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Review Article

Advances in deciphering intratumoral versus peritumoral heterogeneity of infiltrating lymphocytes in pancreatic cancer: a spatial perspective

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal malignancies, characterized by a profoundly immunosuppressive and spatially heterogeneous tumor microenvironment. Recent research has focused on the distinct topographic distribution of tumor-infiltrating lymphocytes (TILs) across intratumoral and peritumoral compartments. This review synthesizes the latest advances, delineating the distribution, functional states, and ontogeny of region-specific TIL subsets, and dissects how spatial heterogeneity fuels disease progression and resistance to immunotherapy. We further explore the reciprocal crosstalk between immunosuppressive stroma and lymphocyte heterogeneity, highlight prevailing technical and conceptual challenges, and outline emerging technologies poised to shape the next phase of discovery. This work provides a comprehensive roadmap to accelerate translation in PDAC immuno-oncology.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal solid malignancies; 2023 data indicate a five-year survival rate of only 12 % in the United States and approximately 10 % in the Chinese population, with both incidence and mortality continuing to rise worldwide [1]. Immune checkpoint inhibitors—such as anti-PD-1/PD-L1 antibodies—have produced durable responses in multiple solid tumors, yet they confer minimal benefit in PDAC. A principal obstacle is the profoundly immunosuppressive tumor immune microenvironment (TIME) characteristic of this disease [2,3].

The spatial distribution of tumor-infiltrating lymphocytes has emerged as a defining biological feature of the tumor microenvironment. In PDAC, lymphocytes are compartmentalized into distinct intratumoral and peritumoral niches, and this spatial heterogeneity correlates tightly with tumor behavior and patient survival [4,5]. Recent studies integrating single-cell RNA sequencing, spatial transcriptomics, and multiplex immunohistochemistry have uncovered functional zonation of T and B cells within tertiary lymphoid structures (TLS). Notably, artificial-intelligence-guided spatial mapping now enables precise quantification of TIL density and its prognostic impact in PDAC, laying the groundwork for next-generation immunoscore systems [6].

Clinical investigations further link therapeutic resistance to these spatial immune signatures. PDAC typically presents as an immunologically "cold" tumor, hallmarked by a paucity of immune infiltration and aberrant checkpoint expression [7,8]. This immunosuppressive milieu arises through multifactorial mechanisms, including extracellular-matrix-mediated immune sequestration, hypoxia-driven metabolic reprogramming, and an intricate immunoregulatory network forged by cancer-associated fibroblasts [9–12]. Importantly, the anatomical positioning of CD103+CD8+ T cells within PDAC directly parallels their functional state, implying that location-dependent checkpoint expression patterns may underlie treatment failure [13]. Deciphering these spatially resolved features therefore offers a strategic framework to overcome resistance to immunotherapy [14,15].

Why spatial immunology matters in PDAC: The spatial distribution of tumor-infiltrating lymphocytes (TILs) is a defining biological feature that correlates tightly with tumor behavior and patient survival. Understanding intratumoral versus peritumoral heterogeneity is essential for developing effective immunotherapy strategies, as the anatomical positioning of immune cells directly parallels their functional state and therapeutic responsiveness.

What previous reviews missed: Most previous reviews focused on the

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overall immunosuppressive nature of PDAC without adequately addressing the spatial compartmentalization of immune cells and its functional implications. The integration of emerging spatial technologies with clinical translation has not been comprehensively reviewed.

Specific aim of this review: Our study aims to synthesize the latest advances in understanding spatial heterogeneity of TILs in PDAC, delineate the distribution, functional state, and ontogeny of region-specific TIL subsets, and provide a roadmap for translating spatial immunology research into clinical practice.

The evolving therapeutic landscape for pancreatic cancer has witnessed significant advances in recent years. Immunonutrition and prehabilitation have emerged as important adjuncts to pancreatic cancer surgery, improving patient outcomes through enhanced immune function and reduced postoperative complications [16]. Despite these advances, immunotherapy continues to face challenges in PDAC. Comprehensive analyses have revealed why immunotherapy keeps failing in pancreatic cancer, with a focus on the tumor immune microenvironment and predictive biomarkers [17]. The present and future of personalized medicine in pancreatic cancer offers new hope, with tailored therapeutic approaches based on molecular profiling and immune signatures [18]. Recent studies have highlighted the prognostic significance of the cachexia index in cancer patients, emphasizing the importance of addressing malnutrition in cancer care [19]. Major clinical trials, including CheckMate 032, the PRINCE trial, and emerging neoadjuvant immunotherapy approaches, have provided valuable insights into the challenges and opportunities of immunotherapy in PDAC [20,21].

Methods for literature search

This is a narrative review following narrative review guidelines. We conducted a comprehensive literature search across multiple databases including PubMed, Web of Science, and Scopus. Search terms included: "pancreatic cancer", "pancreatic ductal adenocarcinoma", "tumor-infiltrating lymphocytes", "spatial heterogeneity", "tumor immune microenvironment", "immunotherapy", "spatial transcriptomics", "multiplex immunohistochemistry", and "tertiary lymphoid structures". Inclusion criteria were: studies published in English, focusing on spatial heterogeneity of tumor-infiltrating lymphocytes in pancreatic cancer, using spatial transcriptomics, multiplex immunohistochemistry, or other spatial analysis methods. We primarily focused on literature from 2020–2025 with the inclusion of seminal earlier works. Exclusion criteria included case reports, non-peer-reviewed articles, and studies not focusing on PDAC. The literature selection and synthesis process involved independent review by multiple authors, with disagreements resolved through discussion (Figs. 1-5).

