC is commonly described in terms of three major patterns: Inflamed, immune-desert and immune-excluded phenotypes. These

terms provide a practical framework for characterizing the localization of immune cells relative to tumor-cell nests and interpreting potential differences in ICI responses (6,8,13,14) (Fig. 1; Table SI). However, they should be regarded as operational, context-dependent spatial states rather than fixed, mutually exclusive biological categories defined by universal thresholds. Different phenotypes may coexist within the same tumor, and their classification may vary according to the sampled region, disease stage, prior treatment, tissue processing, marker selection, analytical platform, and thresholding method (8,14,15) (Fig. 2).

The inflamed phenotype is operationally characterized by the presence of immune cells, particularly CD8⁺ T cells, within tumor-cell nests or in close spatial proximity to malignant epithelial cells (8,13,14). This pattern is often accompanied by enhanced antigen-presentation activity, IFN-associated transcriptional programs, and increased immune-cell interactions within the tumor parenchyma (6,13,16). Inflamed tumors are generally more likely to harbor pre-existing antitumor immune activity and may respond more favorably to ICIs than non-inflamed tumors. Nevertheless, abundant intratumoral T-cell infiltration does not necessarily translate into durable clinical benefit, because infiltrating T cells may remain functionally exhausted or metabolically compromised, while local costimulatory and antigen-presentation programs may be inadequate (6,16). The inflamed phenotype should therefore be interpreted as a state of spatial immune infiltration rather than as a definitive indicator of functional antitumor immunity or ICI responsiveness.

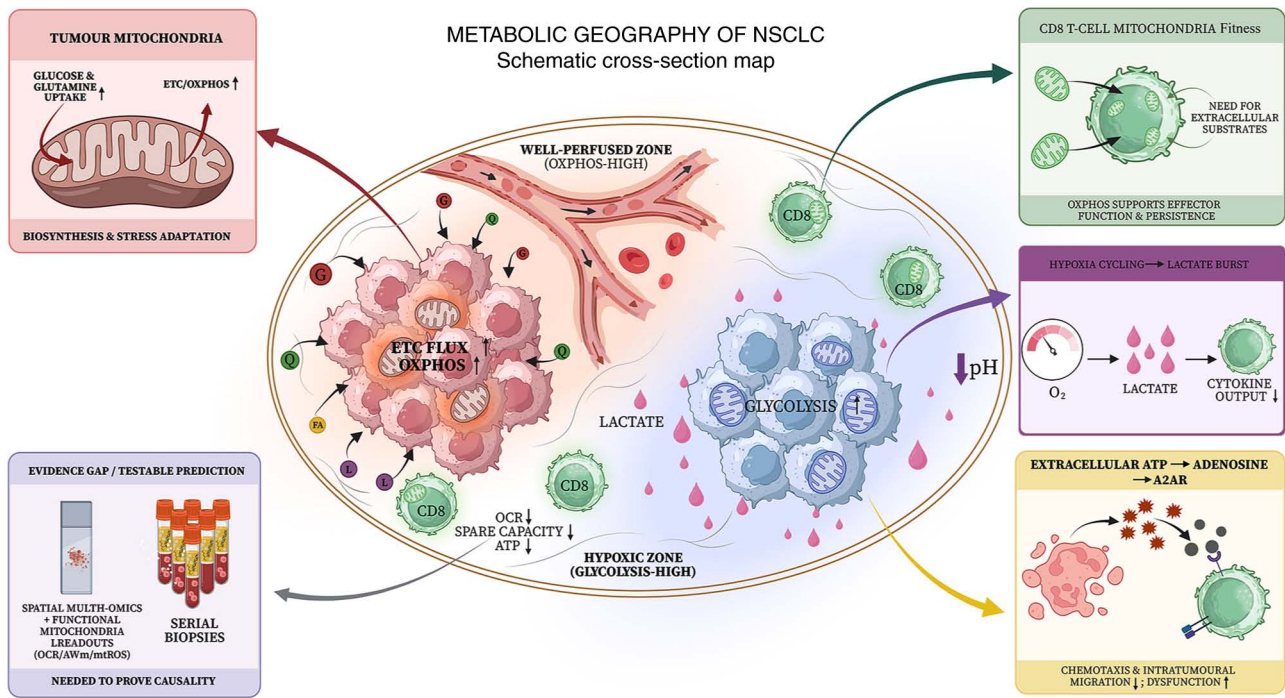


Figure 2. Metabolic geography of NSCLC: Spatial heterogeneity of tumor metabolism and potential immune consequences. Tumor regions within NSCLC exhibit substantial metabolic heterogeneity, with distinct bioenergetic states potentially influencing the local immune microenvironment. This schematic illustrates a conceptual spatial map integrating tumor-cell mitochondrial activity, metabolic adaptation and immune-cell responses across different tumor niches. Highly proliferative tumor regions may exhibit increased glucose and glutamine utilization, enhanced ETC activity, and elevated OXPHOS, supporting biosynthetic demands and stress adaptation. By contrast, poorly perfused or hypoxic regions are characterized by increased glycolytic activity, lactate accumulation, extracellular acidification and altered immune-cell function. The upper right panel illustrates potential effects of tumor-associated metabolic conditions on CD8⁺ T-cell mitochondrial fitness. Although oxidative metabolism is essential for effector T-cell persistence and function, nutrient limitation and altered metabolite availability within tumor regions may impose metabolic constraints on infiltrating immune cells. Hypoxia-associated metabolic remodeling may further influence immune-cell behavior through lactate accumulation, reduced cytokine production, and altered signaling pathways. In addition, extracellular ATP metabolism and adenosine generation may contribute to immunoregulatory states through purinergic signaling, including A2AR-mediated pathways. The proposed model highlights tumor metabolic demand as a potential upstream pressure that may contribute to spatial immune dysfunction. However, direct evidence demonstrating local substrate competition between tumor cells and immune cells in human NSCLC remains limited. Spatial multi-omics approaches combined with functional mitochondrial measurements and serial sampling strategies will be required to establish causal relationships between tumor metabolic states and immune-cell impairment. This figure summarizes candidate metabolic constraints and spatial associations rather than a universally established causal hierarchy between tumor metabolism and immune exclusion. NSCLC, non-small cell lung cancer; ETC, electron transport chain; A2AR, A2A receptor; OXPHOS, oxidative phosphorylation; OCR, oxygen consumption rate; mtROS, mitochondrial reactive oxygen species.

The immune-desert phenotype is characterized by sparse immune-cell infiltration throughout both tumor cell nests and the surrounding stromal compartments (8,14,15). Operationally, this phenotype is identified by low densities of intratumoral and stromal effector immune cells, particularly CD8⁺ T cells, although the precise quantitative thresholds vary among studies. Immune-desert tumors frequently exhibit impaired immune priming and recruitment, including reduced dendritic-cell (DC) activity, antigen-presentation capacity, IFN signaling, or chemokine expression (13,16). Clinically, the scarcity of preexisting effector cells may limit the capacity of PD-(L)1 blockade alone to elicit an effective antitumor response. However, a desert-like appearance may result from several distinct biological processes and may also be influenced by sampling bias. Therefore, low immune-cell density alone should not be interpreted as evidence of a specific underlying mechanism.

The immune-excluded phenotype is characterized by the presence of immune cells that remain predominantly confined to stromal compartments, invasive margins, or peritumoral regions, with limited penetration into tumor cell nests (8,14,17). Unlike immune-desert tumors, immune-excluded tumors

may contain substantial numbers of T cells and other immune populations, but their spatial distribution limits direct contact with malignant cells. This pattern is frequently associated with enrichment of CAFs, ECM deposition and remodeling, transforming growth factor- β -related programs, abnormal vasculature and hypoxia-associated barriers (17). Nevertheless, these features should be regarded as common correlates rather than universal defining mechanisms because the relative contributions of stromal, vascular, chemotactic and tumor-intrinsic factors vary among tumors.

These phenotypes can be evaluated using conventional immunohistochemistry, multiplex immunofluorescence, imaging mass cytometry, digital pathology, whole-slide image analysis, and spatial transcriptomic or proteomic approaches (6,8,13,14). Relevant measures include immune-cell densities within tumor and stromal compartments, distances between immune and malignant cells, localization relative to tumor-stroma boundaries, and spatially resolved immune or stromal gene-expression programs. However, no universally accepted quantitative thresholds currently distinguish inflamed, immune-desert and immune-excluded NSCLC across analytical platforms. Throughout the present review,

these terms are therefore used as practical spatial frameworks for organizing evidence rather than as validated clinical diagnostic categories. Pending prospective comparisons and standardized spatial assessment, their potential value should be considered complementary to that of established biomarkers such as PD-L1 expression and TMB.

4. Cell type-specific mitochondrial mechanisms in the NSCLC immune microenvironment

For analytical clarity, mitochondria-related processes within the NSCLC TIME are considered according to the principal cellular or tissue compartments in which they are measured or proposed to operate. This compartment-resolved approach is important because malignant, immune, stromal and vascular states vary substantially among regions within the same tumor. Moreover, the biological consequences of a mitochondrial signal may depend on its cellular source, magnitude, duration and surrounding microenvironmental context (6,8,14,17-19). Accordingly, the following subsections separately address tumor-cell mitochondrial metabolism and redox signaling, T-cell mitochondrial fitness, DC and myeloid innate sensing, the stromal and vascular context, TCA metabolite-linked epigenetic regulation, and intercellular mitochondrial transfer.

However, these mechanisms should not be interpreted as one-to-one determinants of the inflamed, immune-desert, or immune-excluded phenotypes. In human NSCLC, evidence is relatively strong for several spatial associations among immune-cell localization, stromal organization, and treatment response. By contrast, direct cell type-resolved measurements of mitochondrial function and interventional evidence remain limited (6,8,14,17-19). Throughout this section, relatively well-established spatial associations were therefore distinguished from preclinical mechanistic evidence and hypothesis-generating extrapolations (Fig. 3).

Tumor-cell mitochondrial metabolism and redox stress signaling. NSCLC cells do not uniformly rely on glycolysis alone. Spatial mapping studies have revealed substantial intratumoral heterogeneity in mitochondrial organization and bioenergetic activity. Better-perfused regions tend to exhibit greater oxidative metabolic capacity, whereas poorly perfused and hypoxic regions are more commonly associated with glycolytic programs (20,21). Metabolic analyses of patient samples have further shown that glucose-derived carbon can contribute substantially to the TCA cycle in biologically aggressive NSCLC (22). These findings indicate that tumor-cell mitochondrial metabolism varies spatially and may create regional differences in the demand for oxygen and carbon substrates.

In tumor cells with relatively high OXPHOS activity, electron transport chain (ETC) flux supports ATP production, biosynthesis and adaptation to metabolic stress. Respiratory activity is also required to sustain biosynthetic pathways essential for lung cancer growth and survival (21,23). Moreover, lactate can modulate ETC activity independently of its direct oxidation as a carbon substrate, further illustrating the metabolic plasticity of the respiratory chain (24). Under glucose-limited conditions, NSCLC cells, including drug-resistant cell models, can activate adaptive metabolic programs that sustain survival

and proliferation (25). Collectively, these findings support the existence of tumor-cell states characterized by both high metabolic demand and metabolic plasticity.

These observations, however, do not in themselves demonstrate direct substrate competition between tumor and immune cells in human NSCLC. Although the cited studies establish spatial bioenergetic heterogeneity, metabolic adaptation and altered substrate utilization, they do not simultaneously measure tumor-cell OXPHOS activity, local substrate depletion, and mitochondrial dysfunction in neighboring T cells within the same tissue regions (20-25). Tumor-cell substrate consumption should therefore be regarded as a candidate upstream metabolic pressure rather than an established proximal cause of immune-desert or immune-excluded architecture. The potential effects of these tumor-cell metabolic states on mitochondrial fitness in neighboring T cells are discussed in the following section.

