



Spatial Heterogeneity of TMEs in cHL and PDAC: Mechanistic Insights and Therapeutic Perspectives

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Abstract

The tumor microenvironment (TME) is a critical niche that influences tumor progression and the development of therapeutic resistance. Spatial omics technologies enable in situ investigation of cellular ecology and intercellular interactions within the TME, addressing the lack of spatial context associated with bulk and single-cell sequencing approaches. Classical Hodgkin lymphoma (cHL) and pancreatic ductal adenocarcinoma (PDAC) exhibit distinct TME characteristics: cHL is characterized by dense immune-cell infiltration accompanied by functionally ineffective immune responses, whereas PDAC represents a prototypical immune-cold tumor. This review summarizes recent advances in spatial omics research on cHL and PDAC, with particular emphasis on how cellular heterogeneity, immune-cell distribution, stromal architecture, and metabolic niches collectively influence tumor pathobiology. Finally, several emerging therapeutic strategies, including oncolytic viruses, local immune agonists, and advanced drug-delivery systems, are discussed for their potential to remodel the TME and enhance immunotherapeutic efficacy.

Keywords:

classical hodgkin lymphoma; pancreatic ductal adenocarcinoma; tumor microenvironment; spatial omics; tumor progression

1. Introduction

Spatial multi-omics technologies are increasingly being applied to the characterization of pathological tissue sections, enabling researchers to generate insights that extend beyond those obtained from conventional histopathology, epidemiological studies, and animal models. These advances have contributed to a more comprehensive understanding of the tumor microenvironment (TME), particularly by preserving information on cellular localization and tissue architecture. In 2020, *Nature Methods* recognized spatial transcriptomics as the “Method of the Year,” highlighting its ability to generate single-cell-scale transcriptomic information while preserving tissue spatial architecture. This technology enables the simultaneous analysis of cellular localization and intercellular relationships within tissues, thereby addressing the limitations of traditional single-cell sequencing and bulk transcriptomic approaches, which lack spatial context or provide aver-

aged molecular signals [1]. Similarly, other spatial omics technologies, including spatial proteomics and mass spectrometry imaging, enable in situ analysis of the spatial distribution and functional heterogeneity of proteins and metabolites while preserving tissue architecture. These approaches facilitate the identification of functional alterations that may not be captured by single-cell transcriptomic analyses alone [2,3]. These spatial omics techniques have been applied to classical Hodgkin lymphoma (cHL) and pancreatic ductal adenocarcinoma (PDAC) to identify specific cellular populations, analyze their spatial organization, and characterize metabolic features within the TME (Figure 1).

There is substantial heterogeneity in patient responses to immunotherapy [4]. While some patients experience significant clinical improvement [5], others derive little or no therapeutic benefit [6]. To better understand this heterogeneity, researchers are increasingly classifying

tumors according to distinct characteristics of the immune landscape within the TME. A pan-cancer immunogenomic analysis encompassing 33 cancer types from The Cancer Genome Atlas (TCGA), including more than 10,000 samples, identified six distinct immune subtypes: wound healing, interferon-gamma (IFN- γ) dominant, inflammatory, lymphocyte depleted, immunologically quiet, and transforming growth factor-beta (TGF- β) dominant [7]. Another TCGA-based study analyzed data from 33 cancer types and classified tumors as immunologically “hot” or “cold.” Immunologically “hot” tumors are characterized by increased infiltration of CD8+ T cells and activated NK cells, elevated T-cell proliferation and cytotoxic activity scores, and reduced abundance of M2 macrophages. Immune “cold” tumors are

characterized by reduced CD8+ T-cell infiltration, enrichment of M2 macrophages, and an overall immunosuppressive microenvironment. Hot tumors are often associated with improved survival outcomes, whereas cold tumors are associated with poorer survival outcomes [8]. Classifying tumors based on their immune characteristics can help predict responses to immunotherapy, and researchers increasingly design preclinical antitumor efficacy studies and guide clinical trial strategies using the “hot” and “cold” tumor paradigm [9,10]. For example, “cold” tumors can be converted into “hot” tumors through combination therapies or tumor microenvironment modulation, thereby enhancing the efficacy of immune checkpoint inhibitors (ICIs) [11].

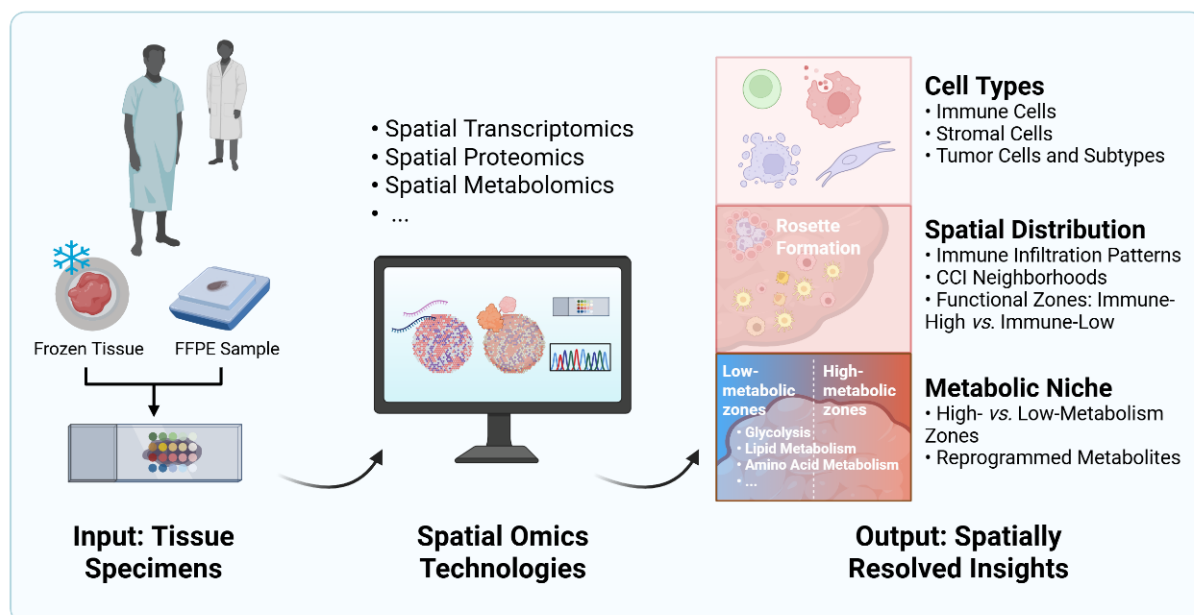


Figure 1: Schematic overview of the spatial omics workflow applied to cHL and PDAC. Tissue specimens, including frozen tissues and FFPE samples, are processed for spatial transcriptomics, spatial proteomics, and spatial metabolomics analyses, enabling interrogation of molecular features while preserving spatial information. These technologies allow integrated visualization of cell types, their spatial distribution, and metabolic niches within the TME. created with <https://BioRender.com>.

Hodgkin lymphoma (HL) is a malignancy of the lymphatic system that most frequently originates in the cervical lymph nodes. In 2020, its global incidence and mortality rates were 0.98 and 0.26 per 100,000 people, respectively [12]. According to the World Health Organization, HL is classified into two main subtypes: classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) [13]. Among these subtypes, cHL accounts for the vast majority of cases, representing approximately 95%, whereas NLPHL is relatively rare, comprising about 5% of all HL diag-

noses [14,15]. Classical Hodgkin lymphoma (cHL) is characterized by extensive infiltration of inflammatory and immune cells, reflecting a highly immune-active tumor microenvironment; however, it concurrently exhibits prominent immunosuppressive features [16,17]. In contrast, pancreatic ductal adenocarcinoma (PDAC) is the most prevalent form of pancreatic cancer, accounting for more than 90% of cases [18]. The majority of PDAC tumors exhibit an “immune-cold” phenotype, which is believed to contribute to therapeutic resistance and poor clinical outcomes [19,20].

This review focuses on two tumor types with distinct immune microenvironments: cHL, characterized by dense immune-cell infiltration with immunosuppressive features, and PDAC, characterized by low effector immune-cell infiltration and limited response to im-

muno-therapy. This article first summarizes recent advances in spatial omics studies of the TME in cHL and PDAC (Table 1) and subsequently discusses emerging adjuvant therapeutic strategies for TME modulation.

Table 1: Comparative overview of spatial TME features in cHL and PDAC.