Core-subset profiles of infiltrating lymphocytes in pancreatic cancer

Heterogeneity of CD8⁺ cytotoxic T cells

PDAC contains CD8+ T-cell subsets that can recognize tumor neoantigens via their T-cell receptors (TCRs). Single-cell transcriptomics reveal pronounced clonal heterogeneity among tumor-infiltrating CD8+ T cells, with TCR diversity mirroring functional state [22,23]. A fraction of these cells display individualized neoantigen reactivity and measurable anti-tumor activity [24,25]. Within the PDAC niche, however, they are typically functionally quenched, exhibiting impaired effector capacity and up-regulated exhaustion markers [26]. scRNA-seq further resolves multiple CD8+ T-cell substrates—effector-memory, exhausted, and regulatory-like—co-existing within the same lesion [27].

Polarisation of CD4+ helper and regulatory T cells

CD4+ T cells in PDAC show conspicuous functional polarization. Single-cell profiling detects admixed Th1, Th2, Th17, and Treg subsets within the tumor mass [28]. Th1 cells secrete IFN-γ and exert anti-tumor effects, whereas Th2 cells can foster progression. Th17 representation is markedly expanded, promoting inflammation and angiogenesis through IL-17. The polarization pattern is spatially encoded: intratumoral areas are dominated by suppressive subsets, whereas peritumoral zones harbor larger effector fractions.

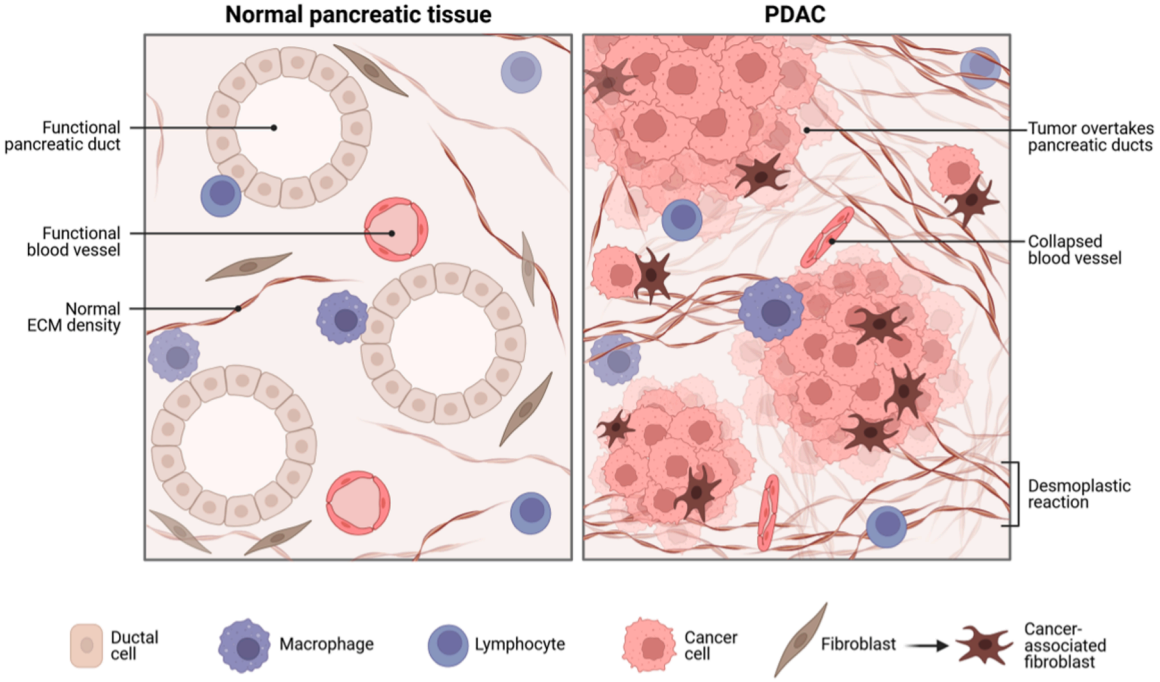

Fig. 1. Overview of the TME in pancreatic cancer.