In addition to regulating bioenergetic demand, tumor-cell mitochondria participate in redox signaling. mtROS arise primarily from electron leakage at respiratory-chain complexes I and III. Their production is influenced by membrane potential, substrate redox state and changes in oxygen availability, including hypoxia-reoxygenation (25,26). Depending on their abundance, duration and cellular context, mtROS may act as signaling mediators or cause oxidative damage (27,28). Accordingly, the proposed 'biphasic threshold' of mtROS is best regarded as a conceptual framework rather than a universally established spatial rule. Moderate redox signaling may facilitate cellular adaptation or inflammatory signaling, whereas sustained or excessive oxidative stress can damage macromolecules and reinforce stress-response programs.

The consequences of tumor-cell redox signaling are further shaped by tumor-intrinsic antioxidant capacity and molecular context. Broad manipulation of oxidative stress or antioxidant pathways has yielded inconsistent effects across experimental cancer models and may, in some contexts, promote rather than inhibit tumor progression (29). In NSCLC, transcriptional states that phenocopy deleterious Kelch-Like ECH-associated protein 1 (KEAP1) alterations have been associated with clinical outcomes after immunotherapy (30), whereas KEAP1/STK11 alterations also define distinct stress responses and therapeutic vulnerabilities (31). These findings underscore that redox biology cannot be interpreted independently of tumor genotype and cellular state. Importantly, they do not establish either low or high tumor-cell mtROS as a sole determinant of any specific spatial immune phenotype.

Overall, tumor-cell mitochondrial activity may contribute to spatially heterogeneous metabolic and redox constraints through changes in oxygen consumption, substrate demand, respiratory-chain activity and oxidative stress. Current evidence from human NSCLC supports the existence and clinical relevance of regional metabolic heterogeneity, but direct causal links among tumor-cell mitochondrial states, immune-cell positioning, and ICI response remain insufficiently defined. Future studies should integrate spatial bioenergetic and metabolomic mapping (18,20) with multiplexed immune imaging (19) and cell type-resolved measurements of mitochondrial function to determine whether tumor-cell metabolic states act as upstream drivers, amplifiers, or concomitant features of spatial immune organization.

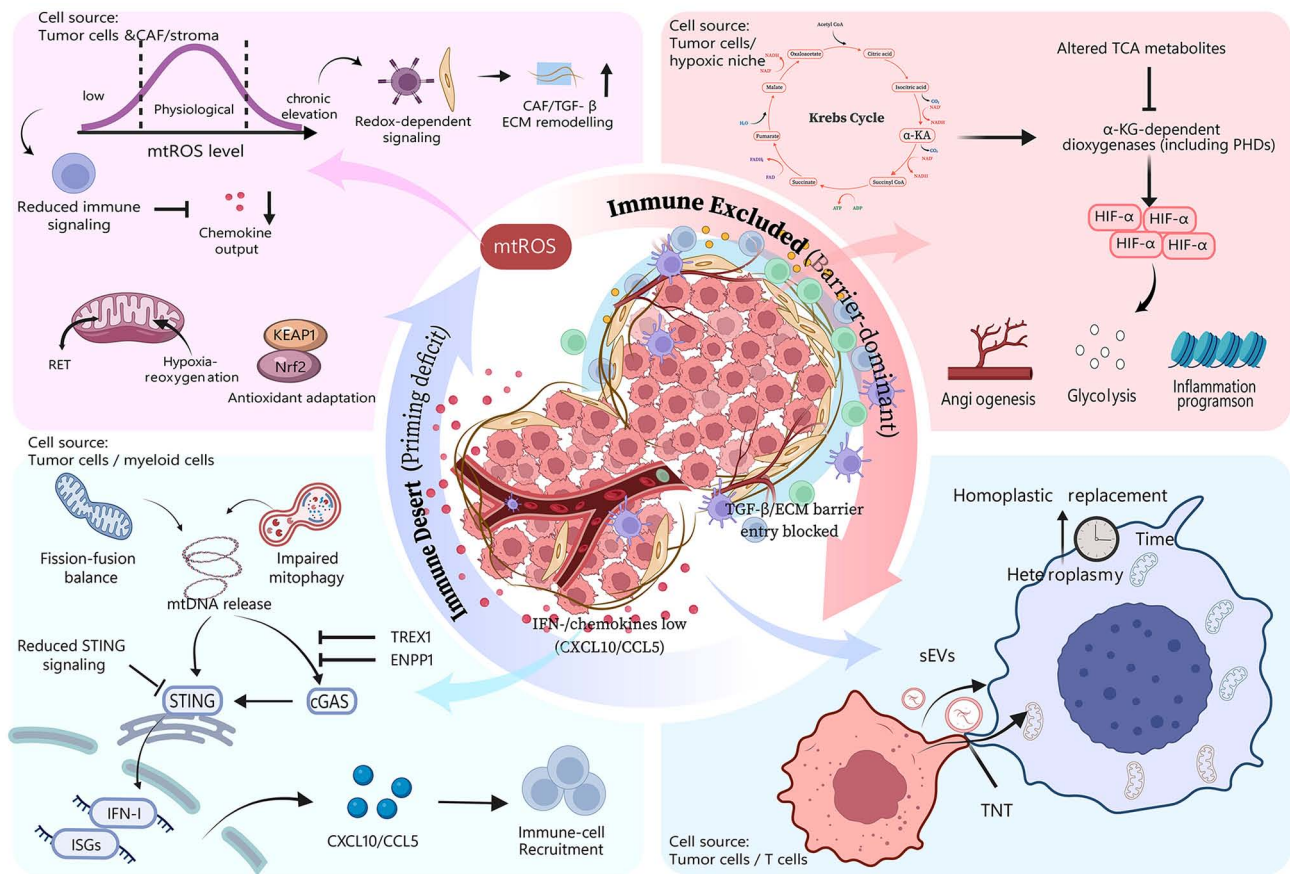


Figure 3. Mitochondria-associated mechanisms potentially shaping spatial immune phenotypes in NSCLC. Mitochondrial alterations may influence spatial immune organization in NSCLC through multiple interconnected mechanisms, including redox regulation, metabolic remodeling, mitochondrial danger signaling, and intercellular mitochondrial communication. The schematic summarizes candidate mitochondria-associated pathways that may contribute to immune-excluded and immune-desert phenotypes. In the upper left panel, altered mtROS levels are depicted as a context-dependent regulator of cellular signaling. Moderate mitochondrial redox activity may support physiological signaling, whereas persistent mtROS elevation may induce redox-dependent signaling alterations, antioxidant adaptation (for example, KEAP1-nuclear factor erythroid 2-related factor 2 pathway), and stromal remodeling involving CAFs and ECM components. These processes may influence immune-cell function and spatial immune organization. In the upper right panel, tumor-associated metabolic remodeling, including alterations in TCA cycle metabolites and α -KG-dependent dioxygenase activity, may regulate HIF- α stabilization and downstream hypoxia-associated programs, such as angiogenesis, glycolytic adaptation and inflammatory transcriptional responses. These metabolic changes represent potential microenvironmental constraints rather than directly established drivers of immune exclusion. In the lower left panel, mitochondrial stress-associated signals, including mtDNA release caused by altered mitochondrial dynamics or impaired mitophagy, may activate cGAS-STING-mediated innate immune signaling. Subsequent induction of type I IFN-related genes and chemokines, including CXCL10 and CCL5, may facilitate immune-cell recruitment. However, the spatial consequences of mtDNA sensing remain context dependent and may vary according to tumor type, immune composition and signaling status. In the lower right panel, intercellular mitochondrial communication mediated by TNTs or sEVs is illustrated as a potential mechanism influencing mitochondrial heterogeneity and immune-cell fitness. Current evidence for mitochondrial transfer in cancer immunity is predominantly derived from experimental models, and its contribution to spatial immune phenotypes in human NSCLC requires further investigation. The illustrated pathways represent a combination of relatively established mitochondrial signaling mechanisms, preclinical observations and hypothesis-generating models. The connections between mitochondrial alterations and spatial immune phenotypes should therefore be interpreted as potential regulatory relationships rather than universally validated causal pathways. NSCLC, non-small cell lung cancer; mtROS, mitochondrial reactive oxygen species; CAFs, cancer-associated fibroblasts; ECM, extracellular matrix; TCA, tricarboxylic acid; α -ketoglutarate; α -KG, α -ketoglutarate; HIF- α , hypoxia-inducible factor- α ; IFN, interferon; TNTs, tunneling nanotubes; sEVs, small extracellular vesicles.

T-cell mitochondrial fitness and dysfunction. The activation, effector activity, and long-term persistence of CD8⁺ T cells depend on tightly coordinated mitochondrial bioenergetics and quality-control mechanisms. Beyond ATP generation, mitochondrial OXPHOS, spare respiratory capacity, membrane potential, and redox homeostasis collectively support cytokine production, cytotoxicity, and sustained T-cell function under conditions of metabolic stress (32). Together, these characteristics define mitochondrial fitness, a functional property that is conceptually distinct from T-cell abundance or the extent of spatial infiltration.

Evidence from lung cancer further indicates substantial heterogeneity in the mitochondrial states of tumor-infiltrating

CD8⁺ T cells. A CD38-high CD8⁺ T-cell subset exhibiting features of mitochondrial adaptation has been identified in lung cancer, suggesting that certain T-cell populations may preserve bioenergetic programs that facilitate persistence within the TME (5). However, the identification of such subsets does not suggest that mitochondrial fitness is uniformly maintained in inflamed tumors, nor does it establish any single T-cell marker as a universal indicator of functional competence across patients.

Within the TME, T cells are simultaneously exposed to nutrient deprivation, hypoxia, extracellular acidification and immunosuppressive metabolites. Experimental evidence indicates that glucose restriction can reshape mitochondrial

signaling and alter T-cell differentiation programs (33). Studies in a non-NSCLC model have additionally shown that selective suppression of glutamine metabolism in tumor cells can enhance antitumor T-cell activity, supporting the broader principle that competition for metabolic substrates between malignant and immune cells can influence immune function (34). Nevertheless, direct evidence demonstrating glutamine competition between tumor cells and T cells within spatially resolved regions of human NSCLC remains scarce.

Lactate and adenosine constitute two additional metabolic constraints capable of compromising T-cell mitochondrial fitness and effector activity. Lactate accumulation and extracellular acidification can inhibit T-cell glycolysis, cytokine production and migration, although the magnitude of these effects varies according to metabolite concentration, duration of exposure, and T-cell differentiation state (35,36). In parallel, extracellular ATP released by stressed, hypoxic, or dying cells can be sequentially converted to adenosine by ecto-nucleotidases expressed across tumor, stromal and immune compartments. In NSCLC, CAFs and other cellular populations may contribute to CD73-dependent adenosine production and signaling (37,38). Activation of the A2A receptor by adenosine can suppress T-cell metabolic activity and chemotaxis, whereas experimental blockade or disruption of this pathway can partially restore T-cell function (36,39). However, because some of these functional observations were obtained in models other than NSCLC, they should be regarded as mechanistic evidence supporting biological plausibility rather than as direct demonstrations of spatial immune regulation in human NSCLC.

These metabolic stressors are unlikely to operate in isolation. Nutrient limitation, lactate accumulation, extracellular acidification, adenosine signaling, hypoxia and high tumor-intrinsic metabolic activity frequently converge within the same tissue regions. Their combined effects may impair T-cell OXPHOS, reduce spare respiratory capacity and ATP generation, disturb redox homeostasis, and ultimately compromise functional persistence. To date, however, studies of human NSCLC have not directly established a complete causal sequence in which tumor-cell substrate consumption creates localized nutrient depletion, subsequently induces mitochondrial dysfunction in adjacent T cells, and thereby determines their spatial distribution or responsiveness to immune checkpoint inhibition (6,40). Metabolic competition should therefore be viewed as a biologically plausible and experimentally testable framework rather than an established patient-level mechanism.

