

Table SI. Operational definitions and assessment of spatial immune phenotypes in non-small cell lung cancer.

Spatial immune phenotype	Operational definition	Main spatial compartment assessed	Dominant immune-cell pattern	Common assessment methods and readouts	Threshold status	Clinical interpretation and major caveats
Inflamed phenotype	Immune cells, particularly CD8 ⁺ T cells, are present within tumor cell nests or in close spatial proximity to malignant epithelial cells (8,13-14).	Intratumoral compartment and tumor cell nests	Relatively abundant intratumoral CD8 ⁺ T cells, frequently accompanied by dendritic cells, antigen-presentation activity, interferon-related programs, and immune-cell interactions within the tumor parenchyma (6,13,16).	IHC, mIF, imaging mass cytometry, digital pathology, whole-slide image analysis, and spatial transcriptomic or proteomic profiling; relevant readouts include intratumoral immune-cell density, immune-tumor cell distance, and immune-cell neighborhood composition (6,8,13,14).	No universally accepted quantitative threshold; cutoffs for intratumoral immune-cell density and tumor proximity are study- and platform-specific.	Generally associated with pre-existing antitumor immune activity and a greater likelihood of ICI benefit. However, immune infiltration does not necessarily indicate functional competence, because T cells may remain exhausted, metabolically impaired, or insufficiently supported by antigen presentation and co-stimulation
Immune-desert phenotype	Immune cells are globally sparse in both tumor cell nests and surrounding stromal compartments (8,14,15).	Tumor nests and stromal compartments	Low densities of intratumoral and stromal effector immune cells, particularly CD8 ⁺ T cells; dendritic cells and other antigen-presenting populations may also be limited or functionally impaired (13,16).	IHC, mIF, digital pathology, spatial transcriptomics, and immune-cell deconvolution; relevant readouts include total and compartment-specific immune-cell density, immune-related transcriptional signatures, and dendritic-cell or antigen-presentation programs (6,8,13,14).	No universal threshold; definitions of “low infiltration” vary according to the immune-cell marker, tissue area, cohort distribution, and analytical platform.	Frequently associated with weak pre-existing immune priming and reduced benefit from ICI monotherapy. However, a desert-like pattern may reflect low antigenicity, impaired recruitment, treatment effects, regional heterogeneity, or sampling bias and should not be attributed to a single mechanism on the basis of cell density alone

Immune-excluded phenotype	Immune cells are present but remain predominantly confined to stromal compartments, invasive margins, or peritumoral regions, with limited penetration into tumor cell nests (8,14,17).	Tumor-stroma interface, invasive margin, peritumoral stroma, and tumor cell nests	CD8 ⁺ T cells and other immune populations accumulate outside tumor nests; this pattern is frequently accompanied by cancer-associated fibroblast enrichment, extracellular matrix remodeling, abnormal vasculature, or hypoxia-associated barriers (17).	mIF, imaging mass cytometry, spatial transcriptomics, digital pathology, and whole-slide image analysis; relevant readouts include stromal-to-intratumoral immune-cell ratios, distance to the tumor-stroma boundary, immune-cell penetration depth, and CAF/ECM-associated spatial programs (6,8,13,14).	No universal threshold; definitions of tumor boundaries, stromal regions, and penetration distance vary among studies and platforms	Frequently associated with restricted immune-tumor contact and reduced ICI responsiveness. CAF, TGF-beta, ECM, vascular, and chemotactic abnormalities are commonly associated mechanisms but are not universal defining features. Excluded, inflamed, and desert-like regions may coexist within the same tumor
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CAF, cancer-associated fibroblast; ECM, extracellular matrix; ICI, immune checkpoint inhibitor; IHC, immunohistochemistry; mIF, multiplex immunofluorescence; NSCLC, non-small cell lung cancer. These phenotypes are presented as operational and context-dependent spatial frameworks rather than mutually exclusive or universally thresholded clinical diagnostic categories.

Table SII. Evidence matrix linking mitochondrial processes to spatial immune phenotypes in non-small cell lung cancer.