Feature	cHL	PDAC
Immune phenotype	Immune “hot” but immunosuppressed tumor [16,17]	Prototypical immune “cold” tumor [19,20]
Tumor cells	Rare HRS cells derived from germinal center B cells [21,22]	Malignant ductal epithelial cells [23]
Key genetic drivers	Genomic instability and constitutive signaling activation [22,24]	KRAS-driven oncogenesis and metabolic reprogramming [25]
Major immune infiltrates	T cells, macrophages, dendritic cells [21]	Macrophages, Tregs, MDSCs; scarce effector T cells [26,27]
T-cell status	Exhausted phenotype (PD-1, LAG-3, TIM-3) [28,29]	Exhausted T cells enriched at invasive front (TIGIT–NECTIN) [30,31]
Immunosuppression	Mediated by Tregs, M2-TAMs, IL-10, TGF- β , IDO [17,32]	Driven by hypoxia, dense stroma, Tregs, M2-TAMs [33]
Spatial immune pattern	PD-L1 ^{high} tumor regions and myeloid-rich niches [34]	Immune cells confined to stroma (“immune-excluded”) [35]
CAF spatial heterogeneity	PDGFRB ⁺ and FAP ⁺ CAFs linked to prognosis [36]	myCAFs near tumor core; iCAFs in distal stroma [37–39]
Spatial metabolism	Nil (not systematically characterized) *	High- and low-metabolic zones with metabolic reprogramming [40–42]
Therapy response (spatial)	HRS antigen presentation shapes immune cell localization [43]	KRAS G12D inhibition induces IFN γ signaling and CD8 ⁺ T-cell recruitment [44]
Immunotherapy response	Highly sensitive to PD-1 blockade [5,45,46]	Poor response to ICI monotherapy [6]
Prognosis	Generally favorable [45,46]	5-year survival <10% [47]

* Note: In the initial staging of classical Hodgkin’s lymphoma (cHL), fluorodeoxyglucose positron emission tomography–computed tomography (FDG PET-CT) detects abnormal glucose uptake in lymph nodes, extranodal organs, and bone marrow, even without CT abnormalities, indirectly suggesting high metabolic activity in cHL regions and providing a hypothesis for future spatial metabolism studies [48].

2. Literature Search Strategy

This review used PubMed as the primary database to identify relevant studies. The review included studies published between January 2000 and February 2026. Where appropriate, the review also incorporated seminal landmark studies to provide historical context for the development of cancer immunology and tumor microenvironment research. Additionally, the reference lists of key publications were manually screened to identify further relevant studies.

To summarize advances in spatial omics technologies and their application in TME research, keyword combinations included “spatial transcriptomics,” “spatial proteomics,” “spatial omics,” “digital spatial profiling,” and “tumor microenvironment.” These searches aimed to identify key methodological advances and review articles describing spatially resolved techniques and their applications in cancer biology.

For the cHL-related analysis, search terms included “classical Hodgkin lymphoma,” “tumor microenvironment,” “immune microenvironment,” “spatial transcriptomics,” “spatial profiling,” and “single-cell RNA sequencing.” These searches aimed to identify studies describing immune cell composition, immune checkpoint regulation, and the spatial organization of immune cells in cHL.

For PDAC, keyword combinations included “pancreatic ductal adenocarcinoma,” “pancreatic cancer,” “tumor microenvironment,” “spatial transcriptomics,” and “single-cell RNA sequencing.” The review particularly emphasized studies investigating matrix architecture, immunosuppressive mechanisms, and spatially resolved cellular interactions within the PDAC TME.

In addition, targeted searches were conducted to identify studies investigating therapeutic strategies to

modulate the tumor microenvironment (TME). Search terms included “oncolytic virus,” “STING agonist,” “TLR agonist,” “CD40 agonist,” “nanoparticle drug delivery,” as well as “hyaluronan” and “hyaluronidase.” Relevant clinical trials, translational studies, and recent review articles were prioritized.

3. cHL: A Hot but Suppressed Immune Microenvironment

3.1. Introduction to cHL

cHL lesions contain rare malignant Hodgkin and Reed–Sternberg (HRS) cells surrounded by a rich infiltrate of immune cells [21]. HRS cells are rare tumor cells of germinal center B-cell origin that lose the typical B-cell program and exhibit constitutive activation of multiple signaling pathways [22]. HRS cells display highly aberrant gene expression and genomic instability [24], along with multiple genetic alterations that contribute to immune evasion [49]. In addition, they secrete cytokines and chemokines that shape an immunosuppressive tumor microenvironment (TME), promoting the recruitment of regulatory T cells (Tregs) and myeloid-derived suppressor cells, thereby further inhibiting anti-tumor immunity [32]. The hallmark histological features of cHL—including marked inflammation, eosinophil infiltration, and fibrosis or sclerosis—are thought to be driven, at least in part, by HRS cells through their expression of surface molecules and secretion of diverse cytokines and chemokines [50]. Notably, HRS cells are often surrounded by CD4⁺ T cells that form functional immunological synapses through HLA-II and CD58 interactions and express immune regulatory markers such as PD-1 and CTLA-4, potentially contributing to tumor survival and immune evasion [51,52].

Unlike many solid tumors, cHL is characterized by a dense immune infiltrate. Although HRS cells are rare, the surrounding microenvironment is predominantly composed of non-malignant immune cells, including T cells, macrophages, and dendritic cells [21]. HRS cells produce IL-6, contributing to the establishment of a highly inflammatory microenvironment [53]. They can also secrete CCL5 and induce stromal cells to do the same, thereby promoting the recruitment of CD4⁺ T cells and eosinophils and reinforcing local inflammatory responses [54]. Despite this pronounced inflammatory milieu, concurrent mechanisms that suppress effective anti-tumor immunity are also present, resulting in a functional paradox in cHL biology.

Tumor-infiltrating T cells in cHL commonly express exhaustion and inhibitory markers, including PD-

1, LAG-3, and TIM-3, consistent with a functionally impaired yet potentially reversible T-cell compartment [28, 29]. Expanded regulatory T cells (Tregs) and M2-like tumor-associated macrophages (TAMs) contribute to a persistent immunosuppressive microenvironment through the production of IL-10, TGF- β , and IDO, as well as direct cell–cell interactions, thereby suppressing effector T-cell activity and facilitating immune evasion [17]. Moreover, HRS cells secrete multiple immunomodulatory factors that collectively suppress effector T-cell activity, promote regulatory T-cell expansion, and drive TAM polarization toward an immunosuppressive M2-like phenotype [32].

Despite the complex and sometimes paradoxical nature of the cHL TME, clinical outcomes for many patients with cHL are generally favorable. Conventional chemotherapy regimens, including ABVD (Adriamycin, Bleomycin, Vinblastine, Dacarbazine) and BEACOPP (Bleomycin, Etoposide, Adriamycin, Cyclophosphamide, Vincristine, Procarbazine, Prednisone), remain effective for treating HL; however, these regimens are associated with both short-term and long-term adverse effects. More recently, targeted therapies, including antibody–drug conjugates such as brentuximab vedotin and immune checkpoint inhibitors such as pembrolizumab and nivolumab, have demonstrated clinical benefit in relapsed or refractory HL, providing additional therapeutic options and improving patient outcomes [45]. In the context of immune checkpoint blockade (ICB), pembrolizumab in combination with the GVD regimen (gemcitabine, vinorelbine, doxorubicin) has demonstrated high efficacy and favorable tolerability in patients with relapsed or refractory cHL [5]. A phase II trial evaluated PET-adapted nivolumab administered either as monotherapy or in combination with NICE (ifosfamide, carboplatin, etoposide) in relapsed/refractory cHL as a bridge to autologous stem cell transplantation (AHCT) (NCT03016871). The regimen was well tolerated, achieved high response rates, and enabled most patients to proceed to AHCT, with two-year progression-free and overall survival of 72% and 95%, respectively [46].