Importantly, spatial infiltration and mitochondrial competence represent interconnected but non-equivalent dimensions of antitumor immunity. An inflamed tumor may harbor abundant CD8⁺ T cells while those cells remain metabolically compromised, functionally exhausted, or unable to sustain cytotoxic activity. Conversely, low T-cell density may arise from defective priming or impaired recruitment rather than from an intrinsic mitochondrial defect in the T cells that do reach the tumor. Accordingly, mitochondrial fitness is best considered a potential determinant of sustained T-cell function after immune-cell recruitment, rather than a universal mechanism governing the initial formation of a particular spatial immune phenotype.

DC and myeloid mitochondrial sensing. Effective antitumor immunity requires more than the physical presence of T cells; it also depends on efficient antigen presentation, DC activation, costimulatory signaling, and productive T-cell priming. DC functional states can influence the magnitude of benefit derived from PD-L1 blockade, suggesting that immune checkpoint inhibition is less likely to generate durable adaptive immunity when local priming circuits are inadequate (41). Mitochondrial stress may intersect with these processes through the release of mtDNA and subsequent engagement of cytosolic DNA-sensing pathways in tumor and myeloid compartments.

When released from mitochondria into the cytosol, mtDNA can act as a damage-associated molecular pattern. Experimental studies have demonstrated that mitochondrial fragmentation, altered membrane permeability, disrupted cristae architecture, mitochondrial permeability transition, and defective mitophagy can promote the cytosolic accumulation of mtDNA or other damaged mitochondrial components (42-46). Much of this mechanistic evidence, however, has been generated in non-NSCLC settings, including other malignancies and inflammatory disease models. These findings establish the biological plausibility of stress-induced mtDNA release but do not, in themselves, demonstrate that this mechanism is a dominant determinant of immune organization in human NSCLC.

Once cytosolic, double-stranded DNA can be recognized by cyclic GMP-AMP synthase (cGAS), which catalyzes the production of cyclic GMP-AMP (cGAMP). cGAMP subsequently activates stimulator of IFN genes (STING), triggering downstream signaling through mediators such as TANK-binding kinase 1 (TBK1) and inducing type I IFNs, IFN-stimulated genes, and chemokines including C-X-C motif chemokine ligand 10 (CXCL10) and C-C motif chemokine ligand 5 (CCL5). These outputs can promote innate immune activation and facilitate immune-cell recruitment. Importantly, however, the cytosolic DNA that activates this pathway cannot automatically be assumed to originate from mitochondria. Nuclear DNA damage, chromosomal instability, replication stress and therapy-induced DNA damage can likewise generate cytosolic DNA and activate cGAS-STING signaling (47-50). This distinction is particularly important in the setting of radiotherapy and other DNA-damaging treatments, in which mitochondrial and nuclear DNA may simultaneously contribute to pathway activation (48).

The cellular compartment in which cGAS-STING signaling occurs is equally important. Tumor cells may sense cytosolic DNA and produce cGAMP, whereas downstream STING activation and IFN production may occur predominantly in neighboring DCs or macrophages. Cancer-cell-intrinsic cGAS activity has been associated with tumor immunogenicity (49), while lung cancer models have demonstrated immune-dependent antitumor effects mediated by STING activation in trans (51). Collectively, these findings support a compartmentalized signaling model in which tumor-derived signals are transferred to myeloid cells that execute downstream innate immune responses. Nevertheless, the relative contributions of tumor-cell cGAS, tumor-cell STING, DC STING, macrophage STING, and intercellular cGAMP transfer differ across experimental systems and remain insufficiently defined in human NSCLC specimens.

SCIENTIFIC MECHANISM: STING-MEDIATED T CELL PRIMING SPLIT

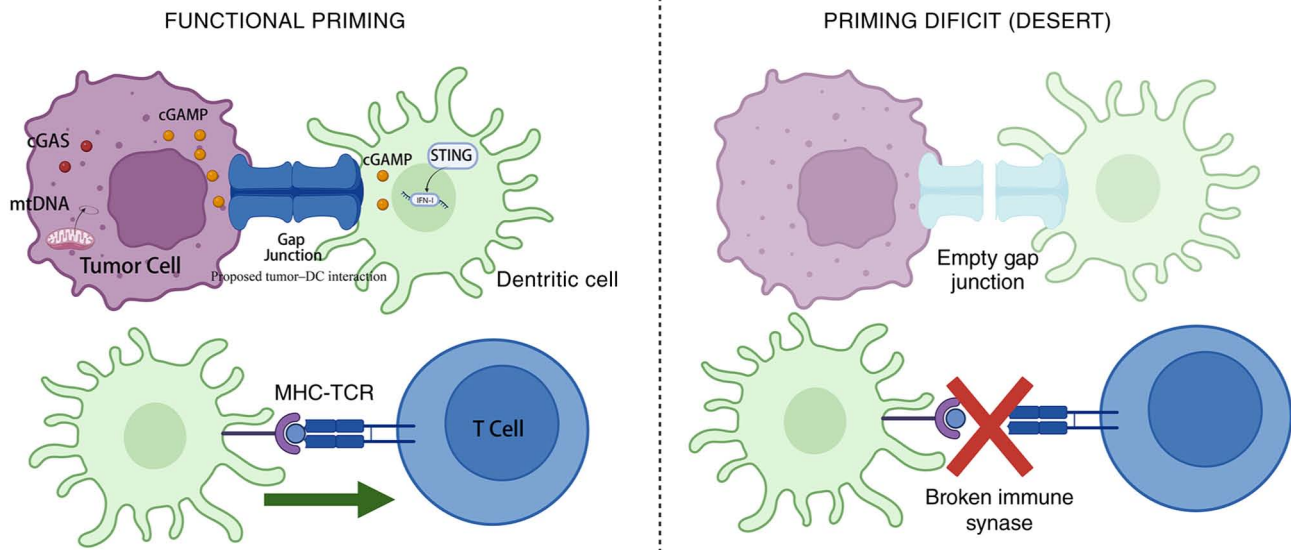


Figure 4. Defective tumor-DC cGAS-STING coupling as a candidate mechanism of priming failure in immune-desert NSCLC. Schematic illustration of a proposed model describing how impaired tumor-DC cGAS-STING coupling may contribute to immune-desert phenotypes in NSCLC. In a functional priming state, mitochondrial stress-associated mtDNA release may contribute to cytosolic DNA sensing by cGAS and subsequent cGAMP production in tumor cells. Tumor-derived cGAMP can then be transferred to DCs through proposed intercellular communication mechanisms, including gap-junction-mediated transfer, leading to STING activation, type I interferon signaling, DC maturation and productive T-cell priming. By contrast, disruption of this tumor-DC innate sensing axis, potentially caused by reduced mtDNA/cGAS signaling, impaired cGAMP transfer, or defective downstream STING activation, may result in insufficient DC activation and reduced T-cell priming. This schematic represents a conceptual framework rather than an established causal mechanism, and the contribution of mtDNA-driven cGAS-STING signaling to immune-desert formation in human NSCLC requires further spatial and functional validation. DC, dendritic cell; cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; NSCLC, non-small cell lung cancer; mtDNA, mitochondrial DNA; MHC, major histocompatibility complex; TCR, T cell receptor.

NSCLC-specific studies provide several complementary, although mechanistically distinct, lines of evidence. In preclinical NSCLC models, rocaglamide (RocA) activated cGAS-STING-associated signaling, increased the expression of CXCL10 and CCL5, and enhanced natural killer-cell infiltration and antitumor activity (52,53). These findings provide mechanistic support for the ability of mitochondrial damage-associated signaling to promote immune recruitment; however, because the evidence remains preclinical, it should not be interpreted as clinical validation of either RocA or mtDNA-directed therapeutic strategies. Separately, loss or reduced expression of STING has been associated with clinical and prognostic characteristics in NSCLC (54,55). STK11/LKB1-deficient NSCLC has likewise been linked to attenuated STING/type I IFN/CD8⁺ T-cell programs and diminished responsiveness to immunotherapy (54,55). Together, these observations support the clinical relevance of impaired innate immune signaling but do not establish mtDNA release as the initiating event in individual tumors.

Epigenetic regulation and other tumor-intrinsic mechanisms may further modulate the magnitude of pathway activation. Reduced STING expression, transcriptional silencing, negative regulators of cGAS-STING signaling, and extracellular degradation of cGAMP can each attenuate downstream IFN and chemokine production (50,53). Consequently, comparable levels of mitochondrial or nuclear DNA stress may produce markedly different immune consequences depending on the functional competence of the cGAS-STING pathway in tumor and myeloid cells.

Overall, defective tumor-myeloid innate sensing may contribute to inadequate immune priming and recruitment in a

subset of immune-desert NSCLC. However, spatially resolved studies that simultaneously define the source of cytosolic DNA, cell type-specific activation of cGAS and STING, DC maturation, chemokine production, T-cell recruitment and clinical response to ICIs remain lacking. The mtDNA-cGAS-STING axis should therefore be regarded as a high-priority candidate mechanism supported by molecular and preclinical evidence, rather than as a universal proximal driver of the immune-desert phenotype (Fig. 4).

Stromal and vascular mitochondrial context. Direct evidence linking stromal-cell- or endothelial-cell-intrinsic mitochondrial programs to the spatial organization of antitumor immunity in human NSCLC remains scarce. By contrast, the spatial distribution of CAFs, ECM organization, and vascular and hypoxic features has been more directly associated with impaired T-cell infiltration (7). Mitochondrial processes within these compartments are therefore better regarded as context-dependent modulators of stromal and vascular barriers rather than established primary drivers of the immune-excluded phenotype.

CAFs in lung cancer exhibit substantial phenotypic and spatial heterogeneity, with distinct fibroblast states contributing to different microenvironmental conditions and therapeutic responses (55). Spatial analyses of human lung tumors have further demonstrated that CAF localization and matrix-associated programs at the tumor-stroma interface are associated with T-cell exclusion (17). Transforming growth factor- β (TGF- β) signaling can promote fibroblast activation and ECM remodeling, whereas experimental inhibition of TGF- β -associated