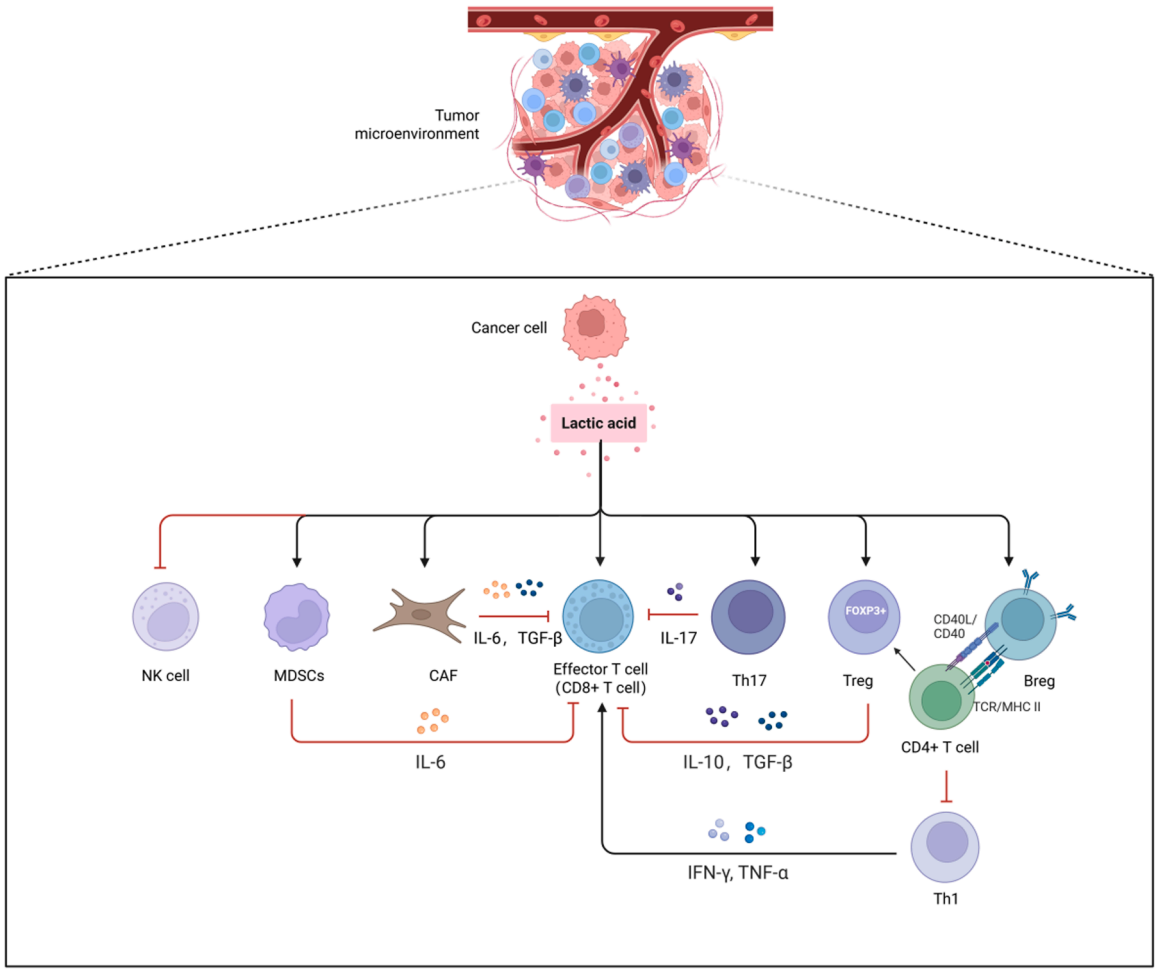

Fig. 2. The relationship between cancer cells and various immune cells.

Features of bystander lymphocytes

A sizeable pool of "bystander" lymphocytes resides at the PDAC margin. Although physically separated from malignant cells, they display a distinct activation signature and divergent TCR repertoire relative to intratumoral counterparts [29]. The population contains quiescent neoantigen-specific T-cell precursors competent to differentiate into effector cells. Spatial metrics show activation waning with distance from the tumor core [30]. FOXP3+ Tregs are dense within tumors and metastatic lymph nodes, whereas CD8+ T cells are sparse; proximity to neoplastic cells is inversely related to CD8+ abundance and prognostic value [31]. At the invasive front, CD8+ T cells accumulate but remain functionally muted; the central tumor is rendered an immune desert by CAF-derived IL-6 and TGF-β, hypoxia, and acidosis, which together heighten T-cell apoptosis and enable immune escape [32].

Immunosuppressive signature of FOXP3+ regulatory T cells

FOXP3+ Tregs are cardinal architects of PDAC immunosuppression. Mass-cytometry shows high CTLA-4 and PD-1 expression; these cells dampen immunity through contact-dependent inhibition, secretion of IL-10 and TGF-β, and competitive IL-2 consumption. scRNA-seq uncovers a Treg-specific transcriptional programme centered on sustained immune-modulatory gene activity. Tregs are markedly enriched in the tumor core, a distribution that aligns with and probably underpins their suppressive potency.

Attributes of additional immune subsets

Beyond T cells, PDAC hosts B cells, NK cells, and myeloid populations that weave an intricate interaction network. B cells can contribute anti-tumor immunity via antibody production and antigen presentation, yet frequently differentiate into IL-10-secreting Bregs that restrain immune responses [33]. NK-cell cytotoxicity and cytokine output are blunted by microenvironmental cues. Tumor-associated macrophages reinforce suppression through IL-6 and other soluble mediators. Single-cell co-mapping reveals striking co-localization among these subsets, forming functional units that collectively sculpt the immunosuppressive landscape of PDAC.

Spatial heterogeneity

Phenotypic and functional profile of intratumoral TILs

Single-cell transcriptomics reveal marked phenotypic and functional diversification among intratumoral T cells in PDAC. Within the tumor core, CD8+ T cells display an exhausted signature, with high expression of PD-1, TIM-3, and related inhibitory receptors [34]. Although residual cytotoxic potential is detectable, proliferative capacity and effector output are severely curtailed. FOXP3+ regulatory T cells, enriched in the same region, reinforce immunosuppression through IL-10 and TGF-β secretion [35]. B cells frequently organize into tertiary lymphoid structures (TLS), which may modulate local anti-tumor immunity [36].