3.2. Spatial Omics Reveals the Immuno-Geography of cHL

The immune microenvironment of cHL is highly complex, comprising both immunosuppressive and inflammatory components. Characterizing the composition and spatial organization of these cells requires methodologies capable of quantifying cell populations while preserving their spatial context within tissue architecture. Spatial biology techniques enable such analyses at both

single-cell and tissue-region levels. Recent studies using spatial transcriptomics with the GeoMx Digital Spatial Profiler have demonstrated that the cHL microenvironment comprises transcriptionally and spatially distinct niches, including PD-L1-high tumor regions enriched for exhausted T cells and myeloid-dominant neighborhoods composed of classical monocytes, macrophages, and cDC2s. These niches exhibit distinct chemokine signatures, with the myeloid-rich compartment showing upregulation of CCL18, CCL13, CCL24, CCL26, and granulocyte-recruiting chemokines CXCL1 and CXCL6, thereby forming spatial microarchitectures that cannot be resolved using non-spatial approaches. Notably, enrichment of a cDC2–monocyte–macrophage signaling axis is associated with early treatment failure, establishing a spatially defined prognostic signature [34].

High-dimensional spatial proteomics has demonstrated substantial spatial heterogeneity among HRS cells in cHL. Analysis of more than 23 million cells identified 92 distinct HRS cellular states. Antigen-presentation programs in HRS cells, particularly *B2M*, *MHC-I*, *MHC-II*, and *PD-L1*, appeared to shape the surrounding microenvironment. *MHC-I*–positive HRS cells are frequently localized within niches enriched for CD8⁺ T cells and macrophages, whereas multinucleated HRS aggregates tend to form T-cell-excluded regions populated by TIM-3⁺ macrophages and Tregs, suggesting immune evasion mediated through spatial organization [43]. Multiplex immunofluorescence and automated multispectral imaging have further demonstrated that HRS cells expressing tumor necrosis factor receptor superfamily member 9 (TNFRSF9/CD137) are frequently co-localized with exhausted T cells and monocytic myeloid-derived suppressor cells (mMDSCs), highlighting a potential role in the establishment of immunosuppressive niches and identifying CD137 as a potential immunotherapeutic target [55].

The impact of viral infection on spatial immune organization has been characterized using combined spatial transcriptomics and multiplex imaging approaches. These analyses show that Epstein–Barr virus (EBV)-positive cHL contains PD-1^{high}/TIGIT^{high} CD8⁺ T-cell clusters, CXCR3⁺ cytotoxic T cells, and CD163^{high} macrophages within inflammatory niches enriched for T-cell receptor (TCR) and B-cell receptor (BCR) signaling, as well as chemokines such as CXCL9, CXCL10, and CXCL13 [56]. In addition, HRS cells in EBV-positive cHL exhibit retained and markedly enhanced expression of antigen-presentation machinery, more frequently displaying a B2M⁺/MHC-I⁺/MHC-II⁺ “triple-positive” phenotype [43]. In contrast, HIV/EBV co-infected tumors exhibit reduced T-cell activation, decreased PD-1^{high}/TIGIT^{high} CD8⁺ T-cell populations, impaired

TCR signaling, and extensive extracellular matrix remodeling, reflecting a distinct spatial pattern of immune dysfunction [56].

Spatial profiling of CD4⁺ T-cell biology has further revealed that rosette-forming CD4⁺ T cells surrounding HRS cells constitute highly specialized niches enriched for OX40, PD-1, and CTLA-4 transcripts and proteins. These cells are distinct from other CD4⁺ T-cell subsets and indicate a checkpoint-enriched microenvironment shaped by close proximity to tumor cells [52]. A complementary DSP study demonstrated that CD4⁺ T-cell exhaustion and regulatory signatures were preferentially concentrated in tumor-rich, PD-L1-high regions and were associated with poorer clinical outcomes, whereas immune-predominant regions retained TH1-like and memory-associated profiles [57].

Beyond immune cells, spatial proteomic analyses of stromal and malignant compartments have identified four cancer-associated fibroblast (CAF) subtypes in cHL, with PDGFRB⁺ CAFs located in proximity to HRS cells consistently associated with poor survival outcomes. The study further identified prognostically unfavorable FAP⁺ CAF and FAP⁺ macrophage populations, and revealed recurrent macrophage-dominant FAP⁺ niches that are associated with adverse clinical outcomes [36]. Although this study employed low-plex multiplex immunofluorescence rather than high-throughput spatial omics approaches, it nevertheless provided important spatial insights into CAF biology in cHL. These findings establish a foundation for future studies integrating transcriptomic and spatial technologies to further refine mechanistic understanding.

A limited number of studies have begun to investigate spatial alterations associated with recurrent cHL. A spatially resolved transcriptomic study revealed an immunosuppressive spatial pattern in relapsed or refractory cases, characterized by reduced non-malignant B-cell populations, enrichment of inhibitory cell types including M2 macrophages, Tregs, and mast cells, and attenuated chemokine and interferon signaling. In contrast, non-relapsed cases preserve immune structures enriched with non-malignant B cells and are associated with improved progression-free survival. However, because this study was primarily based on pretreatment baseline samples, the findings more likely reflect prognostically relevant spatial features than true dynamic changes associated with disease relapse [58]. Another study compared paired biopsy samples obtained at diagnosis and at relapse, demonstrating that different relapse time points are associated with distinct patterns of spatial remodeling. In early relapse cases, the TME remained relatively stable between diagnosis and relapse, particularly through the persistence of macrophage/myeloid cell-enriched ecotypes. In con-

trast, late relapse cases exhibited more pronounced spatial structural changes, including increased infiltration of non-malignant B cells as well as CD4⁺ and CD8⁺ T cells [59].

Based on existing spatial studies, the cHL microenvironment appears to exhibit a relatively stable spatial organizational framework, in which HRS cells establish distinct immune neighborhoods and diverse immune and stromal components form functionally specialized niches within the tissue architecture. These spatial units are not randomly distributed; rather, they are closely associated with immune activation, immunosuppression, and prognostic risk. Despite these advances, spatial research in cHL remains constrained by several limitations, including relatively small patient cohorts, incomplete characterization of stromal and immune cell lineages, and limited whole-transcriptome spatial resolution across full lymph node architecture. In addition, studies examining longitudinal paired samples and dynamic spatial remodeling before and after treatment remain limited, particularly with respect to the systematic characterization of spatial ecological evolution during the development of drug resistance. Because the systematic application of spatial biology to cHL has emerged only recently, the complexity of the TME likely remains incompletely characterized.

4. PDAC: A Cold and Spatially Restricted TME

4.1. Introduction to PDAC

PDAC is characterized by high metastatic potential, late detection, and poor prognosis [23], with a five-year survival rate of less than 10% [47]. Although surgical resection remains the only potentially curative option, only approximately 15–20% of patients are eligible for surgery at initial diagnosis [60]. Currently, first-line chemotherapy regimens used for PDAC (NALIRIFOX, FOLFIRINOX, and GEM-NABP) have not demonstrated decisive differences in overall efficacy; however, they differ substantially in toxicity profiles, resulting in distinct types and severities of adverse reactions [61]. Furthermore, PDAC has consistently demonstrated limited clinical responses to immune checkpoint blockade (ICB) monotherapy. This resistance reflects the immunosuppressive characteristics of the tumor microenvironment [6]. Accordingly, PDAC is generally regarded as an “immune-cold” tumor. Substantial research efforts are therefore focused on developing strategies to remodel this immune-hostile microenvironment and enhance tumor sensitivity to immunotherapy.

Dense profibrotic stroma is a key histological hallmark of PDAC [62]. The combination of desmoplastic

stroma, vascular compression, and hypoperfusion forms a physiological barrier characteristic of PDAC and represents a major contributor to its resistance to systemic therapy. Patient survival is significantly and inversely correlated with the extent of extracellular matrix deposition in primary tumors [63]. The combination of gemcitabine with IPI-926, a stromal-depleting agent targeting tumor-associated stromal tissue, has been shown to improve drug delivery efficiency [64]. Hypoxia is also a key determinant of PDAC progression and treatment resistance, as it promotes tumor growth, metabolic reprogramming, and epithelial–mesenchymal transition while simultaneously enhancing immunosuppression. Hypoxia activates pancreatic stellate cells and fibroblasts, increases collagen deposition, and induces chemokine secretion, thereby promoting the recruitment of myeloid-derived suppressor cells and B cells. These cells, together with regulatory T cells (Tregs) and M2 macrophages, suppress effector T-cell activation and promote T-cell exhaustion [33]. In addition, PDAC development is driven by a range of oncogenic mutations, among which KRAS mutations are particularly critical. Constitutive activation of downstream signaling pathways enables KRAS to drive tumor cell proliferation, reprogram cellular metabolism, promote immune evasion, and contribute to therapeutic resistance. Therefore, KRAS activation is widely recognized as a fundamental molecular mechanism underlying PDAC initiation, disease progression, and associated adverse clinical outcomes [25].