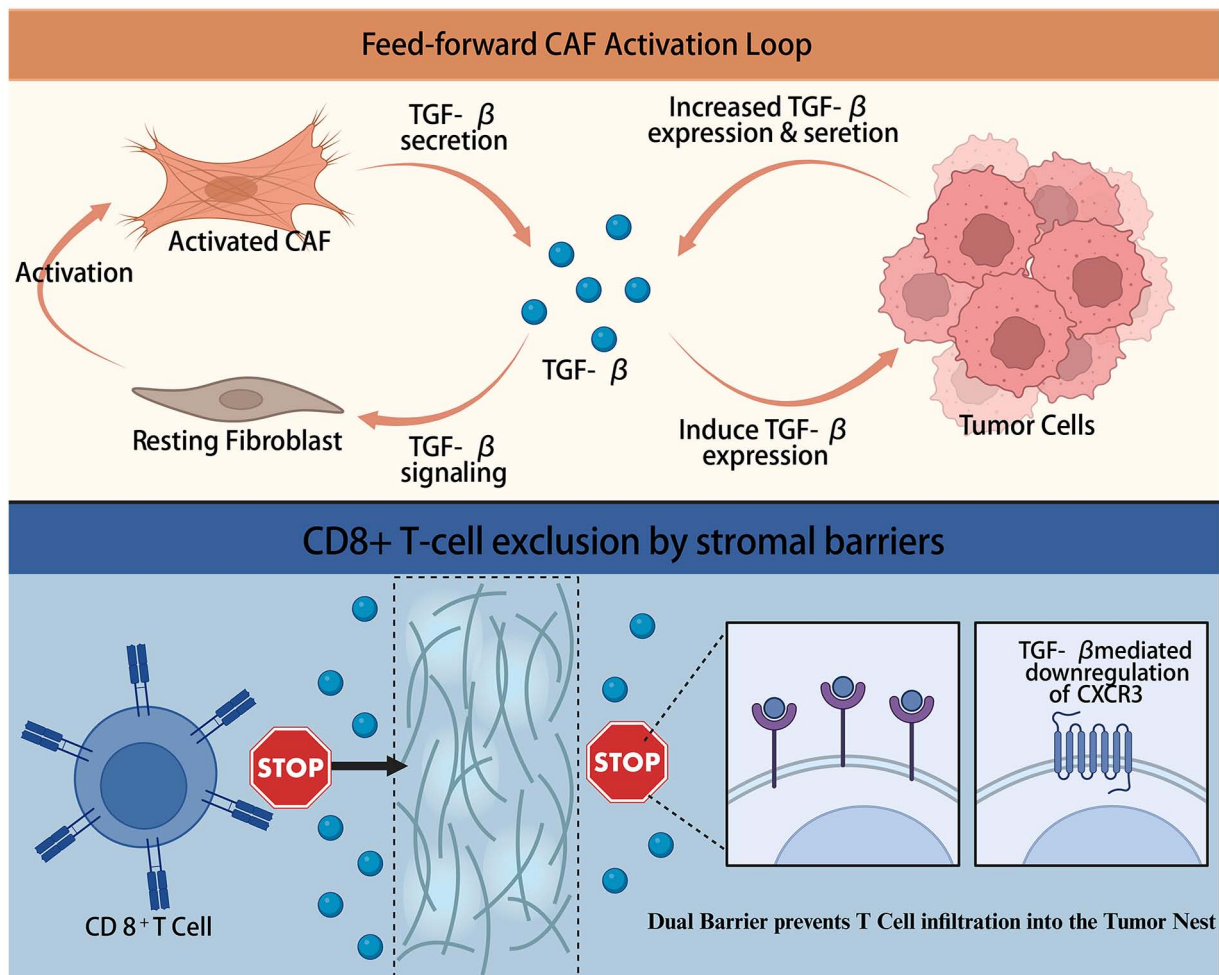


Figure 5. Feed-Forward CAF Activation and dual stromal barriers in the immune-excluded phenotype of NSCLC. Schematic model illustrating how TGF- β -associated CAF activation may contribute to the immune-excluded phenotype in NSCLC. TGF- β signaling promotes the transition of resting fibroblasts into activated CAFs, while CAF-derived TGF- β may further reinforce stromal remodeling programs within the tumor microenvironment, forming a potential feed-forward regulatory loop. This process may contribute to dual barriers restricting CD8⁺ T-cell infiltration. First, ECM deposition, crosslinking and densification generate a physical barrier that limits immune-cell penetration into tumor nests. Second, TGF- β -mediated suppression of T-cell function and downregulation of migration-associated receptors such as CXCR3 may impair chemokine-guided recruitment. Together, these mechanisms may restrict T-cell entry into tumor nests and contribute to maintenance of the immune-excluded spatial phenotype. CAF, cancer-associated fibroblast; NSCLC, non-small cell lung cancer; TGF- β , transforming growth factor beta.

stromal programs can enhance the efficacy of PD-L1-directed therapy in selected models (56). These observations are consistent with a self-reinforcing relationship in which TGF- β drives fibroblast activation, while activated fibroblasts in turn maintain TGF- β -rich, matrix-dense microenvironments.

The resulting stromal barrier may operate through two complementary mechanisms. First, collagen-rich, remodeled ECM can impose a physical constraint on CD8⁺ T-cell penetration and migration from stromal regions into tumor-cell nests (17,57). Second, TGF- β -associated signaling can directly impair T-cell proliferation and effector function and may disrupt migration-related programs, thereby diminishing T-cell responsiveness to chemotactic cues. Together, these structural and signaling barriers may confine immune cells to the tumor margin or stromal compartments and prevent effective contact with malignant cells (Fig. 5).

Abnormal vasculature and hypoxia may impose an additional layer of spatial restriction. Dysregulation of hypoxia-inducible factor-1 α and vascular endothelial growth factor-dependent pathways has been documented in

NSCLC, including treatment-resistant and epidermal growth factor receptor (EGFR)-mutant disease (58). Poor perfusion and regional hypoxia may coincide with extracellular acidification, lactate accumulation and adenosine-generating pathways involving tumor, stromal and immune-cell populations (35,37,38). Collectively, these conditions can intensify metabolic stress and may further compromise immune-cell trafficking and sustained effector function. However, direct evidence showing that endothelial-cell mitochondrial dysfunction itself is sufficient to drive immune exclusion in human NSCLC is currently unavailable.

Mitochondrial redox signaling may further interact with these stromal and vascular processes. Chronic oxidative stress can accompany hypoxia, impaired perfusion, lactate accumulation and fibroblast activation, while mtROS exert strongly context- and cell type-dependent effects (28). In experimental systems, oxidative stress can promote stress-response pathways and tissue-remodeling programs. In patient tumors, however, oxidative stress, CAF accumulation, ECM remodeling, hypoxia and vascular abnormalities frequently occur

simultaneously, making their temporal sequence and causal relationships difficult to resolve.

Accordingly, mtROS and other mitochondrial metabolic alterations should not be portrayed as universal proximal causes of stromal immune exclusion. A more evidence-based interpretation is that mitochondrial redox and metabolic states may reinforce or sustain pre-existing barriers associated with CAFs, ECM remodeling, hypoxia, or vascular dysfunction in selected tumors. At present, the strongest evidence from human lung cancer supports a spatial association between CAF/matrix programs and T-cell exclusion (17), whereas the contribution of cell type-specific mitochondrial dysfunction remains largely mechanistic and hypothesis-generating.

Future investigations should integrate spatially resolved assessments of CAF states, ECM architecture, vascular organization, hypoxia, and immune-cell localization with cell type-specific measurements of mitochondrial function and redox status. Longitudinal and treatment-paired analyses will be particularly important for determining whether mitochondrial alterations precede stromal barrier formation, arise as secondary adaptations, or predominantly contribute to maintenance of the immune-excluded state.

TCA metabolites and epigenetic regulation as hypothesis-generating links. Among the proposed mitochondrial mechanisms linking tumor metabolism to spatial immune phenotypes, the relationship among TCA cycle metabolites, epigenetic regulation and immune architecture remains particularly speculative in human NSCLC. Although the biochemical coupling between mitochondrial metabolism and chromatin regulation is well established, direct evidence linking defined metabolite alterations to specific spatial immune states in patient tumors remains limited.

α -Ketoglutarate (α -KG) functions as an essential cofactor for multiple α -KG-dependent dioxygenases, including enzymes involved in DNA and histone demethylation and the regulation of hypoxia-inducible factors (59). Reduced α -KG availability, or relative accumulation of metabolites such as succinate and fumarate, can inhibit these enzymes and thereby alter chromatin organization and hypoxia-responsive signaling. JmJc-domain-containing histone demethylases constitute one important class of mediators through which mitochondrial metabolic state may be coupled to transcriptional regulation (60–63).

In NSCLC, however, current evidence more strongly supports broad alterations in metabolic flux, hypoxic signaling, and epigenetic reprogramming than a recurrent, canonical oncometabolite-driven phenotype. Patient-derived studies have shown that glucose-derived carbon can make a substantial contribution to the TCA cycle in aggressive NSCLC (22), while multimodal and multi-omics analyses have revealed extensive epigenetic and transcriptional reprogramming across lung tumors (62,63). These observations support the biological plausibility of metabolism-chromatin coupling but do not demonstrate that any specific TCA metabolite directly determines an inflamed, immune-desert, or immune-excluded phenotype.

A more cautious framework is that altered TCA-associated metabolic states may reinforce pre-existing transcriptional and microenvironmental programs rather than initiate them.

By modulating α -KG-dependent enzymes, chromatin accessibility, and hypoxia-responsive pathways, changes in TCA metabolites could promote the persistence of angiogenic, glycolytic, inflammatory, or stromal-remodeling states. Epigenomic state transitions have also been observed during lung adenocarcinoma progression in experimental models (64), supporting the broader concept that chromatin programs can stabilize tumor-cell phenotypes over time. Whether TCA metabolite alterations function as upstream drivers, downstream consequences, or parallel adaptations of these states, however, remains unresolved.

The cellular compartment in which these effects occur is another major source of uncertainty. Metabolite-dependent epigenetic regulation may operate in tumor cells, fibroblasts, endothelial cells, myeloid cells, or T cells, and the functional consequences are unlikely to be equivalent across these populations. Bulk-tissue measurements cannot reliably distinguish whether a given metabolite or epigenetic signature originates from malignant or non-malignant compartments. Associations between TCA-related signatures and immune phenotypes should therefore not be interpreted as evidence of a tumor-cell-intrinsic causal mechanism in the absence of cell type-resolved validation.

At present, large-scale studies that simultaneously quantify TCA metabolites, α -KG-dependent enzyme activity, chromatin states, and the spatial distribution of immune cells within the same NSCLC tissue regions are lacking (62–66). Most available human evidence is cross-sectional and correlative, with few longitudinal or interventional datasets demonstrating that manipulation of a TCA metabolite-epigenetic axis is sufficient to reprogram a defined spatial immune phenotype.

TCA metabolites are therefore best regarded as potential stabilizers or modifiers of established immune and microenvironmental states rather than confirmed initiating drivers of cold-tumor formation in NSCLC. This framework provides a biologically plausible and experimentally testable link between mitochondrial metabolism and epigenetic persistence, but its relevance to spatial immune organization will require direct validation in cell type-resolved, spatially annotated human specimens.

Intercellular mitochondrial transfer as an emerging and context-dependent mechanism. Intercellular mitochondrial transfer has emerged as a form of non-cell-autonomous metabolic communication within the TME. Unlike suppression mediated by soluble metabolites, this process entails the transfer of intact mitochondria, mitochondrial components, or mtDNA-containing material between donor and recipient cells. Its biological effects are neither inherently beneficial nor detrimental but instead depend on the direction of transfer, the identities of the donor and recipient cells, the functional integrity of the transferred mitochondria, the route of transfer, and the capacity of recipient cells to maintain mitochondrial quality control.

The most direct evidence relevant to NSCLC comes from Ikeda *et al* (67), who identified shared mtDNA mutations in cancer cells and TILs from clinical specimens and subsequently demonstrated the transfer of cancer-cell-derived mitochondria to T cells in experimental systems. Their findings implicated both direct transfer through tunneling nanotubes (TNTs) and indirect transfer via small extracellular vesicles (EVs).

Disruption of direct cell contact, TNT formation, or small-EV release reduced mitochondrial transfer, suggesting that these routes may operate in parallel rather than as functionally interchangeable pathways (67). However, pharmacological inhibition of TNT- or EV-associated pathways can also perturb other cellular processes, and EVs carry a broad range of additional immunomodulatory cargo. Thus, route-inhibition experiments alone cannot ascribe all downstream immune effects specifically to mitochondrial transfer.

In the same study, the acquisition of mitochondria harboring pathogenic mtDNA variants was associated with reduced mitochondrial membrane potential ($\Delta\Psi_m$), altered ROS production, metabolic dysregulation, senescence-associated phenotypes, impaired effector activity, and defective memory formation in recipient T cells (67). Over time, transferred mutant mtDNA could shift from heteroplasmy toward homoplasmic replacement. This progression appeared to reflect not only repeated transfer but also differences in mitochondrial quality control: Endogenous T-cell mitochondria remained susceptible to stress-induced mitophagy, whereas cancer-cell-derived mitochondria were comparatively resistant to clearance. Notably, the mitophagy-inhibitory molecule implicated in this process was USP30 rather than ubiquitin-specific protease 27. USP30 was co-transferred with cancer-cell mitochondria and antagonized Parkin-dependent mitophagy, while pharmacological inhibition or RNA-interference-mediated suppression of USP30 partially reduced the persistence of transferred mitochondria and their progression toward homoplasmy (67).