Mitochondrial process	Primary cell/compartment	Proposed spatial phenotype link	Main biological output	Evidence source / model	Evidence type	Spatial and human NSCLC evidence	Evidence-weighted interpretation	Major limitations
Tumor-cell OXPHOS and substrate demand	Malignant epithelial cells; perfused and hypoxic tumor regions	May contribute to metabolic constraints associated with immune-poor or immune-excluded regions	Regional oxygen and carbon-substrate demand; ETC activity; biosynthetic and stress-adaptive capacity	Human lung-cancer spatial bioenergetic and metabolomic studies (21,22); NSCLC and cancer-cell mechanistic studies (23-25)	Human spatial/metabolic association plus mechanistic support	Regional mitochondrial and metabolic heterogeneity is demonstrated in human lung cancer/NSCLC (21,22,28), but local substrate depletion and neighboring T-cell dysfunction were not co-measured	Relatively established tumor metabolic heterogeneity; the link to a defined immune phenotype remains hypothesis-generating	No direct <i>in situ</i> demonstration of tumor-T-cell substrate competition; perfusion, hypoxia and oncogenic state are confounders
Lactate, acidosis and adenosine signaling	Tumor cells, CAFs and immune cells; extracellular tumor and stromal compartments	May impair T-cell function in inflamed or immune-excluded regions and reinforce immune-poor states	Reduced glycolysis, cytokine production, chemotaxis, mitochondrial biogenesis and OXPHOS maintenance in T cells	NSCLC/lung-cancer microenvironment studies (35,37,38); T-cell functional studies (31,32)	Mixed human NSCLC association and mechanistic evidence	NSCLC-specific evidence supports lactate and CD73/adenosine-rich contexts (29,30,33); direct spatial coupling to T-cell mitochondrial function is limited	Preclinical mechanistic support with NSCLC contextual evidence	Effects depend on concentration, exposure duration and T-cell state; hypoxia and nutrient limitation usually coexist; some functional data are non-NSCLC

T-cell mitochondrial fitness and metabolic resilience	Tumor-infiltrating CD8+ T cells	May support functional maintenance in inflamed tumors; loss of fitness may produce infiltrated but ineffective immune states	OXPHOS, spare respiratory capacity, membrane potential, redox homeostasis, cytokine production and persistence	Lung-cancer CD38-high mitochondrial-adapted CD8+ T-cell study [5]; T-cell mitochondrial review/mechanistic work (20,26,32); NSCLC TIL landscape (34)	Emerging human association with mechanistic support	Human lung/NSCLC studies support heterogeneous T-cell states (5,34), but spatially resolved mitochondrial functional measurements remain sparse	Emerging human association; not a validated spatial classifier	T-cell abundance does not equal mitochondrial competence; marker-defined subsets may not generalize; directionality relative to infiltration is unclear
mtROS and redox signaling	Tumor cells and immune/myeloid cells; potentially stromal and vascular cells	Moderate signaling may support inflammatory programs, whereas chronic oxidative stress may amplify immune suppression or exclusion	Redox-sensitive signaling, macromolecular damage, stress adaptation, inflammatory transcription and tissue-remodeling programs	Respiratory-chain and ROS biology (25-28); spatial/clinical NSCLC context (30,31); antioxidant intervention evidence (29)	Mechanistic and clinical-context evidence; phenotype mapping is mainly inferential	KEAP1/NRF2-related redox states are clinically relevant in NSCLC (46,47), but in situ mtROS is usually inferred rather than directly measured with spatial immune architecture	Hypothesis-generating, cell type- and threshold-dependent model	Biphasic effects; indirect ROS readouts; strong cell-type and genotype dependence; oxidative stress is collinear with hypoxia, perfusion and stromal remodeling
mtDNA-cGAS-STING signaling across tumor and myeloid compartments	Tumor cells, dendritic cells and macrophages	Pathway competence may support priming and recruitment; attenuation may contribute to immune-desert states	cGAMP generation, STING/TBK1 signaling, type I interferon, CXCL10/CCL5, DC/myeloid activation and T-cell priming	mtDNA release mechanisms (48-52); NSCLC models (53,54); human NSCLC STING studies (55); DC/PD-L1 response (41,71); cancer-cell cGAS and pathway regulation (79,80)	Preclinical mechanistic evidence plus human clinical association	NSCLC evidence supports STING-related clinical and immune associations [54,55,86], but no human spatial study integrates DNA source, cell-specific cGAS/STING activity, DC priming and ICI outcome	High-priority candidate mechanism; not a universal proximal driver	Cytosolic DNA may derive from mtDNA or nuclear DNA damage/CIN; tumor and myeloid roles differ; cGAMP transfer routes and pathway competence vary