To further understand the pathogenesis of PDAC and its therapeutic resistance, detailed characterization of the composition and functional features of the tumor microenvironment is essential. The TME of PDAC comprises a diverse array of components, including cancer-associated fibroblasts (CAFs), macrophages, neutrophils, mast cells, and lymphocytes [26]. Advances in single-cell sequencing have enabled systematic mapping of interactions between PDAC tumor cells and diverse microenvironmental cell types. For example, receptor–ligand interactions between T cells and malignant ductal cells, such as CCL5-SDC1/4, may promote tumor cell migration [65]. Regulatory T cells (Tregs), through interactions with malignant ductal cells, contribute to the establishment of an immunosuppressive TME [27]. Metabolic crosstalk between CAFs and tumor cells is also observed in PDAC. Loss of SETD2 in tumor cells drives the emergence of lipid-rich CAF subpopulations via BMP2 signaling and ectopic enrichment of H3K27ac. These CAFs support tumor cell mitochondrial oxidative phosphorylation through lipid provision, thereby promoting tumor progression [66].

4.2. Decoding the Spatial Complexity of PDAC through Omics

Although single-cell sequencing and conventional methods can characterize the composition and functional states of the TME, they provide limited spatial context. This limits the comprehensive resolution of spatial heterogeneity and in situ interactions among diverse cell types in complex tissues. Spatial omics technologies provide new opportunities to dissect the PDAC TME and generate insights into its biological mechanisms and therapeutic responses.

4.2.1. Tumor Cells and the Spatial Heterogeneity of Precancerous Lesions

The morphological evolution of PDAC begins with pancreatic intraepithelial neoplasia (PanIN) and, with progressive histological grading, ultimately advances to invasive adenocarcinoma [67]. Studies of the healthy pancreas may also provide insights into PDAC initiation and TME remodeling. Analysis of healthy donor pancreata has shown that PanIN lesions are commonly present in most individuals and are surrounded by enriched macrophages, CD4⁺ T cells, and highly heterogeneous fibroblasts. Their microenvironment is markedly distinct from that of the normal pancreas, and their epithelial cells are transcriptionally highly similar to cancer cells [68], with associated high expression of repetitive RNAs [68], and are associated with high repetitive RNA expression [69]. This study is the first to apply spatial omics to characterize the immune and stromal composition of early lesions in the healthy human pancreas, providing new insights into the cellular origins of PDAC.

Following tumor establishment, spatial omics analyses have further revealed pronounced intratumoral heterogeneity in PDAC tissues. Spatial transcriptomic analyses have demonstrated pronounced spatial heterogeneity among PDAC tumor cells. Tumor cell subpopulations exhibiting distinct proliferative, differentiation, and stress states are spatially segregated, with certain regions showing low-grade morphological features, while others are enriched for highly proliferative cells, particularly at the tumor invasive front [30]. In addition, the study identified a transitional epithelial cell subpopulation (Ep_VGLL1) positioned between the classical and basal-like subtypes. These cells occupy intermediate spatial regions between the two populations, exhibit both epithelial characteristics and basal-like invasive properties, and are associated with poor prognosis [37].

4.2.2. Spatial Distribution and Dysfunction of Immune Cells

Spatial technologies have revealed the immune microarchitecture underlying the characterization of PDAC as an “immune desert.” Studies have shown that although immune cells such as T cells and macrophages are present within tumor tissues, they are largely confined to stromal regions, whereas dendritic cells (DCs) and fibroblasts cluster at tumor margins, forming an immune-excluded spatial pattern [35]. In addition, exhausted T cells and regulatory T cells (Tregs) are enriched at the tumor invasive front, where they establish a localized immunosuppressive microenvironment in concert with immune checkpoint molecules expressed by tumor cells (such as TIGIT–NECTIN) [30,31]. In liver metastases, regions in which basal-like tumor cells co-localize with myofibroblastic CAFs (myCAFs) exhibit more pronounced immunosuppressive features, including exclusion of plasma cells and T and B cells, whereas lung metastases enriched for classical subtype tumor cells display more active immune infiltration [70].

Clinically, high monocyte and CD68⁺ macrophage infiltration are independent adverse prognostic factors [71]. At the microenvironmental level, however, macrophages in PDAC exhibit substantial functional heterogeneity, and their spatial distribution is closely associated with local signaling networks. Studies have shown that IL-1 β ⁺ TAMs in PDAC are not randomly distributed but are enriched in fibroblast-rich regions surrounding the tumor core. These regions also exhibit elevated expression of inflammatory, hypoxia-related, and angiogenesis-associated gene programs. These TAMs are cooperatively induced by prostaglandin E2 (PGE2) and tumor necrosis factor (TNF). In close proximity to tumor cells, they may promote inflammatory reprogramming and pathogenic transformation, thereby contributing to early tumorigenesis and poor prognosis [13]. On the other hand, M1-like macrophages inhibit tumor cell lipid metabolism, proliferation, and invasion via the IRF7/RPS18 axis and, through their spatial proximity to tumor cells, exert antitumor effects [72]. Integration of spatial transcriptomics with single-cell data has further revealed zoned distributions of distinct TAM subtypes (including IL-1 β ⁺, FOLR2⁺, SPPI⁺ populations) within tumor tissues, suggesting that their functional heterogeneity is tightly regulated by local cell–cell interactions [73].

In addition, treatment-induced stress can further modulate the state of TAMs. For instance, in murine pancreatic ductal adenocarcinoma (PDAC) models, gemcitabine treatment leads to increased TAM infiltration, accompanied by upregulation of CSF1 and CCL2. Inhibi-

tion of CSF1R or CCR2 alters TAM accumulation and associated immune responses, thereby enhancing the antitumor efficacy of gemcitabine [74]. Moreover, conditioned medium derived from gemcitabine-treated tumor cells promotes macrophage migration and invasion and upregulates M2-associated markers, including arginase-1 and TGF- β 1. IL-8 levels increase significantly after gemcitabine treatment, and IL-8 neutralization partially reduces macrophage recruitment but does not fully reverse their M2-like polarization state [75].

4.2.3. Spatial Functional Heterogeneity of CAFs

CAFs in PDAC are not only abundant [66] but also exhibit pronounced spatial and functional heterogeneity: within the tumor center (TS), CAFs predominantly exert immunosuppressive effects, whereas at the tumor margin (ATT), fibroblasts are more associated with immune-enhancing functions [38].

PDAC mainly contains two well-described fibroblast subtypes: myofibroblastic CAFs (myCAFs) and inflammatory CAFs (iCAF) [39]. Spatial transcriptomics data show that myCAFs are predominantly enriched in tumor-proximal regions, where they co-localize with basal-like tumor cells, form a stromal barrier, and contribute to immune exclusion [37,70]. Similarly, in residual tumors following neoadjuvant therapy, interactions between myCAFs and tumor cells are enhanced, particularly through enrichment of IL-6 family signaling pathways, which may contribute to chemoresistance [76]. In addition, myCAFs colocalize with regions exhibiting disulfidoptosis-associated activity. Although this observation suggests a potential role for myCAFs in regulating disulfidoptosis-related tumor phenotypes, direct *in vivo* or *in vitro* experimental evidence confirming this function remains lacking [77]. In contrast, iCAFs are more widely distributed in regions distant from tumor cells and, during PDAC progression, can even replace pancreatic islets within the tumor stroma [38].

In recent years, spatial molecular imaging at single-cell resolution has further elucidated regulatory mechanisms underlying the myCAF phenotype, demonstrating that high repetitive RNA expression is associated with enhanced inflammatory signaling, activation of epithelial-mesenchymal transition-related gene programs in cancer cells, and the transition of myCAFs toward an iCAF state [69]. Experimental inhibition of macropinocytosis *in vivo* has been reported to promote a shift from myCAF-like to iCAF-like states, thereby remodeling the tumor stroma. This remodeling—including increased iCAF abundance, reduced collagen deposition, enhanced immune cell infiltration, and expanded vasculature—

enhances the sensitivity of PDAC tumors to both immunotherapy and chemotherapy [39].