These findings provide mechanistic support for a model in which tumor-to-T-cell mitochondrial transfer contributes to impaired antitumor immunity. In melanoma and NSCLC cohorts, tumor mtDNA mutations were also associated with poorer outcomes after PD-1 blockade (66). However, tumor mtDNA mutation status should not be interpreted as a specific biomarker of mitochondrial transfer, because such mutations may affect tumor biology through multiple independent mechanisms. Furthermore, the prevalence, spatial distribution and quantitative contribution of tumor-to-T-cell mitochondrial transfer across human NSCLC remain incompletely defined. Current clinical evidence therefore supports an association and a biologically plausible mechanism but does not establish mitochondrial transfer as a universal cause of T-cell dysfunction or resistance to immune checkpoint inhibition.

Mitochondrial exchange between cancer and immune cells may also occur in the reverse direction. A single-cell genomic study reported predominantly T-cell-to-cancer-cell mitochondrial transfer and inferred donor-recipient relationships using mitochondrial single-nucleotide variants, raising the possibility that cancer cells can acquire mitochondrial material from neighboring T cells to enhance their metabolic fitness (67). Because genomic inference does not directly visualize the movement of intact organelles in each transfer event, these observations should be distinguished from transfer demonstrated through imaging and functional perturbation. Nevertheless, when considered alongside the tumor-to-T-cell transfer described by Ikeda *et al* (67), these findings suggest that mitochondrial exchange within the TME can be bidirectional and strongly context dependent.

The functional competence of the transferred mitochondria is also a key determinant of biological outcome. Baldwin *et al* (68) showed that nanotube-mediated transfer of metabolically

competent mitochondria from bone-marrow stromal cells to CD8⁺ T cells increased mitochondrial respiration and spare respiratory capacity and promoted T-cell expansion, tumor infiltration, resistance to exhaustion, and antitumor activity in experimental models. By contrast, transfer of dysfunctional mitochondria failed to confer comparable benefits (68). These observations indicate that mitochondrial transfer can either compromise or enhance T-cell function depending on the cellular source and bioenergetic quality of the transferred organelles. The biological significance of mitochondrial transfer therefore cannot be inferred solely from its occurrence.

Several methodological limitations should also be considered when interpreting evidence of intercellular mitochondrial transfer. Shared mtDNA variants can facilitate donor-recipient lineage tracing but may be confounded by cellular admixture, sequencing depth, clonal expansion, or technical contamination. Fluorescent mitochondrial labels can identify transferred mitochondrial material without necessarily establishing the movement of intact, functionally competent organelles. Likewise, TNT and EV-release inhibitors lack complete pathway specificity, and distinct EV populations may transport mitochondrial proteins, mtDNA fragments, partial organelles, or other bioactive cargo. Future studies should therefore integrate mtDNA lineage tracing, live-cell imaging, ultrastructural confirmation, functional assessment of mitochondrial activity, and route-specific genetic perturbation rather than relying on any single detection strategy.

Overall, intercellular mitochondrial transfer is best regarded as an emerging, context-dependent mechanism capable of modifying T-cell mitochondrial fitness and antitumor immunity in selected tumors. Current evidence is strongest for the experimental feasibility of transfer and its functional consequences, whereas spatially resolved data defining its prevalence and causal contribution in human NSCLC remain limited. Therapeutic strategies aimed at disrupting TNT- or EV-mediated transfer, overcoming USP30-dependent resistance to mitophagy, or supplying metabolically competent mitochondria to therapeutic T cells are conceptually promising but remain preclinical. These approaches should therefore be considered separately from established ICI therapies in the translational discussion (Table SII).

5. Mitochondrial regulation across spatial immune phenotypes in NSCLC

The forementioned mitochondrial processes are unlikely to function independently. Within individual NSCLC lesions, tumor-cell metabolism, immune-cell mitochondrial fitness, innate immune sensing, stromal architecture, vascular function and intercellular mitochondrial communication may interact to influence both the spatial distribution and functional state of antitumor immune cells. The relative importance of these processes is likely to differ not only between patients but also among spatially distinct regions within the same tumor.

Within this framework, mitochondrial alterations can be considered in relation to the three commonly recognized spatial immune phenotypes: Immune-desert, immune-excluded and inflamed tumors. These phenotypes are not necessarily discrete or mutually exclusive, and transitions between them may occur during tumor progression or in response to therapy.

The following sections therefore examine how mitochondrial and microenvironmental mechanisms may contribute to each spatial phenotype without assuming a fixed one-to-one correspondence between any single biological process and a particular immune state.

Immune-desert phenotype. The immune-desert phenotype is characterized by sparse immune-cell infiltration within both tumor nests and the surrounding stromal compartment. Biologically, this pattern is more consistent with inadequate initiation of the cancer-immunity cycle than with a simple inability of already activated T cells to enter the tumor. Deficient antigen presentation, attenuated type I IFN and chemokine signaling, and limited DC abundance or functional maturation may collectively impair T-cell priming and subsequent recruitment (14,16,41).

The functional state of intratumoral DCs is particularly relevant to the efficacy of immune checkpoint blockade. PD-L1 blockade can enhance DC-mediated co-stimulation by releasing CD80 from PD-L1-dependent sequestration, thereby promoting CD28 signaling and T-cell priming. In patients with NSCLC or renal cell carcinoma (RCC) treated with atezolizumab, a DC-related gene signature was associated with improved overall survival (41). These observations suggest that PD-(L)1 blockade is more likely to be effective when a functional antigen-presenting and costimulatory circuit is already present, at least to some extent. Conversely, checkpoint inhibition alone may have limited efficacy when tumors contain few functional DCs and lack an established pool of tumor-reactive T cells.

Mitochondria-related innate sensing may intersect with this priming defect through the cGAS-STING pathway. Cytosolic DNA sensing in tumor or myeloid cells can induce type I IFNs and chemokines, thereby promoting DC activation and immune-cell recruitment. Preclinical NSCLC studies have shown that pharmacological or treatment-induced activation of STING-associated signaling can increase the expression of chemokines such as CXCL10 and CCL5 and enhance immune-cell infiltration (51,52). Conversely, reduced STING expression has been associated with adverse clinical features in NSCLC (55). However, as aforementioned, cytosolic DNA can arise from mitochondrial damage, nuclear DNA damage, chromosomal instability, or replication stress. Attenuated STING signaling should therefore not be attributed automatically to insufficient mtDNA release.

Tumor genotype provides one example of how this pathway can be disrupted. In KRAS-driven lung cancer models, loss of STK11/LKB1 induced epigenetic silencing of STING and impaired responses to cytosolic double-stranded DNA. Restoration of STING reactivated downstream TBK1-IRF3 signaling in these models (69). Subsequent clinical and translational studies have linked STK11/LKB1 deficiency to impaired STING, type I IFN and CD8⁺ T-cell programs, as well as reduced benefit from immunotherapy (54,69,70). Nevertheless, this represents a genotype-specific mechanism of immune attenuation rather than a universal explanation for the immune-desert phenotype in NSCLC.

The contribution of mtROS is less clearly defined. Moderate redox signaling can participate in inflammatory and innate immune responses, whereas excessive antioxidant buffering or

altered mitochondrial signaling could, in principle, attenuate these pathways. However, direct spatial evidence demonstrating that reduced mtROS in tumor or myeloid cells causes an immune-desert phenotype in human NSCLC is lacking. The proposed relationship between diminished mitochondrial danger signaling and defective immune initiation should therefore remain hypothesis-generating rather than being framed as a defined mtROS threshold.

Taken together, the immune-desert phenotype likely results from multiple convergent deficiencies, including limited tumor-antigen recognition, inadequate DC abundance or maturation, attenuated IFN and chemokine signaling, tumor-intrinsic suppression of innate immune sensing, and insufficient T-cell recruitment. Impairment of the tumor-myeloid cGAS-STING axis may contribute to selected tumors, but current evidence does not establish mtDNA release or mitochondrial dysfunction as the dominant initiating cause of this spatial phenotype.

Immune-excluded phenotype. In immune-excluded tumors, lymphocytes are present at the invasive margin or within stromal compartments but exhibit limited penetration into malignant cell nests. This spatial pattern indicates that immune-cell recruitment has occurred to some extent, whereas subsequent trafficking through the tumor stroma and productive interactions with cancer cells remain restricted (14,17). Compared with immune-desert tumors, the dominant constraint is therefore less likely to reflect a complete failure of immune priming and more likely to arise from the organization and functional properties of the stromal and vascular interface.

Among the mechanisms associated with immune exclusion, the strongest spatial evidence in human lung tumors relates to CAF organization and ECM programs. Integrated single-cell and multiplex imaging analyses have identified distinct CAF populations positioned around or within tumor structures and associated with dense, aligned ECM deposition and peripheral localization of T cells (17). These findings indicate that the effects of CAFs cannot be inferred from their overall abundance alone; their molecular states, spatial distribution, and relationship to matrix architecture are also important determinants of immune organization. The primary human lung tumor study supporting this association is included as reference (17).

TGF- β signaling may reinforce this spatial barrier by promoting fibroblast activation and matrix remodeling while simultaneously suppressing antitumor immune responses. Activated CAFs can sustain a self-reinforcing stromal state through the production of TGF- β , ECM components and additional regulatory mediators (55,56). The resulting barrier has both structural and functional dimensions: Dense or highly aligned matrix can restrict T-cell migration, whereas TGF- β -rich conditions can suppress T-cell proliferation and effector activity and reduce responsiveness to local chemotactic signals. Together, these processes provide a plausible basis for the accumulation of CD8⁺ T cells outside, rather than within, tumor-cell nests.

Abnormal vasculature and regional hypoxia may further strengthen this excluded state. Disorganized or dysfunctional tumor vessels can impair perfusion, promote hypoxia and acidosis, and disrupt the conditions required for leukocyte adhesion, extravasation and tissue entry. HIF-1 α - and

VEGF-associated vascular programs have been described in NSCLC, including treatment-resistant and EGFR-mutant contexts (58). A previous analysis of two human NSCLC cohorts further linked vascular leakage to spatially distinct stromal niches, immune evasion and poor responses to immunotherapy (71). These findings support the clinical relevance of vascular abnormalities in immune exclusion, although they do not establish endothelial mitochondrial dysfunction as the direct causal mechanism.

Metabolic conditions within poorly perfused stromal regions may impose an additional functional barrier. Hypoxia, lactate accumulation, extracellular acidification and CD73-mediated adenosine signaling can impair T-cell migration and the maintenance of effector function (35,37,38). These metabolic pressures may therefore reduce the likelihood that T cells retained near the tumor boundary remain sufficiently motile and functionally competent to traverse the stromal compartment. Nevertheless, metabolic dysfunction alone does not account for the characteristic spatial geometry of immune exclusion, which is more directly supported by CAF organization, matrix architecture and vascular abnormalities.

Mitochondrial redox signaling should likewise be interpreted within this broader microenvironmental context. Chronic oxidative stress may coexist with hypoxia, lactate accumulation, fibroblast activation, and vascular dysfunction and may reinforce tissue-remodeling or immunosuppressive programs in selected tumors (28,58). However, these processes are highly interdependent in patient tissues. Current evidence is insufficient to determine whether altered mtROS precedes CAF activation and matrix remodeling, arises secondarily from hypoxia and impaired perfusion, or primarily contributes to maintaining an already established stromal barrier.

The immune-excluded phenotype is therefore best understood as the product of interacting stromal, vascular, metabolic and immune constraints. Evidence from human lung tumors most directly supports the spatial association between specific CAF/matrix programs and T-cell marginalization (17), whereas mitochondrial and redox processes are more appropriately regarded as potential modifiers or amplifiers of this architecture. Defining their causal contribution will require spatially matched assessments of CAF identity, ECM organization, vascular function, tissue oxygenation, mitochondrial activity and T-cell localization before and after therapeutic intervention.

Inflamed phenotype. The inflamed phenotype is characterized by the accumulation of immune cells, particularly CD8⁺ T cells, within or in close proximity to tumor-cell nests. This spatial pattern is frequently accompanied by active antigen-presentation machinery, IFN-associated signaling, and chemokine programs that promote immune-cell recruitment and retention (14,16,19,40,72). Compared with immune-desert and immune-excluded tumors, inflamed tumors are generally more likely to harbor a pre-existing antitumor immune response that can be further enhanced by immune checkpoint inhibition. Nevertheless, the presence of intratumoral T cells does not necessarily indicate that these cells remain metabolically competent or capable of sustaining effective tumor control.