CAF-TGF-beta-ECM-associated stromal restriction	Cancer-associated fibroblasts and extracellular matrix at the tumor-stroma interface	Directly associated with immune-excluded spatial organization	Matrix deposition, remodeling and densification; physical restriction; suppression of T-cell migration and effector activity	TGF-beta/PD-L1 combination model (16); lung-cancer fibroblast states (17); human lung-tumor spatial CAF/matrix study (18); collagen-immune review (74)	Human spatial association plus preclinical mechanistic evidence	Human lung-tumor spatial evidence directly links CAF positioning and matrix programs with T-cell exclusion (18)	Relatively established spatial association; mitochondrial involvement remains unproven	CAF heterogeneity; multiple stromal and tumor programs coexist; the direction and magnitude of mitochondrial/red ox contribution are uncertain
Vascular dysfunction and hypoxia	Endothelial/vascular compartment and poorly perfused tumor regions	May support immune exclusion or immune-poor regions by limiting trafficking and increasing metabolic stress	Poor perfusion, hypoxia/HIF-VEGF programs, altered nutrient/oxygen delivery, lactate and adenosine accumulation	Human spatial/metabolic NSCLC studies (21,22,28,29); NSCLC HIF-1alpha/VEGF-dependent phenotype (73)	Human association with mechanistic context	Regional perfusion and metabolic heterogeneity are documented (21,22,28); direct endothelial mitochondrial measurements linked to immune localization are lacking	Relatively established microenvironmental context; endothelial mitochondrial mechanism is hypothesis-generating	Strong collinearity with CAF/ECM, tumor metabolism and treatment resistance; limited cell type-resolved vascular mitochondrial data
TCA metabolite-epigenetic coupling	Potentially tumor, stromal, endothelial and immune cells	May stabilize or modify pre-existing inflamed, immune-desert or immune-excluded states	Regulation of alpha-KG-dependent dioxygenases, DNA/histone demethylation, HIF stabilization and persistent transcriptional programs	Human NSCLC metabolic and multi-omics studies (28,59,66); biochemical/epigenetic evidence (61-64); lung-adenocarcinoma epigenomic transitions (65)	Biochemical mechanism and multi-omics association; limited direct spatial evidence	NSCLC shows metabolic and epigenetic reprogramming (28,59,66), but studies jointly measuring metabolites, chromatin and immune-cell location are lacking	Hypothesis-generating extrapolation; more plausible as a stabilizer than an initiating driver	Bulk tissue obscures cell of origin; canonical IDH/SDH oncometabolite states are uncommon in NSCLC; no direct longitudinal/interventional spatial validation

Tumor-to-T-cell mitochondrial transfer	Tumor cells as donors; TILs as recipients; TNT and small-EV routes	May generate infiltrated but dysfunctional T-cell states and contribute to ICI resistance	Transfer of abnormal mitochondria or mutant mtDNA; heteroplasmy/homoplasmy, impaired OXPHOS, altered ROS, senescence-like dysfunction and defective memory	Human NSCLC specimens plus experimental transfer and perturbation study (67); conceptual synthesis (68)	Emerging human association with direct mechanistic support	Shared tumor/TIL mtDNA variants and experimental transfer were reported (67), but prevalence and spatial contribution across human NSCLC are unknown	Emerging, context-dependent mechanism rather than a general driver	mtDNA mutation is not a transfer-specific biomarker; TNT/EV perturbations are not fully specific; route, cargo and quality-control effects are difficult to separate
Transfer of metabolically competent mitochondria to T cells	Stromal donor cells and CD8 ⁺ T-cell recipients in experimental systems	May enhance T-cell metabolic resilience and antitumor function	Increased respiration and spare respiratory capacity; reduced exhaustion; improved expansion, infiltration and antitumor activity	Nanotube-mediated healthy mitochondrial transfer study (69); perspective (70)	Preclinical mechanistic evidence	No established human NSCLC spatial or clinical evidence	Preclinical proof of principle; biological effect depends on donor and mitochondrial quality	Uncertain delivery, persistence, scalability and safety; potential off-target transfer; no validated clinical biomarker or NSCLC trial