4.2.4. Spatial Reprogramming of Metabolism and Signaling Pathways

The integration of spatial metabolomics and transcriptomics has revealed regional differences in metabolic activities within PDAC. Studies have identified distinct high- and low-metabolic zones within PDAC tissues, with macrophages and specific ductal cell populations closely interacting with tumor cells in high-metabolic regions. In these malignant areas, carbohydrate, amino acid, lipid, and nucleotide metabolic pathways undergo marked reprogramming, including enhanced glycolysis consistent with the Warburg effect, increased glutamine utilization, and reduced fatty acid metabolism [40]. Moreover, tumors with high sphingolipid metabolism (SM) exhibit poorer prognosis, whereas SM-low tumors display a more active immune microenvironment [41]. Glycerolipid metabolism (GLM) is broadly suppressed within tumor regions, and spatial transcriptomics has, for the first time, visualized the spatial distribution of this signaling pathway [42].

4.2.5. Spatiotemporal Evolution of PDAC and Treatment-Associated Microenvironmental Remodeling

Spatial omics studies not only reveal treatment-induced remodeling of the tumor microenvironment (TME) but also characterize the spatial dynamics of PDAC throughout disease progression.

Spatial omics studies have revealed structural alterations during the transition from precursor lesions to invasive carcinoma. Clonal differences are observed between PanIN and PDAC. Compared with normal tissue, both lesions exhibit increased T-cell infiltration; however, PDAC displays a higher proportion of regulatory T cells (Tregs) and a greater degree of T-cell exhaustion. Spatial analysis demonstrates increased lymphocyte proximity in PDAC, with a higher frequency of mature tertiary lymphoid structures (TLS), whereas regions adjacent to PanIN are predominantly characterized by immature TLS [78]. Similarly, during the progression from intraductal papillary mucinous neoplasm (IPMN) to invasive PDAC, high-grade IPMN exhibits a relatively uniform clonal structure, whereas invasive PDAC demonstrates more pronounced clonal heterogeneity and a higher proportion of CAFs. The two entities also differ in immune cell composition, with invasive PDAC showing an increased proportion of M2 macrophages, accompanied by enhanced spatial interactions among tumor, immune, and stromal compartments [79]. When the disease progresses to metastasis

sis, significant differences emerge between primary and metastatic lesions. Primary tumors exhibit pronounced desmoplasia and fibrosis, characterized by enrichment of myofibroblastic cancer-associated fibroblasts (myCAFs), regulatory T cells, and tumor-associated macrophages. This stromal remodeling is accompanied by T-cell infiltration and upregulation of immune checkpoint pathways. In contrast, metastatic lesions exhibit reduced CAF infiltration, a higher proportion of malignant cells, and enrichment of cell cycle-related pathways. Primary and metastatic lesions also differ in glycolytic activity and amino acid metabolism. Multiple ecotypes may coexist within the same patient, reflecting pronounced spatial heterogeneity [80].

Spatial omics studies further demonstrate that different treatment strategies remodel the tumor microenvironment at the levels of cellular composition, functional states, and cell–cell interactions. Following treatment of PDAC patients with the KRAS G12D inhibitor MRTX1133, tumor regions exhibit upregulation of IFN γ signaling and MHC class II antigen-presentation genes, accompanied by increased recruitment of CD8 $^{+}$ T cells and reduced neutrophil infiltration, indicating a shift in the local immune microenvironment from a suppressive to an activated state [44]. Following neoadjuvant chemotherapy, tumor cell abundance decreased, whereas ligand–receptor communication between CAFs and tumor cells, particularly IL-6 signaling, was enhanced and may have contributed to chemoresistance [76]. Moreover, although global gene expression differences between neoadjuvant-treated and surgery-only groups were not marked, favorable responders in the neoadjuvant group exhibited upregulation of *MFAP4* and *EGR3*, stronger B- and T-cell-associated signals, and higher CD3/CD4/CD8 infiltration [71].

Spatial subtyping studies also report an increase in neural progenitor-like and neuroendocrine-like malignant programs after treatment. Multicellular community analysis identified treatment-enriched communities composed of specific malignant programs, CAF subtypes, and CD8 $^{+}$ T cells. Different treatment regimens produce distinct spatial and immunological patterns: chemoradiotherapy plus losartan (CRTL) samples show a higher proportion of CD8 $^{+}$ T cells and greater effector molecule expression than chemoradiotherapy (CRT), whereas immunomodulatory CAF programs are more prominent in CRT [81]. Beyond these treatment-associated changes, spatial and

single-cell analyses of resistant tumors reveal enrichment of specific cell subsets. In gemcitabine-resistant PDAC, PRRX2 $^{+}$ epithelial cells and SPP1 $^{+}$ TAMs are significantly enriched and co-localize in the tumor core, exhibiting evident intercellular communication. High PRRX2 expression is associated with migration, invasion, and drug resistance, whereas PRRX2 downregulation reduces proliferation, migration, invasion, and gemcitabine resistance [82]. Similarly, in tumors resistant to FOLFIRINOX or gemcitabine plus nab-paclitaxel, fibroblast subsets exhibit the most pronounced changes: inflammatory CAFs (iCAFs) increase approximately threefold compared with untreated tumors, whereas myCAF abundance remains comparable across treatment groups [30].

In summary, spatial omics studies have revealed the structural complexity and regional organization of the PDAC tumor microenvironment across multiple levels. Tumor cell subtypes, immune cells, and CAFs exhibit distinct spatial distributions within the tissue, accompanied by corresponding differences in their functional states. Following therapeutic intervention, the spatial localization and interactions of specific cell populations are altered, including immune cell redistribution, enhanced CAF–tumor communication, and enrichment of distinct tumor programs.

5. Adjunctive Therapeutic Approaches

The TME not only exhibits immune dysregulation but also displays complex tissue architectural features. Collectively, these features influence immune cell infiltration and modulate the overall therapeutic response. In recent years, increasing attention has focused on adjunctive therapeutic strategies that act locally within tumors to enhance treatment efficacy by remodeling the TME, augmenting local immune responses, and improving drug delivery. A range of adjunctive strategies is being developed, including local immune activation, optimized drug delivery, and novel delivery systems designed to enhance drug accumulation at tumor sites while reducing systemic toxicity. In addition, modulation of tumor tissue architecture to enhance the infiltration and penetration of therapeutic agents and immune cells into tumors has emerged as an important area of research. These strategies may help overcome multiple barriers within the TME and improve the efficacy of antitumor therapy (Figure 2).

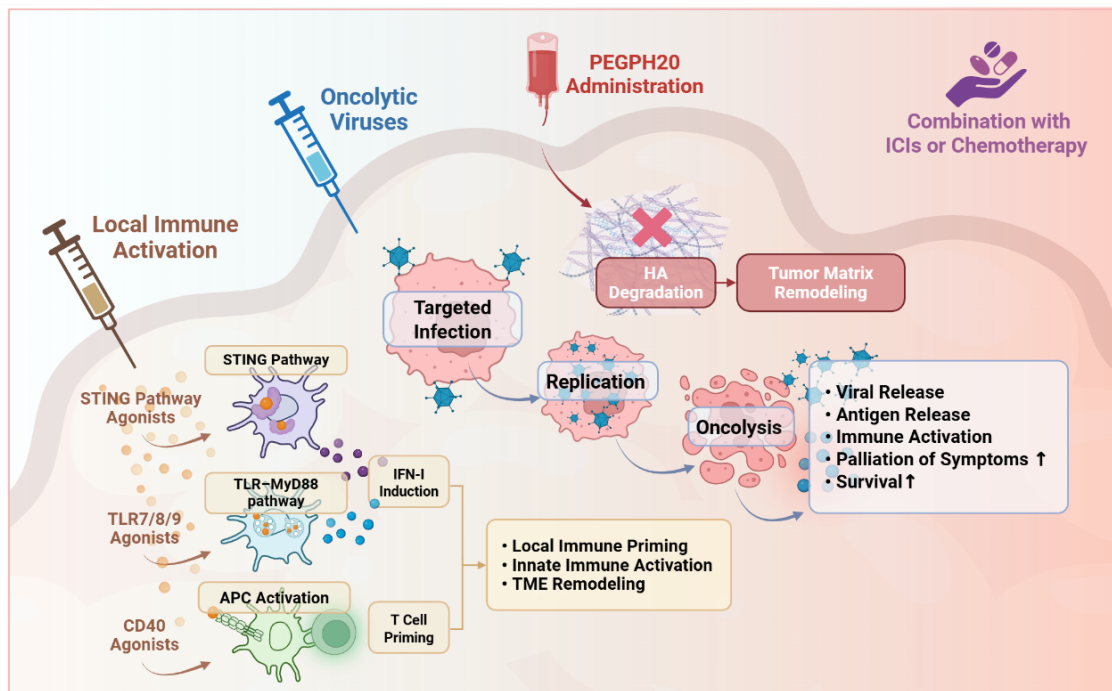


Figure 2: Schematic illustration of representative adjunctive strategies used to enhance therapeutic responses by remodeling the TME. Oncolytic viruses promote immune activation, alleviate tumor-related symptoms, and improve survival outcomes. Local immune activation strategies, including STING pathway agonists, TLR7/8/9 agonists, and CD40 agonists, can induce local immune priming, activate innate immunity, and drive tumor TME remodeling. Structural modulation via HA degradation (e.g., PEGPH20) reduces physical barriers and improves drug delivery. These effects help transform immunologically “cold” tumors into a more inflammatory and responsive state, and provide a mechanistic basis for their rational combination with ICIs or chemotherapy. The detailed mechanisms are elaborated in the main text. Created with <https://BioRender.com>.