Maintenance of an inflamed state requires coordinated and persistent interactions among tumor-antigen recognition, DC

activation, T-cell co-stimulation, local chemokine production and effector-cell persistence. In human NSCLC, increased tumor-cell calreticulin (CRT) expression has been associated with greater infiltration by mature DCs and effector-memory T cells, as well as with favorable clinical outcomes (73). These findings support the importance of immunogenic tumor-cell signals and effective antigen presentation in establishing a T-cell-rich microenvironment. However, CRT expression should be regarded as an associated feature rather than a sufficient marker defining the inflamed phenotype.

DCs may also determine whether a pre-existing immune response can be effectively amplified by PD-L1 blockade. PD-L1 expressed on DCs can sequester CD80 through cis interactions, thereby reducing the availability of CD80 for CD28-mediated T-cell co-stimulation. Blocking PD-L1 can relieve this constraint and enhance DC-dependent T-cell priming (41). Thus, the therapeutic responsiveness of an inflamed tumor depends not only on the abundance of infiltrating T cells but also on the preservation of effective antigen presentation and costimulatory signaling. Mechanistically, the relevant interaction is CD80-CD28 rather than CD75-CD25.

Mitochondrial fitness constitutes an additional functional dimension of the inflamed phenotype. Activated CD8⁺ T cells require coordinated OXPHOS, mitochondrial quality control, redox homeostasis, and metabolic flexibility to sustain cytokine production, cytotoxicity, and persistence under tumor-associated stress (32). However, these properties cannot be assumed to be uniformly preserved among T cells within an inflamed tumor. Spatial infiltration and mitochondrial competence are related but distinct biological features, and substantial metabolic heterogeneity may exist even among neighboring T-cell populations.

A lung cancer study identified CD38^{hi} and CD38^{int} CD8⁺ T-cell populations with distinct mitochondrial and functional characteristics (5). CD38^{hi} CD8⁺ T cells exhibited higher expression of exhaustion-associated markers together with dysregulated mitochondrial bioenergetics. Their abundance also differed between peripheral and central tumor regions, and their association with response to anti-PD-1 therapy varied according to spatial location. These observations indicate that, even in T-cell-infiltrated lung tumors, mitochondrial status cannot be inferred solely from T-cell density or expression of a single surface marker. Instead, spatial context should be integrated with direct functional assessment of mitochondria when interpreting the biological significance of individual T-cell subsets.

A recent clinical study involving patients with NSCLC and clear cell RCC further illustrates the complexity of mitochondrial functional readouts. Tumor-associated CD8⁺ T-cell clones with high $\Delta\Psi_m$ exhibited increased OXPHOS-related signatures but simultaneously expressed stress- and exhaustion-associated programs. Moreover, the corresponding TMRE-high signature was negatively associated with clinical benefit from ICIs (74). Although the study was not confined to NSCLC, these findings caution against interpreting elevated $\Delta\Psi_m$ or increased oxidative activity as a direct surrogate for superior T-cell fitness.

T cells within inflamed tumors also remain subject to substrate limitation, hypoxia, lactate accumulation,

extracellular acidification and adenosine-mediated suppression (33,35-39). These metabolic constraints can reduce spare respiratory capacity, disrupt mitochondrial redox homeostasis, and compromise sustained effector function without necessarily preventing T-cell infiltration. Consequently, some inflamed tumors may retain abundant intratumoral lymphocytes while exhibiting impaired cytokine production, defective memory formation, or progressive T-cell exhaustion. This distinction provides a biological basis for the clinical observation that an inflamed spatial phenotype does not invariably translate into durable benefit from immune checkpoint inhibition.

Intercellular mitochondrial transfer may provide an additional mechanism through which an initially inflamed microenvironment becomes functionally compromised. Tumor-derived mitochondria harboring pathogenic mtDNA variants can be transferred to TILs, leading to metabolic dysfunction, senescence-associated phenotypes, and impaired effector and memory functions (66,67). In melanoma and NSCLC cohorts, tumor mtDNA mutations were also associated with poorer outcomes following immune checkpoint inhibition (66). However, tumor mtDNA mutation status is not a specific surrogate for mitochondrial transfer, and the prevalence, spatial distribution and quantitative contribution of this process in human NSCLC remain incompletely characterized. Mitochondrial transfer should therefore be considered an emerging mechanism that may contribute to dysfunction in selected T-cell-infiltrated tumors rather than a universal feature of the inflamed phenotype.

Overall, the inflamed phenotype is best conceptualized as a spatial state in which immune-cell recruitment and tumor entry have occurred, but the functional quality of the infiltrating immune compartment remains heterogeneous. Preserved DC activity, antigen presentation, costimulatory signaling, and T-cell mitochondrial competence may sustain effective antitumor immunity, whereas metabolic stress, progressive exhaustion, and the acquisition of dysfunctional mitochondria may undermine an otherwise established immune response. Mitochondrial processes are therefore more likely to regulate the persistence and functional efficacy of the inflamed state than to determine T-cell infiltration *per se*.

6. Translational opportunities and limitations

Phenotype-matched therapeutic strategies. The therapeutic relevance of spatial immune phenotypes lies in their potential to identify the stage of the antitumor immune response that is predominantly constrained. Within this framework, immune-desert tumors are primarily characterized by inadequate immune initiation and recruitment, immune-excluded tumors by restricted access of immune cells to malignant cell nests, and inflamed tumors by variable maintenance and functional competence of an already established immune infiltrate. Therapeutic priorities may therefore differ across these spatial states. However, spatial immune phenotypes have not yet been validated as stand-alone biomarkers for treatment selection in NSCLC. They should instead be considered alongside tumor stage, histological and molecular features, PD-L1 expression, genomic alterations, prior therapies, and the patient's overall clinical condition rather than applied as a rigid treatment algorithm (Fig. 6).

Immune-desert tumors: Restoring immune priming and recruitment. In immune-desert tumors, the immediate therapeutic objective is to initiate an effective antitumor immune response rather than merely release inhibitory constraints on a response that is largely absent. PD-(L)1 blockade can amplify pre-existing T-cell activity, but its efficacy may remain limited when DC abundance or maturation, antigen presentation, costimulatory signaling, IFN responses, and chemokine production are inadequate (41). Therapeutic strategies for this phenotype should therefore focus on restoring antigen availability, innate immune activation, DC function, and subsequent T-cell recruitment before or in conjunction with checkpoint inhibition.

Radiotherapy and selected cytotoxic agents may facilitate immune initiation by inducing tumor-cell damage, increasing antigen release, and generating cytosolic DNA and other damage-associated signals. These effects can activate cGAS-STING-related pathways and enhance type I IFN and chemokine production, although the magnitude of the response depends on radiation dose and fractionation, tumor genotype, DNA-degradation pathways, and the functional competence of cGAS-STING signaling in both tumor and myeloid cells (48-50). Clinical experience with chemoradiotherapy followed by checkpoint inhibition demonstrates that immune activation can be successfully integrated with checkpoint blockade in specific NSCLC treatment settings. However, these data do not establish radiotherapy as a universal strategy for converting all immune-desert tumors into immune-responsive states.

More direct restoration of innate immune sensing represents another potential strategy. In preclinical NSCLC models, RocA-induced mitochondrial damage and activation of cGAS-STING signaling increased CXCL10 and CCL5 expression and promoted immune-cell recruitment (52). Treatment-induced STING activation *in trans* has also contributed to immune-dependent antitumor effects in lung cancer models (51). These findings, however, remain predominantly preclinical, and RocA should not be regarded as a clinically validated immune-sensitizing agent for NSCLC.

Early clinical studies of intratumoral STING agonists have demonstrated the feasibility of pharmacological STING activation. Ulevostinag, administered either alone or in combination with pembrolizumab in patients with advanced solid tumors, induced pharmacodynamic changes consistent with STING pathway engagement and showed a manageable safety profile. However, the expansion cohorts were not designed to establish efficacy specifically in immune-desert NSCLC, and the clinical benefit of STING agonism remains uncertain (75). STING agonists and related approaches should therefore be regarded as investigational strategies aimed at initiating or strengthening immune priming, with subsequent checkpoint blockade potentially amplifying the resulting antitumor response rather than independently mediating immune conversion.

DC-directed interventions may provide an additional means of restoring immune priming. Potential approaches include expanding or recruiting cross-presenting DC populations, DC vaccination, inducing immunogenic tumor-cell death, and enhancing antigen presentation (41,76). These strategies, however, differ substantially in their degree of clinical development. Moreover, the presence of a DC-related