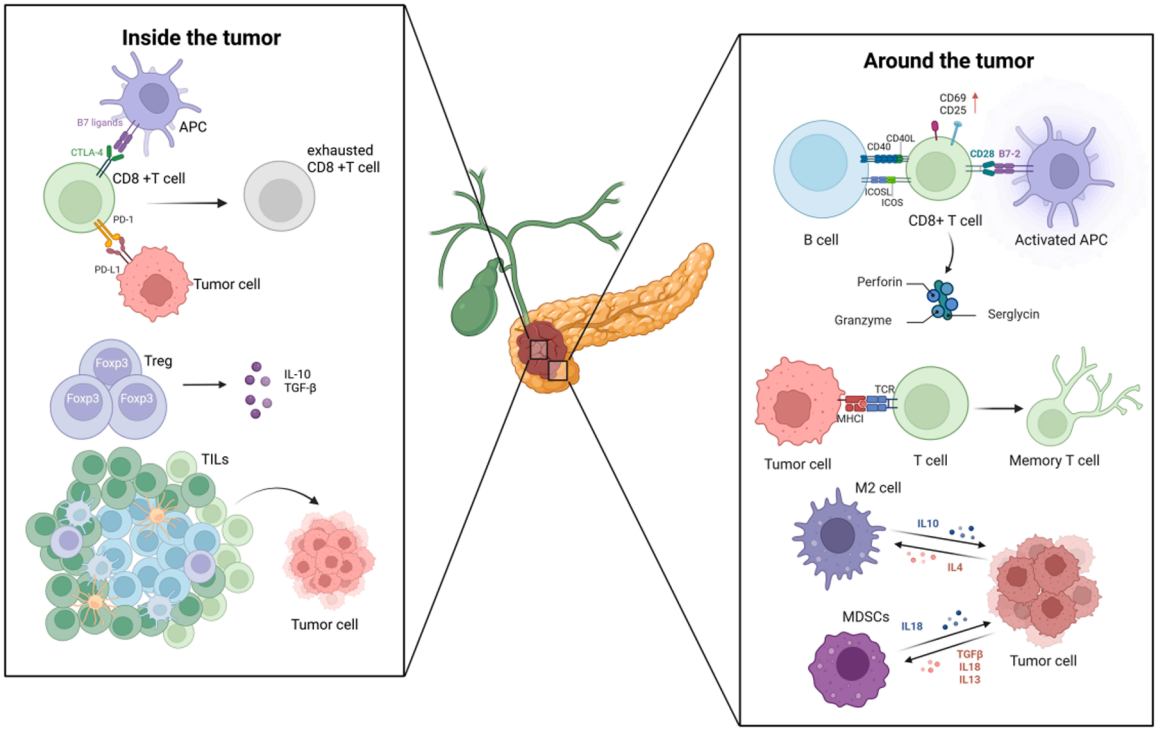

Fig. 3. Spatial distribution patterns within and around pancreatic cancer tumors.