5.1. Oncolytic Viruses

Oncolytic viruses are engineered to selectively replicate in tumor cells while minimizing toxicity to non-tumor tissues. Adenoviruses are among the most commonly used oncolytic viral platforms [83]. Oncolytic viruses exert antitumor effects through direct oncolysis, apoptosis, autophagy, and activation of innate and adaptive immune responses [84]. Moreover, genetically engineered oncolytic viruses can integrate multiple functional modules while retaining the ability to activate the immune system, recruit antitumor T cells, and modulate the TME [85,86]. A study developed a replication-selective oncolytic adenovirus, ICOVIR17, engineered to express the hyaluronidase PH20. This construct demonstrated enhanced antitumor activity in murine xenograft models of human melanoma, irrespective of intratumoral or intravenous administration. A single dose induced tumor regression in approximately 60% of cases; however, overall responses were predominantly partial, with complete regression occurring rarely. The authors further noted that the mouse-derived tumor stroma may restrict viral dissemination within tumors, thereby limiting therapeutic efficacy [87]. In subsequent studies, the application of this

technology in glioblastoma (GBM) models was further optimized. ICOVIR17 not only enhanced intratumoral viral dissemination via hyaluronidase-mediated hyaluronic acid degradation but also significantly increased infiltration by CD4+ and CD8+ T cells and macrophages. In addition, it upregulated PD-L1 expression and prolonged survival in mice. Notably, combination therapy with anti-PD-1 antibodies resulted in long-term remission in a subset of animals [88].

Oncolytic virus technology has also advanced into clinical evaluation. In a phase III randomized clinical trial involving patients with unresectable advanced melanoma, intratumoral administration of talimogene laherparepvec demonstrated superior clinical responses compared with subcutaneous GM-CSF, including improved median survival, durable response rate, objective response rate, and complete response rate [89]. In a phase II clinical trial (NCT04771676) evaluating the oncolytic adenovirus H101 in patients with malignant ascites, significant tumor lysis was observed, along with expansion of CXCR6+ and GZMK+ CD8+ T cells, enhanced immune checkpoint interactions between CD8+ T cells and macrophages, and prolonged ascites control duration [90]. Clinical trials evaluating oncolytic viruses in

pancreatic ductal adenocarcinoma (PDAC) remain at an early developmental stage. In a phase I trial involving a small cohort of PDAC patients, the oncolytic adenovirus Ad5-yCD/mutTKSR39rep-hIL-12 combined with 5-FC followed by chemotherapy was overall safe and tolerable; in the highest-dose group, some patients showed prolonged survival and disease stabilization, accompanied by increased serum IL-12, IFNG, and CXCL10 [91]. This regimen was primarily designed to assess safety and immunological changes, and its results provide only early clinical evidence of oncolytic virus activity in PDAC. A separate clinical trial evaluating LOAd703 in patients with pancreatic ductal adenocarcinoma (PDAC) reported comparable preliminary findings [92]. These early findings suggest the potential utility of oncolytic virus therapy for PDAC, although further clinical validation is required.

This treatment approach also presents important limitations. For instance, an *in vitro* study has demonstrated that pancreatic ductal adenocarcinoma (PDAC) cells exhibit substantial resistance to vesicular stomatitis virus (VSV). This resistance was attributed to multiple mechanisms, including reduced viral attachment efficiency and activation of intracellular antiviral signaling, particularly the interferon (IFN)–JAK–STAT pathway, which suppresses viral replication. Notably, even with the use of JAK inhibitors, viral infection and oncolytic efficacy were only partially enhanced [93]. Although replicating oncolytic viruses can induce tumor regression *in vivo*, late-stage tumor cells may retain resistance to viral infection. In addition, viral dissemination to non-target tissues indicates that long-term therapeutic outcomes may be unstable, underscoring the need for rigorous biosafety evaluation [94].

5.2. Local Immune Activation Strategies

Local immune activation strategies remodel the TME by activating innate immune sensors and costimulatory pathways.

5.2.1. STING Pathway Agonists

Activation of the stimulator of interferon genes (STING) pathway in tumor-resident DCs induces type I interferon production and initiates adaptive antitumor immune responses [95]. Intratumoral injection of STING agonists induces marked tumor regression across multiple murine tumor models and elicits systemic immune responses capable of eliminating distant metastases while establishing long-term immunological memory [96]. In clinical trials, intratumoral administration of the STING agonist

MIW815 in patients with advanced solid tumors demonstrated a favorable safety profile but limited efficacy as monotherapy. Although peripheral immune activation was observed, the induction of a sustained and effective intratumoral antitumor immune response remained challenging [97]. In a phase II study involving patients with untreated metastatic or unresectable recurrent head and neck squamous cell carcinoma (HNSCC), intratumoral administration of ulevostinag, a cyclic dinucleotide STING pathway agonist, was evaluated as monotherapy and in combination with pembrolizumab. Among 8 patients in the combination group, 4 achieved complete or partial responses, whereas only 1 of 10 patients in the monotherapy group responded, indicating preliminary antitumor activity. However, these findings require validation in larger cohorts to assess response durability and identify potential predictive biomarkers [98]. Additionally, multiple clinical trials evaluating STING agonists are ongoing; however, detailed results have not yet been published. For example, SB-11285, TAK-676, and GSK3745417 are currently being investigated in phase I or phase I/II clinical studies [44,79,80].

5.2.2. TLR7/8/9 Agonists

Toll-like receptors (TLRs) 7, 8, and 9 are endosomal receptors that activate innate immunity by recognizing viral, pathogen-derived, or damage-associated nucleic acids, thereby representing important targets for immunotherapeutic strategies [99,100]. In melanoma murine models, TLR7/8 agonism induces Th1 polarization, promotes the enrichment and activation of NK cells and CD8⁺ T cells, and suppresses tumor growth. Furthermore, combination of TLR7/8 agonists with T cell–targeted immunotherapy enhances antitumor efficacy [101]. In murine models of colon cancer and melanoma, combined administration of TLR7/8 and TLR9 agonists increases tumor-infiltrating CTLs and NK cells and enhances their cytotoxic activity while reducing the frequency of immunosuppressive MDSCs. This results not only in the eradication of large primary tumors but also in the establishment of durable protective immunity [102]. In a neoadjuvant clinical trial for early-stage oral squamous cell carcinoma, local application of the TLR7 agonist imiquimod induced an immune-related major pathological response in 60% of patients and showed favorable recurrence-free survival [103]. In a phase I/II trial involving recurrent breast cancer, local administration of the TLR7 agonist imiquimod in combination with radiotherapy induced local and systemic tumor responses in a subset of patients and demonstrated an acceptable safety and tolerability profile [11].

5.2.3. CD40 Agonists

CD40 is a member of the TNF receptor superfamily and is expressed on both immune and non-immune cells [104]. CD40 signaling activates dendritic cells (DCs), thereby enhancing T cell priming against tumor antigens [105]. In addition, CD40 acts on tumor-associated macrophages (TAMs) within the tumor stroma rather than directly on tumor cells [106], indicating that it modulates the tumor microenvironment (TME) through immune remodeling. In vitro and animal studies have demonstrated that the CD40 agonist KHK2840 potently activates CD40 signaling. When combined with anti-PD-1 antibodies and paclitaxel, it enhances antitumor immune responses involving both the tumor microenvironment (TME) and tumor-draining lymph nodes, while exhibiting favorable tolerability in non-human primate toxicology studies [107]. In a clinical study involving advanced solid tumors, intratumoral administration of the CD40 agonist antibody ADC-1013 demonstrated an acceptable safety profile, accompanied by peripheral B cell activation and pharmacodynamic responses [108]. CD40 continues to demonstrate translational potential; in patients with metastatic pancreatic ductal adenocarcinoma (PDAC), the CD40 agonist APX005M (sotigalimab), in combination with gemcitabine and nab-paclitaxel (with optional nivolumab), showed an acceptable safety profile and evidence of clinical activity, with tumor response signals observed in 58% of patients in dose-escalation cohorts [109].