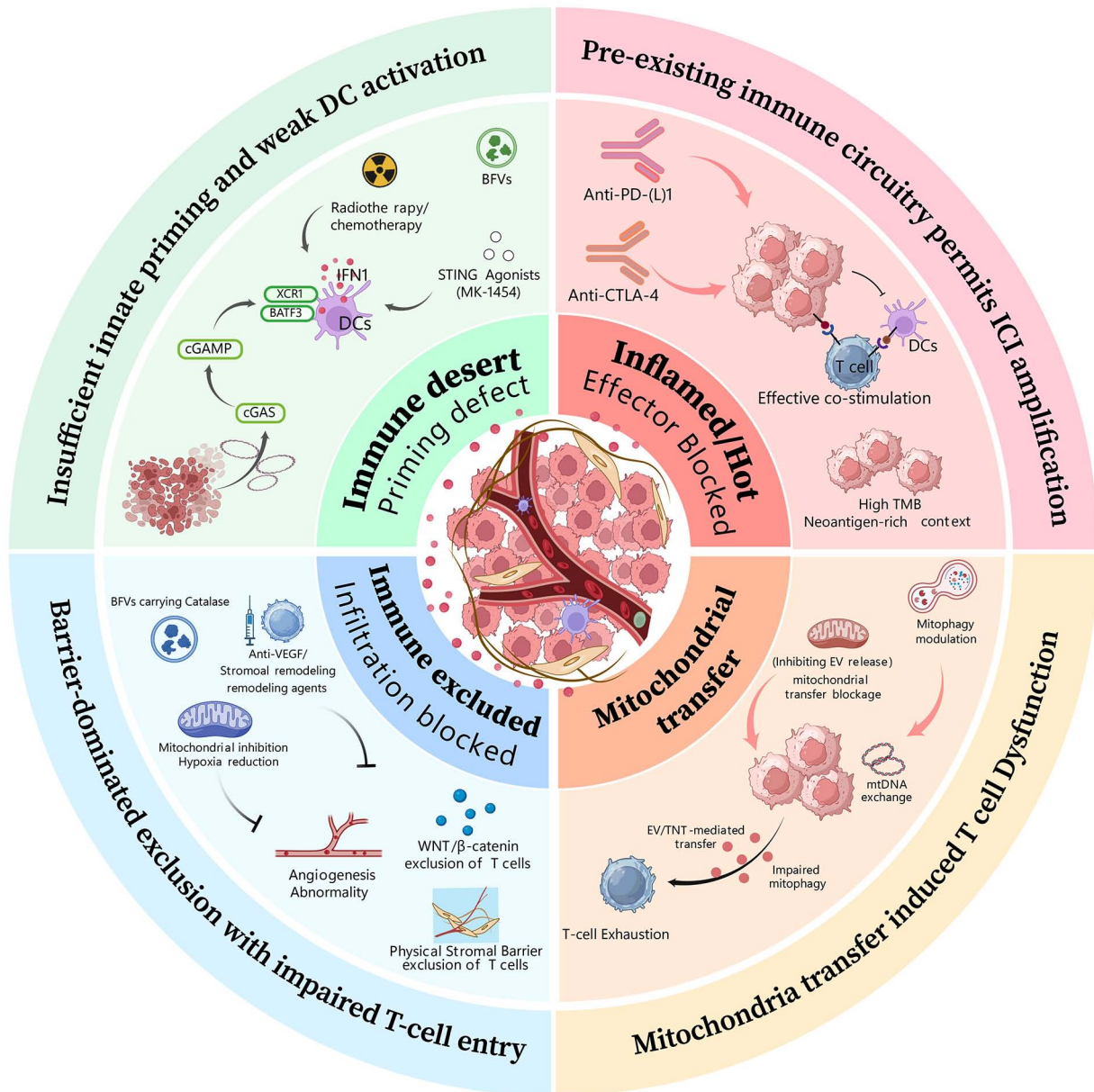


Figure 6. Conceptual framework linking spatial immune phenotypes to potential mitochondria-related intervention directions in NSCLC. This schematic summarizes potential therapeutic strategies associated with distinct spatial immune phenotypes in NSCLC. In immune-desert tumors, impaired innate immune priming and insufficient DC activation may contribute to limited T-cell recruitment. Therefore, approaches targeting innate immune sensing pathways, including cGAS-STING activation and treatment-induced immune stimulation, represent potential strategies to enhance immune initiation. In immune-excluded tumors, stromal barriers, abnormal angiogenesis, hypoxia-associated metabolic alterations, and impaired immune-cell infiltration may restrict T-cell access to tumor nests. Strategies targeting stromal remodeling, vascular abnormalities, or metabolic constraints have therefore been proposed as potential approaches to improve immune-cell penetration, although most remain at preclinical or early translational stages. In inflamed tumors with pre-existing immune activity, immune checkpoint blockade may further enhance antitumor responses by reinforcing existing immune effector functions. However, the clinical benefit of ICIs depends on multiple tumor-intrinsic and microenvironmental factors rather than spatial phenotype alone. The mitochondrial transfer-related module represents an emerging and exploratory mechanism that may contribute to immune dysfunction through mitochondrial exchange between tumor and immune cells, including extracellular vesicle- or tunneling nanotube-mediated processes. These mechanisms remain hypothesis-generating, and their therapeutic implications require further experimental and clinical validation. Overall, this figure provides a conceptual overview of potential mitochondria-related intervention directions across spatial immune contexts rather than a validated phenotype-guided therapeutic algorithm. The maturity of these approaches varies substantially, ranging from clinically established immunotherapy to preclinical and emerging mitochondrial-targeted strategies. NSCLC, non-small cell lung cancer; DC, dendritic cells; ICI, immune checkpoint inhibitor; DCs, dendritic cells; mtDNA, mitochondrial DNA; PD-(L)1, programmed cell death protein 1; CTLA-4, cytotoxic t-lymphocyte associated protein 4; TMB, tumor mutational burden; EV, endothelial vesicle.

signature does not itself establish that a patient will benefit from a specific DC-targeted intervention. Biomarker development should therefore distinguish DC abundance from functional maturation, spatial proximity to tumor cells and T cells, antigen-presenting capacity, and effective CD80-CD28 costimulatory signaling.

Immune-excluded tumors: Relieving stromal and vascular barriers. In immune-excluded tumors, immune priming and recruitment have occurred to some extent, but T cells remain concentrated at the invasive margin or within stromal compartments and fail to establish sufficient contact with malignant cells. The therapeutic priority is therefore to improve

intratumoral access rather than simply enhance peripheral T-cell activation. Strategies targeting vascular abnormalities, hypoxia, CAF-associated programs, TGF- β signaling, and ECM organization may increase the likelihood that recruited immune cells can penetrate tumor nests and maintain their functional capacity after entry (17,55-58).

Vascular normalization is particularly relevant because abnormal angiogenesis and impaired perfusion can simultaneously restrict immune-cell trafficking, exacerbate hypoxia, and intensify metabolic and immunosuppressive stress. The clinical efficacy of atezolizumab combined with bevacizumab and chemotherapy in metastatic non-squamous NSCLC provides a precedent for integrating VEGF inhibition with immune checkpoint blockade and cytotoxic therapy (77). However, patients in this trial were not selected on the basis of a spatially defined immune-excluded phenotype. These findings therefore demonstrate the clinical feasibility of combining antiangiogenic therapy with immunotherapy but do not validate vascular normalization as a phenotype-specific treatment for immune exclusion.

CAF- and matrix-directed interventions may complement vascular strategies. TGF- β -associated fibroblast activation and ECM remodeling can impose both structural and signaling barriers to T-cell entry (17,55-56). Potential approaches include modulation of TGF- β -related pathways, specific CAF states, collagen organization, matrix crosslinking, lysyl oxidase-associated processes, and focal adhesion signaling (56,57). Nevertheless, CAF populations are highly heterogeneous and can exert context-dependent tumor-promoting, immunoregulatory, or tissue-supportive functions. Consequently, indiscriminate fibroblast depletion or non-specific disruption of the ECM may produce unintended biological effects.

Clinical experience further indicates that biologically plausible stromal targeting does not necessarily translate into improved clinical outcomes. In a randomized phase III trial involving treatment-naïve patients with PD-L1-high advanced NSCLC, the bifunctional TGF- β trap/PD-L1 inhibitor bintrafusp alfa failed to demonstrate superior efficacy over pembrolizumab (78). This finding does not exclude a relevant role for TGF- β in selected immune-excluded tumors; rather, it indicates that simultaneous systemic inhibition of TGF- β and PD-L1 is insufficient in the absence of appropriate biological and spatial patient selection. Future trials should therefore evaluate stromal interventions in tumors with directly documented TGF- β -, CAF-, or ECM-associated immune exclusion rather than assuming that all NSCLC tumors share a common stromal barrier.

Modulation of tumor respiration and hypoxia may provide another means of improving the immune-excluded microenvironment. In a window-of-opportunity study involving patients with NSCLC, atovaquone reduced hypoxic tumor volume as measured by hypoxia positron-emission tomography and altered transcriptional programs associated with hypoxia and metabolism (79). These findings provide evidence of a biological effect on tumor oxygenation in patients but do not demonstrate improved responsiveness to ICIs. Atovaquone should therefore be regarded as a clinical proof of principle for hypoxia modulation rather than as an established immune-sensitizing therapy.

Overall, effective treatment of immune-excluded tumors may require coordinated alleviation of vascular, stromal and

metabolic barriers. The optimal intervention is unlikely to be identical across tumors dominated by abnormal angiogenesis, TGF- β -rich fibroblast programs, dense collagen architecture, or severe hypoxia. Spatial profiling may therefore be particularly useful for identifying the predominant barrier requiring intervention and determining whether its modification is followed by increased intratumoral T-cell infiltration.

Inflamed tumors: Amplifying immunity while preserving T-cell function. In inflamed tumors, immune cells have already penetrated tumor nests, making checkpoint inhibition a more direct therapeutic strategy than in immune-desert or immune-excluded states. Nevertheless, an inflamed spatial phenotype does not guarantee durable tumor control. Infiltrating T cells may remain constrained by persistent antigen stimulation, inhibitory receptor signaling, metabolic stress, adenosine accumulation, mitochondrial dysfunction, or inadequate antigen presentation and costimulatory signaling (32,36-41).

PD-(L)1 blockade remains the principal strategy for amplifying a pre-existing antitumor immune response, whereas cytotoxic t-lymphocyte associated protein 4 (CTLA-4)-containing regimens may enhance T-cell priming and clonal expansion in selected clinical or molecular contexts. For example, CTLA-4 blockade has shown the potential to overcome KEAP1/STK11-associated resistance to PD-(L)1 inhibition (80). However, KEAP1 or STK11 status should not be considered equivalent to an inflamed, immune-excluded, or immune-desert spatial phenotype. Molecular genotype, spatial architecture, and immune function should instead be evaluated as complementary dimensions of tumor biology (54,69,70,80).

Metabolic interventions may be considered when immune infiltration is present, but T-cell function remains compromised. The CD73-adenosine-A2A receptor axis can impair T-cell metabolic fitness, migration and effector activity (36-39), providing a rationale for combining adenosine-pathway inhibition with checkpoint blockade. However, such strategies remain investigational and will require biomarkers demonstrating that adenosine signaling constitutes an active suppressive mechanism within the tumor being treated.

Similarly, preserving or restoring T-cell mitochondrial fitness represents a potential complementary therapeutic objective rather than an established treatment category. Strategies that enhance mitochondrial quality control, substrate utilization, redox homeostasis, or the *ex vivo* metabolic fitness of therapeutic T cells may help sustain effector function (32,81). Conversely, tumor-to-T-cell transfer of dysfunctional mitochondria may contribute to impaired antitumor immunity and treatment resistance in selected tumors (66). Interventions targeting mitochondrial transfer and mitochondrial quality control are discussed separately below because they remain predominantly preclinical.

Taken together, phenotype-matched therapy should target the predominant biological bottleneck: Restoring immune priming in immune-desert tumors, facilitating tissue entry in immune-excluded tumors, and sustaining effective antitumor function in inflamed tumors. Because these spatial states may coexist within the same lesion and evolve during treatment, therapeutic selection should ideally be guided by spatially resolved baseline assessment and, where feasible, longitudinal reassessment.

Emerging mitochondria-directed interventions and modular combination strategies. Given the multifaceted roles of mitochondria in tumor metabolism, immune activation and intercellular communication, mitochondria-related processes have emerged as potential therapeutic targets for reshaping spatial immune phenotypes in NSCLC. However, the strength of evidence supporting mitochondria-directed interventions varies substantially across strategies, ranging from early clinical observations of metabolic modulation to predominantly preclinical evidence for mitochondrial regulation of antitumor immunity. Rather than serving as stand-alone therapeutic targets, mitochondrial interventions are more likely to function as complementary modules integrated with established immunotherapeutic strategies. Their therapeutic value may depend on the specific mitochondrial process targeted, the cellular compartment affected, and the predominant spatial immune barrier within the tumor (Table SIII).

Modulation of tumor mitochondrial metabolism and hypoxia. Tumor mitochondrial metabolism contributes not only to cancer-cell survival but also to the formation of metabolically restrictive immune niches through oxygen consumption, nutrient utilization and the accumulation of immunosuppressive metabolites (18,20). Modulating mitochondrial respiration may therefore provide a means of alleviating metabolic constraints within the TME.

Atovaquone provides an example of clinically investigated modulation of mitochondrial metabolism in NSCLC. By inhibiting mitochondrial complex III, atovaquone reduces cellular oxygen consumption and intratumoral hypoxia. A window-of-opportunity study in patients with NSCLC demonstrated that short-term atovaquone treatment reduced hypoxic tumor volume and altered hypoxia-associated transcriptional programs (79). These findings provide human evidence that modulation of mitochondrial respiration can alter the tumor metabolic state; however, they do not establish whether these changes are sufficient to enhance immune-cell infiltration or improve the efficacy of ICIs.

Mitochondrial metabolic intervention should therefore currently be regarded as a strategy for modifying tumor metabolic constraints rather than as an established approach for sensitizing tumors to immunotherapy.

Exploiting mitochondrial danger signaling pathways. Mitochondrial damage can generate immunogenic signals through the release of mtDNA, activation of cGAS-STING signaling, and induction of type I IFN-associated immune responses (50-52). On this basis, pharmacological induction of mitochondrial stress has been proposed as a strategy for enhancing innate immune activation and promoting immune-cell recruitment.

In lung cancer models, RocA-induced mitochondrial dysfunction activated cGAS-STING signaling, increased CXCL10 and CCL5 expression, and promoted immune-cell infiltration (52). Similarly, STING activation in trans has been shown to enhance immune-dependent tumor control through activation of innate immune responses (51).

These findings provide mechanistic and preclinical support for targeting mitochondria-associated danger-sensing pathways. However, STING responses are strongly influenced by tumor genotype, pathway competence, immune-cell composition and treatment context. Mitochondria-associated

STING activation should therefore currently be considered an investigational immunomodulatory strategy rather than a clinically validated approach for converting immunologically cold tumors into inflamed tumors.