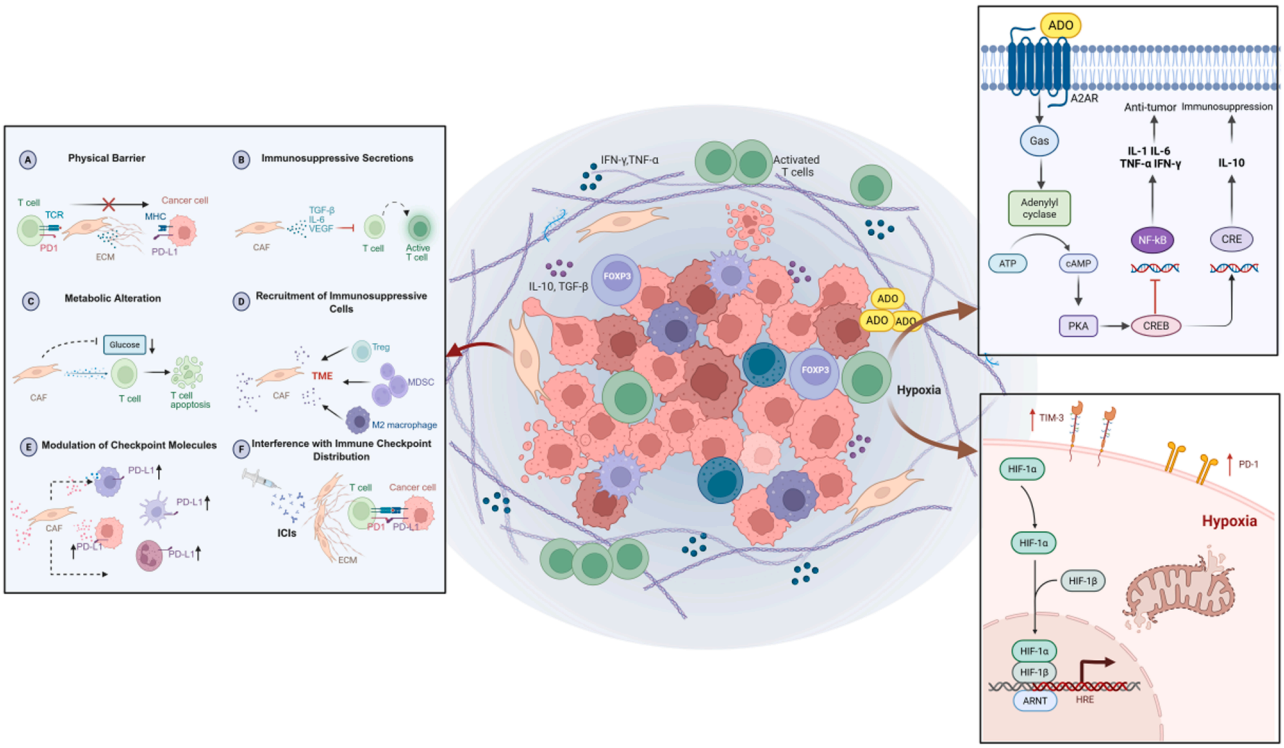

Fig. 4. Regulation of lymphocytes by microenvironmental factors.

Activation status of peritumoral TILs

In contrast to the core, the peritumoral stroma harbors T cells with heightened activation. Multiplex immunohistochemistry shows elevated CD69 and CD25 on CD8+ T cells, together with enhanced proliferation and production of granzyme B and perforin [37]. Memory T-cell subsets are enriched, suggesting that the peritumoral niche is a key site for immunological memory formation. Chemokine gradients—CCL5 and CXCL10 in particular—coincide with lymphocyte accumulation, yet the same region also hosts suppressive cells such as MDSCs, generating a complex regulatory network [38].

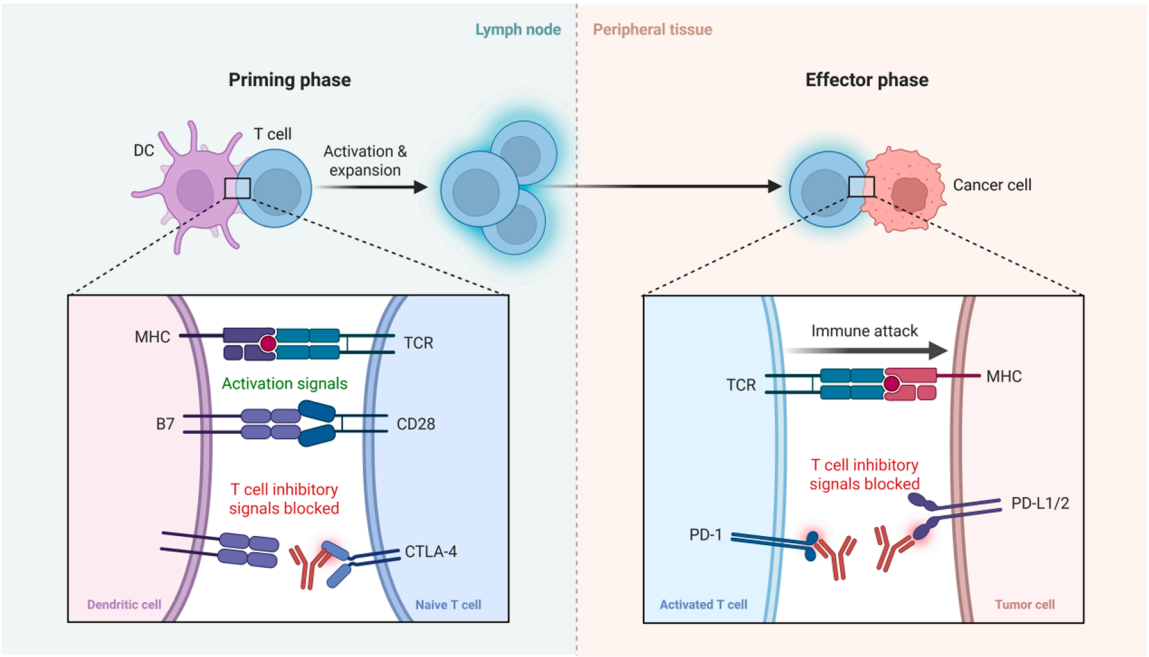

Fig. 5. CTLA-4 inhibitors and PD-1/PD-L1 induced immune restoration.

Anatomy-dependent checkpoint expression

Checkpoint molecules exhibit pronounced spatial patterning in PDAC. scRNA-seq demonstrates high PD-1, CTLA-4, and LAG-3 on intratumoral T cells, whereas peritumoral T cells preferentially express co-stimulatory receptors including ICOS and CD28 [39]. These gradients may reflect local antigen load and inflammatory cues. PD-L1 follows a similar geography: tumor cells within the core express high levels, whereas expression at the invasive margin is comparatively low. Such anatomy-dependent checkpoint profiles likely contribute to the limited efficacy of checkpoint blockade in PDAC [40].

Spatial segregation and the immune barrier at the tumor boundary

Lymphocyte topography also governs the immune barrier that demarcates tumor from adjacent tissue. At the invasive front, CD8+ T-cell density drops sharply, indicative of immune exclusion [41]. Cancer-associated fibroblast-derived extracellular-matrix constituents —collagen in particular—create a physical impediment, while hypoxia-driven metabolic reprogramming fosters a suppressive milieu [42]. The resulting spatial sequestering prevents effector T cells from penetrating the tumor core, establishing an "immune-privileged" phenotype that underlies clinical resistance to immunotherapy [43,44].