Collectively, STING, TLR7/8/9, and CD40 agonists have demonstrated the potential to activate local innate immunity, enhance antitumor responses, and prolong immune memory in preclinical models and early clinical studies. Beyond these three classes, additional innate immune pathways and costimulatory molecules may also serve as novel targets for local immune activation strategies [110–112], suggesting broad therapeutic potential for the treatment of immunologically “cold” tumors.

5.3. Optimization of Administration Strategies and Delivery Platforms

5.3.1. Intratumoral Injection

The concept of intratumoral injection dates back to 1891, when William B. Coley first administered bacterial preparations to treat unresectable sarcomas and observed partial tumor regression [113]. Modern strategies aim to activate local innate immune and costimulatory pathways through intratumoral injection, converting “immune cold” tumors into inflamed phenotypes. This approach can be combined with immune checkpoint blockade (ICB) to en-

hance antitumor immunity and improve therapeutic efficacy [82,95].

In a phase I study, endoscopic ultrasound (EUS)-guided intratumoral injection of the oncolytic virus HF10 combined with erlotinib and gemcitabine in unresectable advanced pancreatic cancer demonstrated the clinical feasibility and safety of this local administration route [114]. A phase 0 clinical study employed Comparative In Vivo Oncology (CIVO) to administer subasumstat (TAK-981) directly into tumors of 12 patients with head and neck cancer. Following tumor resection, spatial transcriptomic and single-cell analyses revealed local inhibition of the SUMO pathway and activation of type I interferon responses, accompanied by a shift of the tumor microenvironment (TME) toward an immune-permissive state [115]. A window-of-opportunity study assessed intratumoral injection of Copaxone® in patients scheduled for curative surgery with percutaneously accessible tumors. The treatment was associated with minimal injection-site adverse effects and induced upregulation of immune-related genes, including pathways linked to response to immune checkpoint blockade (ICB) therapy [116].

Collectively, these clinical studies demonstrate the feasibility and safety of intratumoral injection, as well as its capacity to activate the local immune microenvironment. They further indicate the potential to convert immune-cold tumors into inflamed phenotypes and provide a clinical rationale for combination with immune checkpoint blockade (ICB).

5.3.2. Nanoparticle Modification

Liposomal formulation is a mature and clinically translated anticancer drug delivery strategy widely used in cancer therapy [99,100], for example, Doxil/Caelyx [117], Vyxeos (CPX-351) [118], and Onivyde (NAL-IRI, liposomal irinotecan) [119]. Liposomal modification can improve pharmacokinetics and biodistribution, enhance tumor targeting, and reduce toxicity to normal tissues [103, 104].

Protein-based delivery strategies have been translated from basic research into clinical applications, as exemplified by antitumor agents such as albumin-bound paclitaxel (Abraxane) [120]. In recent years, this technology has also been applied to the development of immunotherapeutic drugs. For example, serum albumin-based nanoparticles functionalized with dual PD-1/PD-L1 aptamers can simultaneously target T cells and tumor cells, enhancing the antitumor effects of PD-1/PD-L1 immune blockade in vitro and in vivo without increasing systemic toxicity in a colorectal cancer animal model [121]. The STING agonist SR-717, when encapsulated in human serum albumin

(HSA)-based protein cages (SH-NPs), exhibits enhanced stability, efficient cellular uptake, superior tumor accumulation, favorable tolerability, and reduced systemic toxicity [122].

In addition, numerous studies have developed anticancer drug-delivery systems based on inorganic nanoparticles, including metal, silica, and iron oxide carriers, to encapsulate or modify chemotherapeutic drugs or immunomodulators and combine imaging, targeted release, and therapeutic functions [123,124].

5.3.3. Targeting Tumor-Stromal Hyaluronic Acid (HA) to Improve Drug Delivery

As previously mentioned, the connective tissue-rich proliferative matrix, vascular compression, and hypoperfusion collectively constitute the physiological barrier characteristics of PDAC. These features are considered key contributors to its reduced sensitivity to systemic therapies [63]. Therefore, targeting extracellular matrix components may enhance antitumor therapy by reducing interstitial pressure and improving drug delivery.

Both preclinical and clinical studies have supported this concept. In an orthotopic mouse model of pancreatic ductal adenocarcinoma (PDAC), administration of pegvorhyaluronidase alfa (PEGPH20), an enzyme that degrades hyaluronic acid in the tumor matrix, resulted in a rapid reduction in interstitial fluid pressure within hours, approaching levels observed in normal pancreatic tissue within 24 hours. PEGPH20 treatment also increases vessel diameter, promoting the formation of widely patent and functional blood vessels. When PEGPH20 was combined with gemcitabine, tumor volume was reduced and median overall survival was improved compared with gemcitabine monotherapy [125]. In a phase II randomized clinical trial, PEGPH20 combined with nab-paclitaxel and gemcitabine improved progression-free survival compared with chemotherapy alone in patients with metastatic pancreatic ductal adenocarcinoma. The greatest benefit was observed in patients with high hyaluronic acid-expressing tumors [126]. A phase III randomized, double-blind, placebo-controlled trial further evaluated the efficacy of PEGPH20 combined with nab-paclitaxel and gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma with high HA expression. In the study, the addition of PEGPH20 improved the objective response rate compared to chemotherapy alone. However, PEGPH20 did not improve overall survival or progression-free survival in this trial [127]. Similarly, a multicenter phase II study evaluating PEGPH20 in combination with pembrolizumab in patients with metastatic PDAC exhibiting high hyaluronic acid expression and resistance to

prior therapy did not demonstrate significant clinical efficacy [128].

Overall, these studies suggest that targeting HA in the tumor matrix can modulate the TME and influence drug delivery; however, clinical efficacy has been inconsistent. This highlights the need for more refined and systematic approaches that incorporate spatial structural features to further optimize intratumoral delivery strategies.

6. Discussion

Spatial omics techniques reveal fundamental differences in the spatial organization of the TME between cHL and PDAC, rather than a shared uniform immunosuppressive pattern [30,34]. cHL is characterized by the coexistence of inflammatory infiltration and immunosuppression. Hodgkin and Reed–Sternberg (HRS) cells, although rare, shape the functional states of surrounding immune cells through distinct spatial microenvironments, including PD-L1–enriched regions, MHC-I–positive antigen-presenting niches, and CD137–associated immunoregulatory foci [34,43,55]. CD4⁺ T cells form distinct “rosette”-like niches around HRS cells and express OX40, PD-1, and CTLA-4, indicating close spatial proximity and enrichment of immune checkpoint molecules within the TME [52]. In PDAC, the spatial organization is characterized by physical compartmentalization and functional exclusion of immune cells. Effector T cells and dendritic cells (DCs) are largely confined to stromal regions or excluded to the tumor periphery [35], whereas the tumor core is enriched with regulatory T cells (Tregs), M2-polarized tumor-associated macrophages (TAMs), and myofibroblastic cancer-associated fibroblasts (my-CAFs) [37], collectively establishing the structural basis of an “immune desert” phenotype. The mechanisms of spatial immune inhibition differ between tumor types: in cHL, immune cells are highly infiltrated yet functionally impaired [32], whereas in PDAC, immune cells are physically excluded by spatial barriers [35].