Targeting pathogenic intercellular mitochondrial transfer. Beyond intracellular mitochondrial regulation, emerging evidence indicates that mitochondria can function as transferable biological units mediating communication between tumor and immune cells.

Tumor-derived dysfunctional mitochondria harboring pathogenic mtDNA alterations can be transferred to T cells through mechanisms such as tunneling nanotubes (TNTs) and EVs, resulting in mitochondrial dysfunction, impaired metabolic adaptation, and reduced immune-cell function (66,67).

From a therapeutic perspective, disrupting pathogenic mitochondrial transfer or restoring mitochondrial quality control represents a potential intervention strategy. Candidate approaches include inhibition of TNT formation, modulation of EV-mediated mitochondrial trafficking, and manipulation of mitophagy-associated pathways.

Among these mechanisms, mitochondrial quality-control regulators such as USP30 may provide potential therapeutic targets by influencing the clearance of dysfunctional mitochondria. However, these strategies remain largely confined to experimental models, and selectively disrupting pathological mitochondrial transfer without interfering with physiological intercellular mitochondrial communication remains a major challenge.

Enhancing T-cell mitochondrial fitness. Because mitochondrial function is closely linked to T-cell persistence, memory formation and sustained effector activity, restoring T-cell mitochondrial fitness represents another potential therapeutic strategy (32).

Experimental studies have investigated approaches such as metabolic optimization of adoptive cell therapies and the delivery of functional mitochondria to immune cells. Transfer of metabolically competent mitochondria has been shown to enhance T-cell respiratory capacity, persistence and functional activity in experimental systems (81).

Although these findings provide a biological rationale for improving immune-cell metabolic fitness, clinical translation remains constrained by challenges related to delivery efficiency, manufacturing feasibility, long-term safety, and identification of patients most likely to benefit.

Modular combination therapy guided by spatial immune barriers. Given the complex and context-dependent roles of mitochondria in both tumor and immune cells, mitochondria-directed interventions are unlikely to replace established immunotherapies. Instead, they may be incorporated as modular components of phenotype-guided combination strategies.

Conceptually, distinct mitochondrial interventions may address different spatial immune barriers. In immune-desert tumors, strategies that enhance mitochondrial danger signaling may promote innate immune activation and immune-cell recruitment. In immune-excluded tumors, metabolic and hypoxia-modulating approaches may help alleviate environmental constraints that limit immune-cell infiltration. In inflamed tumors, interventions designed to preserve T-cell mitochondrial fitness may enhance immune-cell persistence and sustain antitumor activity.

Several emerging platforms, including multifunctional bioengineered vesicles, have been developed to simultaneously modulate immune activation, hypoxia and tumor-cell susceptibility (7). However, these approaches remain experimental, and their clinical translation will require spatially resolved biomarkers capable of identifying the predominant mitochondrial abnormality and guiding selection of the most appropriate intervention modules.

Clinical translation, evidence gaps and future experimental priorities. Although mitochondria-related mechanisms provide a potentially useful framework for understanding spatial immune heterogeneity and resistance to immunotherapy in NSCLC, several challenges must be addressed before these concepts can be translated into clinically actionable strategies. Importantly, current evidence linking mitochondrial alterations to spatial immune phenotypes is derived largely from mechanistic studies, experimental models and correlative analyses. Mitochondrial remodeling should therefore currently be regarded as a potential regulator or modifier of spatial immune organization rather than an established determinant of immune phenotype.

Successful clinical translation will require not only deeper mechanistic understanding but also standardized spatial biomarkers, cell type-specific functional measurements, and prospective validation in human NSCLC cohorts.

Limited causal evidence in human NSCLC. A major challenge is the paucity of direct causal evidence demonstrating that mitochondrial alterations actively drive the establishment of specific spatial immune phenotypes in human NSCLC. Although mitochondrial dysfunction has been associated with immune exclusion, T-cell dysfunction and resistance to ICIs, these associations do not establish whether mitochondrial remodeling is an initiating event, an adaptive response, or a downstream consequence of tumor evolution, immune pressure, or treatment exposure (3,5,12).

For example, mtROS accumulation, mtDNA release and metabolic remodeling have been proposed to modulate immune-cell recruitment and function. However, the biological effects of these mitochondrial alterations are highly dependent on cellular context, spatial localization, magnitude and duration. Accordingly, models of mtROS-mediated immune regulation or mitochondrial stress-induced immune remodeling should currently be regarded as mechanistic hypotheses rather than definitive explanations for the development of spatial immune phenotypes in human NSCLC.

Lack of spatially resolved mitochondrial biomarkers. Current clinical decision-making for NSCLC immunotherapy relies primarily on PD-L1 expression, TMB, actionable genomic alterations and clinical characteristics. Although these biomarkers provide valuable predictive information, they do not fully capture spatial immune organization, metabolic interactions, or cell type-specific mitochondrial states within the TME.

An important unmet need is therefore the development of biomarkers that integrate mitochondrial function with spatial immune architecture. Emerging technologies, including spatial transcriptomics, multiplex imaging, spatial metabolomics and single-cell multi-omics, provide opportunities to

characterize tumor ecosystems at increasingly high spatial and cellular resolution. However, the incorporation of functional mitochondrial measurements into routine clinical workflows remains technically challenging.

Future biomarker strategies should prioritize cell type-resolved mitochondrial features rather than bulk mitochondrial signatures, which may mask biologically opposing effects across tumor, immune and stromal cell populations.

Importantly, spatial immune phenotypes should currently be viewed as complementary to established biomarkers such as PD-L1 and TMB rather than as replacements, because prospective clinical evidence demonstrating their superiority is not yet available (19,82).

Cell-type specificity and therapeutic trade-offs. Another major challenge stems from the shared dependence of tumor, immune and stromal cells on mitochondrial processes. The biological consequences of mitochondrial alterations cannot be reduced to a universal state of ‘mitochondrial dysfunction’, because distinct cellular compartments may exhibit markedly different, or even opposing, responses.

In tumor cells, enhanced mitochondrial metabolism may promote proliferation, invasion and adaptation to metabolic stress. By contrast, mitochondrial oxidative metabolism is essential for CD8⁺ T-cell persistence, memory formation and sustained effector function (32). Broad suppression of mitochondrial activity could therefore inhibit tumor metabolism while simultaneously compromising antitumor immunity.

Similarly, mitochondrial remodeling in DCs, macrophages, CAFs and endothelial cells may affect immune recruitment, stromal organization and vascular function through distinct cell type-specific mechanisms.

Future therapeutic strategies should therefore prioritize selective modulation of disease-associated mitochondrial pathways in defined cellular compartments rather than generalized inhibition of mitochondrial function.

Technical and methodological challenges. The clinical interpretation of mitochondrial alterations is further complicated by substantial technical and methodological heterogeneity. Mitochondrial parameters are highly sensitive to tissue acquisition procedures, ischemia time, preservation conditions, tissue dissociation and analytical platforms. These preanalytical and analytical variables can substantially affect measurements of mitochondrial mass, membrane potential, oxygen consumption and ROS production.

Moreover, individual mitochondrial parameters reflect distinct biological processes and should not be interpreted interchangeably. For example, increased mitochondrial abundance does not necessarily indicate enhanced oxidative capacity, whereas elevated $\Delta\Psi_m$ may reflect either metabolic adaptation or cellular stress, depending on the biological context.

Standardized analytical frameworks that integrate spatial localization with functional mitochondrial assessment are therefore required before mitochondria-associated signatures can be reliably incorporated into clinical stratification strategies.

Future experimental priorities to establish causality. Several experimental priorities should be addressed to move beyond association and establish causal relationships between mitochondrial processes and spatial immune phenotypes.

First, longitudinal studies incorporating pretreatment, on-treatment and post-treatment samples are needed to determine whether mitochondrial remodeling precedes changes in spatial immune organization or instead emerges as a consequence of therapeutic selection.

Second, cell type-specific perturbation approaches are essential for distinguishing the contributions of mitochondrial alterations in tumor, immune and stromal cells. Selective manipulation of mitochondrial pathways in tumor cells and CD8⁺ T cells, for example, could help determine whether observed associations are driven primarily by tumor-cell adaptation, immune-cell dysfunction, or interactions between the two compartments.

Third, spatially resolved functional platforms that integrate spatial transcriptomics, multiplex imaging, metabolomics and measurements of mitochondrial activity will be necessary to directly link mitochondrial states to immune architecture.

Finally, prospective clinical trials incorporating both mitochondrial biomarkers and spatial immune profiling will be required to determine whether mitochondria-associated features can improve patient stratification and inform the selection of combination therapies.

7. Conclusion

ICIs have provided substantial clinical benefit to a subset of patients with advanced NSCLC, but their overall efficacy remains constrained by the spatial organization of the TIME. In general, inflamed tumors are more frequently associated with treatment sensitivity, whereas immune-desert and immune-excluded phenotypes are more commonly associated with primary or acquired resistance. Compared with individual molecular biomarkers, spatial immune phenotypes may provide a more integrated framework for understanding the structural and cellular constraints that shape ICI responsiveness.

At the mitochondrial level, energy production, substrate availability, redox homeostasis, innate immune signaling, and metabolic regulation of gene expression are closely interconnected. Mitochondrial processes may therefore influence T-cell recruitment, infiltration, and functional maintenance through multiple context-dependent mechanisms. Organizing these mechanisms into distinct modules, including substrate competition, mtROS and redox homeostasis, mtDNA release and cGAS-STING signaling, TCA cycle metabolites and epigenetic regulation, mitochondrial transfer, and mitochondrial quality control, may facilitate the development of testable models linking specific mitochondrial programs to features of immune-desert, immune-excluded, and inflamed tumor.

However, the available evidence in human NSCLC remains predominantly correlative or is derived from experimental model systems. In some biological contexts, mitochondrial abnormalities may act as upstream regulators; in others, they may amplify pre-existing immune barriers or simply accompany broader tumor-state changes. Mitochondrial alterations should therefore be interpreted according to the strength and type of supporting evidence, ranging from observational findings in human tumors to functional perturbation and interventional validation. Neither a single biopsy nor an individual biomarker is currently sufficient to establish temporal directionality or causality. A more informative approach will require the integration of spatial multi-omics with cell type-resolved

measurements of mitochondrial function in window-of-opportunity studies and serial-biopsy designs, thereby enabling temporal relationships between mitochondrial remodeling and spatial immune phenotypes to be experimentally defined.

From a therapeutic perspective, cold-to-hot tumor conversion should not be treated as a single-pathway problem. Instead, therapeutic selection should be guided by identification of the dominant biological barrier and subsequent matching of that barrier to an appropriate intervention. Strategies for immune-desert tumors may need to prioritize restoration of immune priming and recruitment, whereas immune-excluded tumors may require disruption of vascular and stromal barriers before effective ICI-based combinations can be achieved. Inflamed tumors, in turn, may benefit from approaches that preserve T-cell metabolic fitness and sustained immune effector function. At the same time, mitochondria-targeted interventions must be evaluated with careful consideration of their context-dependent and potentially bidirectional effects, because systemic modulation of mitochondrial respiration may affect both tumor cells and antitumor immune populations.

Overall, mitochondrial biology does not provide a singular explanation for the development of cold tumors or therapeutic resistance in NSCLC. Rather, it offers a potential framework for integrating spatial immune organization, metabolic adaptation and treatment response. Future studies combining spatial profiling, functional perturbation and longitudinal sampling will be essential to determine whether mitochondrial alterations function as causal drivers, secondary adaptations, or context-dependent modifiers of immune phenotypes. Such efforts may help address the fundamental questions of why cold tumors arise, why they resist therapy, and how they can be converted into more treatment-responsive states, ultimately providing a stronger mechanistic foundation for precision immunotherapy in NSCLC.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

HYL, KS and MSL conceptualized the study and wrote the manuscript. HYL, KS, MSL, KS AND XL reviewed the manuscript. MSL and XL supervised the study. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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