Microenvironmental control of lymphocyte heterogeneity

Extracellular-matrix barriers physically sequester T cells

Excessive extracellular-matrix (ECM) deposition in PDAC forms a dense fibrotic shield that mechanically restricts lymphocyte access to the tumor core [45]. Composed chiefly of collagen types I and III, fibronectin, and hyaluronan, this matrix elevates interstitial pressure and, via integrin signaling, promotes T-cell dysfunction [46]. Over-accumulation of ECM drives "immune exclusion" at the tumor margin, preventing CD8+ T cells from engaging malignant cells [47] and disrupting immunological synapse formation, thereby blunting cytotoxicity.

Hypoxia-driven metabolic reprogramming

PDAC's hypoxic niche activates HIF-1α-dependent metabolic rewiring in lymphocytes [48,49]. At O_2 tensions below 10 mmHg, $CD8^+$ T cells switch from oxidative phosphorylation to glycolysis, incurring mitochondrial damage and reduced IFN-γ secretion [50–52]. Hypoxia concurrently enriches and activates $FOXP3^+$ Tregs that heighten suppression via elevated CTLA-4 and PD-1 [53], while cancer-cell-derived adenosine engages A2A receptors to further curb effector T-cell proliferation and cytotoxicity [54].

Cancer-associated fibroblast (CAF) immunoregulatory networks

Activated CAFs sculpt an intricate immunoregulatory lattice. Secretion of TGF-β and IL-6 steers CD4+ T cells toward Th17 and Treg fates [55,56], whereas CAF-expressed PD-L1 and CD73 directly inhibit CD8+ T cells. Single-cell analyses identify CAF subsets that establish "immune-privileged micro-islands" via the CXCL12–CXCR4 axis, selectively recruiting regulatory B cells and dampening anti-tumor immunity [57,58].

Cytokine and chemokine gradients impose functional polarity

Sharp cytokine gradients polarize lymphocyte function [59,60]. High per-cell concentrations of TGF-β and IL-10 in the core stabilize and expand FOXP3+ Tregs, whereas IFN-γ and TNF-α predominate in the periphery, sustaining transient effector T-cell activity [61,62]. Divergent expression of CCR5 and CXCR3 confines CD8+ T cells to defined anatomical niches, generating functionally zoned microenvironments [63,64]. Gradient-driven IL-1β and IL-23 additionally skew γδ T cells toward a pro-tumor phenotype, intensifying immunosuppression.

Lymphocyte-centric mechanisms of clinical immunotherapy resistance

Epigenetic basis of T-cell exhaustion

T-cell exhaustion is a central pillar of resistance. Epigenetic dysregulation disrupts the cancer–immunity cycle—from antigen

presentation to terminal exhaustion—by silencing tumor antigens, disabling immune cells, and consolidating an immunosuppressive milieu [65]. Chronic antigen stimulation imprints an exhaustion phenotype, compounded by nutrient restriction and additional suppressive cues [66]. Exhausted CD8+ T cells display epigenetic rewiring of PD-1 and antigen-presentation pathways, mediated in part by E3 ubiquitin ligases [67,68]. Decoding the heterogeneity and dynamics of exhausted CD8+ T cells will inform therapies that reprogram rather than simply re-invigorate T-cell differentiation.

Dynamic evolution of the immunosuppressive niche

PDAC's characteristic immunosuppressive microenvironment is a moving barrier to therapy. The landscape integrates increased expression of inhibitory ligands on tumor cells, suppressive immune populations, and soluble immunomodulators released by malignant or stromal cells [69,70]. Cancer-associated fibroblasts add a structural dimension by depositing ECM that physically impedes immune access [71,72]. Progressive reinforcement of TGF-β signaling and cell-cycle-related pathways within this niche steadily erodes immune function, culminating in therapeutic failure [73].

Antigen-presentation defects and faulty immunological synapses

Defective antigen presentation is another key resistance axis. Down-regulation of major histocompatibility complex molecules on tumor cells and sub-optimal processing by antigen-presenting cells curtail the generation of tumor-specific T-cell responses. CHEK2-deficient tumors up-regulate antigen-presentation machinery upon PD-1 blockade, underscoring the importance of intact presentation for therapeutic benefit [74]. Additional layers—antigen heterogeneity, loss of presentation machinery, and impaired immunological synapse formation—synergistically blunt T-cell cytotoxicity and promote escape [75].

Clonal heterogeneity and spatiotemporal immune evasion

Extensive morphological and molecular heterogeneity underpins PDAC resistance. Low mutational burden coupled with broad intra- and inter-tumor diversity offers limited immunogenic substrate [76,77]. Clonal variation drives antigen loss or mutation, a vulnerability exposed during single-antigen CAR-T trials [78,79]. Spatially, an immune barrier at the tumor margin and anatomy-dependent checkpoint expression patterns create distinct escape niches. Targeted RNA-seq combined with multiplex immunofluorescence in non-pathological complete responders highlights a profoundly immunosuppressed microenvironment, providing fresh insight into how clonal heterogeneity begets therapeutic resistance [80].