Cell–cell interactions (CCIs) are prevalent in the TME, and elucidating their complexity is essential for advancing cancer treatment strategies, including the modulation and prediction of therapeutic responses [129]. Although immune-suppressive foci involving FAP⁺ CAFs, TIM-3⁺ macrophages, and Tregs have been identified in cHL [36,43], and PD-L1⁺ HRS cell regions are associated with T-cell depletion in adjacent areas [34], comprehensive spatial colocalization data and definitive evidence of ligand–receptor interactions among CAF–TAM–checkpoint pathways remain limited [34], comprehensive spatial colocalization and definitive evidence of ligand–receptor interactions among CAF–TAM–checkpoint path-

ways remain lacking. In PDAC, relatively well-defined spatially organized cell–cell interactions (CCIs) have been observed: myCAFs co-localize with basal-like tumor cells to form a matrix barrier [37]; IL-1 β ⁺ TAMs are enriched in regions of high fibroblast density [13]; and checkpoint pathways such as TIGIT–NECTIN establish localized inhibitory networks involving Tregs and exhausted T cells at the tumor invasion front [30]. In addition, inflammatory cancer-associated fibroblasts (iCAFs) have been found to be significantly increased in post-treatment pancreatic ductal adenocarcinoma (PDAC) and are associated with chemotherapy resistance [76]. Therefore, although both contexts involve spatial cooperation among CAFs, TAMs, and tumor cells, the cellular composition, spatial organization, and functional characteristics of these interactions are more clearly defined in PDAC. In contrast, the spatial immune regulatory mechanisms mediated by mesenchymal cells in classical Hodgkin lymphoma (cHL) remain to be further elucidated.

Existing studies indicate that cHL and PDAC do not represent different forms of the same spatial mechanism, but rather two distinct spatial organization strategies. Despite high immune cell infiltration [21], spatial analyses indicate that immune dysfunction in classical Hodgkin lymphoma (cHL) arises from functional suppression in adjacent regions. Hodgkin and Reed–Sternberg (HRS) cells impair the activity of spatially neighboring immune cells through PD-L1⁺ enriched regions, CD137⁺ niches, and CAF-associated immunosuppressive foci [43]. In PDAC, physical barriers mediate immune exclusion, including the matrix barrier formed by myCAFs and tumor cells [37], the compartmentalized distribution of TAMs at the tumor–matrix interface [13], and hypoxia-driven metabolic remodeling [33], all of which restrict immune-cell infiltration into the tumor core. A key difference is that immune inhibition in cHL occurs in the context of spatial proximity, whereas immune exclusion in PDAC is driven by spatial separation. This distinction has important therapeutic implications. Classical Hodgkin lymphoma (cHL) may benefit from strategies aimed at restoring the function of spatially proximal immune cells, whereas pancreatic ductal adenocarcinoma (PDAC) requires approaches that first disrupt the stromal matrix barrier to facilitate immune cell infiltration.

Current adjuvant therapeutic strategies, including oncolytic viruses, local immune agonists, and drug delivery systems, can activate antitumor immunity and enhance intratumoral drug accumulation. However, their capacity to remodel the spatial architecture of the tumor microenvironment remains limited. Oncolytic viruses activate both innate and adaptive immune responses through direct tumor cell lysis and the induction of immunogenic cell

death [88]; however, the dense stromal barrier constrains their distribution within solid tumors. Even with treatment using the modified virus ICOVIR17, which expresses hyaluronidase, the tumor stroma in mouse models continues to limit intratumoral viral spread [87]. The resistance of PDAC cells to VSV is also partly attributable to intrinsic antiviral signaling pathways [93]. Local immune agonists, including STING, TLR7/8/9, and CD40 agonists, can induce type I interferon responses at the injection site and modulate the immune microenvironment [101]; however, their effects remain spatially restricted. These agents alone may be insufficient to establish sustained and effective antitumor immune responses within tumors [97]. Nanodrug delivery systems and matrix-targeting approaches, such as PEGPH20, can enhance intratumoral drug penetration and reduce interstitial pressure [125]. However, phase III clinical trials did not demonstrate significant improvements in overall survival or progression-free survival in patients with metastatic pancreatic ductal adenocarcinoma (PDAC) exhibiting high hyaluronic acid expression [127]. This suggests that improving drug accessibility alone is not sufficient to overcome the spatially organized immunosuppressive structure of the tumor microenvironment. In other words, current strategies primarily focus on activating immune responses or enhancing drug delivery but may lack the capacity to directly disrupt or reorganize spatially defined immunosuppressive niches within the TME. For example, in cHL, PD-L1⁺/CD137⁺ immunosuppressive zones are located adjacent to Hodgkin and Reed–Sternberg (HRS) cells; similarly, in pancreatic ductal adenocarcinoma (PDAC), immune barriers are formed by myofibroblastic cancer-associated fibroblasts (myCAFs) and basal-like tumor cells. Future therapeutic approaches should target inhibitory niches identified through spatial omics studies and develop interventions capable of penetrating, remodeling, or eliminating these spatially defined structures within the TME.

7. Conclusions

This review summarizes recent advances in the application of spatial omics technologies to the study of the TMEs of cHL and PDAC. It further highlights fundamental differences in spatial organization between these tumor types, as well as distinct patterns of immune cell distribution. At the therapeutic level, although existing strategies can activate immune responses and enhance drug delivery, they may not effectively remodel established spatially inhibitory structures. Future research should focus on key spatial structures identified through spatial omics studies and develop therapeutic strategies capable of modulating these tissue features, thereby enabling a transition from de-

scriptive spatial analysis to spatially informed therapeutic intervention.

List of Abbreviations

ABVD	Adriamycin, Bleomycin, Vinblastine, Dacarbazine
AHCT	Autologous Stem Cell Transplantation
BCR	B-Cell Receptor
BMP2	Bone Morphogenetic Protein 2
CAF	Cancer-Associated Fibroblast
CCIs	Cell–Cell Interactions
CD40	Cluster of Differentiation 40
cDC2	Conventional Dendritic Cell Type 2
cHL	Classical Hodgkin Lymphoma
CIVO	Comparative In Vivo Oncology
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
CXCR	C-X-C Chemokine Receptor
DAMP	Damage-Associated Molecular Pattern
DC	Dendritic Cell
DSP	Digital Spatial Profiler
EBV	Epstein–Barr Virus
ECM	Extracellular Matrix
EMT	Epithelial–Mesenchymal Transition
FFPE	Formalin-Fixed Paraffin-Embedded
GVD	Gemcitabine, Vinorelbine, Doxorubicin
HA	Hyaluronic Acid
HNSCC	Head and Neck Squamous Cell Carcinoma
HRS	Hodgkin and Reed–Sternberg Cells
ICI	Immune Checkpoint Inhibitor
ICB	Immune Checkpoint Blockade
IDO	Indoleamine 2,3-Dioxygenase
IFN	Interferon
IL	Interleukin
iCAF	Inflammatory Cancer-Associated Fibroblast
JAK	Janus Kinase
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
LAG-3	Lymphocyte-Activation Gene 3
MDSC	Myeloid-Derived Suppressor Cell
MHC	Major Histocompatibility Complex
myCAF	Myofibroblastic Cancer-Associated Fibroblast
NALIRI	FOX Nanoliposomal Irinotecan, Leucovorin, Fluorouracil, Oxaliplatin
NK	Natural Killer Cell
NLPHL	Nodular Lymphocyte-Predominant Hodgkin Lymphoma
OX40	Tumor Necrosis Factor Receptor Superfamily Member 4

PAMP	Pathogen-Associated Molecular Pattern
PD-1	Programmed Cell Death Protein 1
PDAC	Pancreatic Ductal Adenocarcinoma
PD-L1	Programmed Death-Ligand 1
PGE2	Prostaglandin E2
STAT	Signal Transducer and Activator of Transcription
STING	Stimulator of Interferon Genes
TAM	Tumor-Associated Macrophage
TCR	T-Cell Receptor
TGF- β	Transforming Growth Factor Beta
TIGIT	T Cell Immunoreceptor with Ig and ITIM Domains
TIM-3	T Cell Immunoglobulin and Mucin Domain-Containing Protein 3
TLR	Toll-Like Receptor
TME	Tumor Microenvironment
TNF	Tumor Necrosis Factor
TNFRSF9	Tumor Necrosis Factor Receptor Superfamily Member 9
Treg	Regulatory T Cell

Author Contributions

The author is solely responsible for conceptualization, investigation, data curation, writing-original draft, writing-review & editing, and visualization. The author has read and agreed to the published version of the manuscript.

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The author declares no conflicts of interest. The Guest Editor is the academic supervisor of the author. To ensure transparency and avoid any potential conflict of interest, the Guest Editor was not involved in the peer-review process, editorial assessment, or decision-making for this manuscript. The manuscript was handled independently by an alternate editor.

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AI Declaration

During manuscript preparation, the GPT-5 mini model of ChatGPT was used solely for language polishing and

grammar correction. After using this tool/service, the author reviewed and edited the content as necessary and takes full responsibility for the content of the publication.

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