Original reference numbering from the manuscript is retained. Evidence-weighted categories describe the level and type of support and do not imply formal grading or causality. CAF, cancer-associated fibroblast; CIN, chromosomal instability; DC, dendritic cell; ECM, extracellular matrix; ETC, electron transport chain; EV, extracellular vesicle; HIF, hypoxia-inducible factor; ICI, immune checkpoint inhibitor; mtDNA, mitochondrial DNA; mtROS, mitochondrial reactive oxygen species; NSCLC, non-small cell lung cancer; OXPHOS, oxidative phosphorylation; TIL, tumor-infiltrating lymphocyte; TNT, tunneling nanotube; TGF-beta, transforming growth factor-beta.

Table SIII. Mitochondria-directed therapeutic strategies for remodeling spatial immune phenotypes in NSCLC.

Intervention strategy	Target spatial phenotype	Mechanistic rationale	Evidence level	NSCLC-specific evidence	Potential combination strategy	Biomarker considerations	Major limitations/safety concerns
STING agonists	Immune-desert tumors	Activate cGAS-STING signaling, promote type I interferon production and chemokine-mediated immune recruitment	Preclinical	Immune-stimulatory effects reported in NSCLC models; spatial validation remains limited	Combination with ICIs, radiotherapy or chemotherapy	STING activity; IFN-I signatures; CXCL10/CCL5 expression	Inflammatory toxicity; heterogeneous STING activity
Radiotherapy-induced mitochondrial/DNA danger signaling	Immune-desert or poorly inflamed tumors	Induce mitochondrial stress and cytosolic DNA sensing to enhance innate immune activation	Preclinical/clinical evidence	Radiotherapy is established in NSCLC, but mitochondrial mechanisms require validation	Combination with PD-1/PD-L1 blockade	DNA damage response; immune infiltration; inflammatory signatures	Dose and timing uncertainty
CAF/TGF- β pathway inhibition	Immune-excluded tumors	Reduce stromal remodeling, ECM deposition and T-cell exclusion	Preclinical/early clinical	TGF- β -driven stromal barriers are implicated, but phenotype-guided application is unproven	Combination with ICIs	TGF- β activity; FAP; α -SMA; ECM signatures	Systemic toxicity; CAF heterogeneity
Collagen/LOX/ECM remodeling inhibition	Immune-excluded tumors	Reduce physical stromal barriers and improve T-cell penetration	Preclinical	ECM remodeling contributes to exclusion, but clinical validation is limited	Combination with ICIs	Collagen organization; stromal signatures	Effects on normal tissue remodeling
Hypoxia modulation and mitochondrial respiratory targeting	Hypoxia-associated immune exclusion	Alter tumor oxygen consumption and hypoxia-associated immune suppression	Preclinical	Atovaquone-related strategies affect hypoxia parameters; immune sensitization remains unproven	Combination with ICIs or radiotherapy	HIF-1 α ; hypoxia signatures; metabolic profiles	Limited clinical evidence
Mitochondrial metabolic modulation	Metabolically dysfunctional immune niches	Modify respiration, ROS balance and metabolic competition	Preclinical	Spatial immune applications remain exploratory	Combination with ICIs or metabolic therapies	Mitochondrial activity; ROS signatures	May affect immune-cell metabolism

Mitochondrial transfer inhibition or quality-control modulation	Mitochondrial-transfer-associated dysfunction	Target TNT-, extracellular-vesicle- or mitophagy-related mitochondrial exchange	Emerging/preclinical	Direct clinical relevance in NSCLC remains unclear	Combination with ICIs or metabolic therapies	mtDNA heteroplasmy; transfer signatures	Highly experimental; limited safety data
Enhancement of T-cell mitochondrial fitness	Immune-desert/excluded tumors	Improve T-cell persistence and effector function	Preclinical	Supported by cellular therapy studies; spatial application requires validation	Combination with ICIs or cellular therapies	T-cell metabolic state; mitochondrial mass; OXPHOS	Limited clinical translation