Clinical trial outcomes and translational biomarkers

Major immunotherapy clinical trials in PDAC have yielded disappointing results with checkpoint blockade monotherapy. The CheckMate 032 study evaluated nivolumab monotherapy and nivolumab plus ipilimumab with or without cobimetinib in previously treated patients with pancreatic adenocarcinoma [20]. The trial found that nivolumab with or without ipilimumab did not elicit objective responses, although three confirmed partial responses were observed with cobimetinib-containing triplet therapy. The PRINCE trial investigated sotigalimab and/or nivolumab with chemotherapy in first-line metastatic pancreatic cancer, providing important insights into combination immunotherapy approaches [21]. The CCTG PA.7 trial evaluated durvalumab with or without tremelimumab for patients with metastatic pancreatic ductal adenocarcinoma, demonstrating the challenges of dual checkpoint inhibition in this disease [81]. These trials collectively highlight the need for better patient stratification strategies and combination approaches targeting the tumor microenvironment.

Translational biomarkers for immunotherapy response in PDAC remain an active area of investigation. PD-L1 expression, while commonly used in other malignancies, shows spatial heterogeneity in PDAC and inconsistent predictive value [82]. TIL density correlates with prognosis but shows variable predictive utility for immunotherapy response. The presence of tertiary lymphoid structures (TLS) has emerged as a promising biomarker, with TLS presence associating with improved long-term survival in PDAC patients [83]. The Immunoscore, incorporating CD3+ and CD8+ *T*-cell densities, has shown prognostic significance and may guide therapeutic decisions [84]. Emerging spatial biomarkers, including AI-powered spatial analysis of immune phenotypes, offer new opportunities for precision immunotherapy [85]. However, the dynamic nature of the immunosuppressive landscape complicates biomarker development, underscoring the need for refined spatial signatures to guide clinical decisions.

Current limitations and controversies

Spatially resolved studies have markedly advanced understanding of PDAC immunity, yet current spatial-omics platforms remain limited by sub-optimal resolution—both in positional accuracy and in capturing the full spectrum of immune-cell heterogeneity. The desmoplastic stroma unique to human PDAC further complicates technology deployment [86–88].

Second, animal models recapitulate only selected facets of the human PDAC immune milieu. Tertiary lymphoid structures (TLS), readily observed in patients, are poorly reproduced in mice, and the clonal heterogeneity accrued over years of human tumor evolution cannot be compressed into short-term animal experiments [89,90].

Functional studies of PDAC-infiltrating lymphocytes also lack standardization. Variations in tissue-processing protocols, isolation methods, culture conditions, and functional read-outs hinder cross-study comparability. Exhaustion scoring, spatial-boundary definitions, and checkpoint-detection assays remain method-dependent, compromising reproducibility [91–93].

Finally, predictive biomarkers of immunotherapy response remain contentious. PD-L1 expression is spatially heterogeneous; TIL density correlates with prognosis yet shows inconsistent predictive value; TLS presence associates with long-term survival, but their ontogeny and functional contribution are unresolved. The dynamic nature of the immunosuppressive landscape further complicates biomarker development, underscoring the need for refined spatial signatures to guide clinical decisions [94,95].

Also, technical limitations of current spatial-omics platforms present significant challenges. Sub-optimal resolution, both in positional accuracy and in capturing the full spectrum of immune-cell heterogeneity, limits comprehensive analysis [96]. The desmoplastic stroma unique to human PDAC further complicates technology deployment and data interpretation. Animal models recapitulate only selected facets of the human PDAC immune milieu. Tertiary lymphoid structures (TLS), readily observed in patients, are poorly reproduced in mice, and the clonal heterogeneity accrued over years of human tumor evolution cannot be compressed into short-term animal experiments [97]. Lack of standardization in functional studies of PDAC-infiltrating lymphocytes hinders cross-study comparability. Variations in tissue-processing protocols, isolation methods, culture conditions, and functional read-outs compromise reproducibility [98]. Challenges in developing predictive biomarkers persist due to spatial heterogeneity and the dynamic nature of the immunosuppressive landscape. Current immunotherapy clinical trials in PDAC have shown limited success, highlighting the need for better patient stratification strategies based on spatial immune profiling [99].

Future directions

Integrative single-cell spatial multi-omics

PDAC immunity is defined by heterogeneity in composition, spatial positioning, and activation state. Single-cell RNA-seq has already profiled ~80,000 T cells, resolving fine-scale subsets [100,101]. Future work should merge scRNA-seq, spatial transcriptomics, and CyTOF to map lymphocyte location and function at single-cell resolution. Novel computational tools that integrate multi-omic profiles with three-dimensional spatial coordinates will be required to uncover the architectural rules governing immune-cell topography [102–104].

Microenvironment-restricted therapeutic targets

The muted response to checkpoint blockade demands targets tailored to the PDAC niche. CD103+CD8+ tissue-resident memory T cells display distinct localization and activation potential, representing candidate targets [105]. Macrophage–TIL interaction circuits also warrant interrogation. Multiplex immunohistochemistry coupled with spatial statistics should quantify immune–tumor neighborhood relationships and identify prognostically relevant target combinations, with particular attention to TLS organization [106].

Spatiotemporal organoid models

Conventional animal systems inadequately mirror human PDAC immunity. Organoid technology offers a path to model dynamic immune–stromal crosstalk. Co-culture platforms incorporating patient-derived TILs and CAFs, embedded in microfluidic devices that recreate hypoxic gradients and ECM barriers, will enable real-time tracking of immune-cell trafficking and checkpoint expression [107–109]. Such patient-specific "immune-organoids" could pre-screen personalized immunotherapeutic strategies.

AI-guided immune-atlas decoding

Although TIL density is prognostic, clinical translation remains elusive. Deep-learning algorithms already provide spatial maps of TIL abundance [110]. Next-generation computer-vision tools should automate identification and quantification of lymphocyte subsets across intra- and peri-tumoral domains. Neural-network models integrating spatial immune features with clinicopathological variables can generate robust prognostic systems. AI-driven mining of large-scale single-cell datasets will additionally uncover hidden transition states, furnishing new biomarkers to guide PDAC immunotherapy [111].

Promising therapeutic strategies targeting spatial immune heterogeneity

Several promising therapeutic strategies targeting spatial immune heterogeneity in PDAC are emerging. ECM remodeling strategies aim to improve immune cell infiltration by targeting collagen crosslinking enzymes and hyaluronan synthesis [112]. CAF-targeted therapies seek to disrupt the immunosuppressive stroma by reprogramming cancer-associated fibroblasts or depleting specific CAF subsets [113]. Metabolic reprogramming approaches target hypoxia-driven T-cell exhaustion through HIF-1α inhibitors and adenosine receptor antagonists [114]. Combination immunotherapy strategies targeting multiple checkpoints, including PD-1/PD-L1, CTLA-4, and LAG-3, show promise in preclinical models [115]. Neoadjuvant immunotherapy approaches have demonstrated the potential to promote TLS formation and enhance anti-tumor immunity [116]. Personalized vaccine strategies based on spatial immune profiling, including mKRAS-specific vaccines and neoantigen-targeted vaccines, represent innovative approaches [117]. Emerging strategies targeting TLS formation and maturation aim to create organized lymphoid structures that support effective anti-tumor immune responses [118].

AI-guided spatial analysis and clinical translation

Artificial intelligence is transforming spatial immunology research and clinical translation. AI-powered spatial analysis enables automated quantification of TIL density and identification of distinct immune phenotypes with prognostic significance [119]. Deep-learning algorithms can classify tumors into immune-inflamed, immune-excluded, and immune-desert phenotypes, providing valuable prognostic information. The development of next-generation immunoscore systems incorporating spatial features offers improved risk stratification [120]. Challenges for clinical integration include cost-effectiveness considerations, regulatory pathways, and standardization across platforms. Future directions include real-time spatial immune profiling during treatment to monitor response and guide therapeutic decisions [121].

Conclusion

This review has synthesized the major advances in understanding spatial heterogeneity of tumor-infiltrating lymphocytes in pancreatic ductal adenocarcinoma. Key insights include the distinct phenotypic and functional profiles of intratumoral versus peritumoral lymphocytes, with exhausted CD8+ T cells and immunosuppressive Tregs enriched in the tumor core, while activated effector cells predominate at the invasive margin. The mechanisms driving this spatial heterogeneity involve ECM-mediated physical barriers, hypoxia-driven metabolic reprogramming, CAF-derived immunosuppressive signals, and cytokine/chemokine gradients that polarize lymphocyte function across anatomical niches.

The clinical implications of spatial immune profiling are substantial. Integrating spatial immune features into prognostic models can improve patient stratification and guide therapeutic decisions. The identification of immune phenotypes—inflamed, excluded, and desert—provides a framework for personalized immunotherapy approaches. Tertiary lymphoid structures emerge as promising biomarkers for patient selection, while AI-powered spatial analysis offers scalable solutions for clinical implementation.

Current challenges in translating spatial immunology research into clinical practice include technical limitations of spatial-omics platforms, inadequate animal models, lack of standardization in functional assays, and the dynamic nature of the immunosuppressive landscape. Major immunotherapy clinical trials in PDAC have demonstrated the limitations of checkpoint blockade monotherapy, highlighting the need for combination approaches targeting the tumor microenvironment.

Future research priorities should focus on developing integrative spatial multi-omics approaches that combine single-cell transcriptomics, spatial transcriptomics, and proteomics at single-cell resolution. AI-guided immune atlas decoding will enable automated identification of lymphocyte subsets and prognostic modeling. The design of clinical trials incorporating spatial biomarkers for patient stratification and treatment monitoring represents a critical next step. We envision that within the next 5 years, spatial immune profiling will become a standard component of PDAC diagnostic workup, guiding personalized immunotherapy strategies and improving patient outcomes.

Consent for publication

All authors agreed to submit for publication in this journal.

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Availability of data and materials

The datasets used in this study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Ke Zhao: Writing – original draft, Data curation, Conceptualization. **Qiting Yang:** Investigation, Formal analysis. **Yuhan Yan:** Validation, Software, Methodology. **Jiyun Zhu:** Writing – review & editing, Funding acquisition. **Siming Zheng:** Supervision, Funding acquisition.

Declaration of competing interest

The authors all declare that they have no competing interests in this study.

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