GenoMed Connect
ISSN: 3105-3645
2026, Article ID. 2026.0026, Cite as: https://doi.org/10.69709/GenomC.2026.155654

SCIFINITI PUBLISHING

Review Article

OPEN ACCESS

Spatial Profiling of the Head and Neck Squamous Cell Carcinoma Microenvironment: Reshaping Our Understanding and Therapeutic Opportunities

Yu Liu[✉,1] **Xiaotong You**[✉,1] **Chuzi Mo**[✉,1] **Xiaohu Hao**[✉,2,3,*]

[1] Faculty of Dentistry, The University of Hong Kong, Sai Ying Pun, Hong Kong 999077, Hong Kong
[2] Department of Thoracic Surgery and Institute of Thoracic Oncology, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China
[3] Western China Collaborative Innovation Center for Early Diagnosis and Multidisciplinary Therapy of Lung Cancer, Chengdu, Sichuan 610041, China

Article History
Received: November 22, 2025 Accepted: May 30, 2026 Published: June 12, 2026

Abstract
Head and neck squamous cell carcinoma (HNSCC) is characterized by a highly heterogeneous tumor microenvironment (TME), which plays a critical role in disease progression and therapeutic resistance. The emergence of spatial omics and multimodal imaging approaches, including spatial transcriptomics, proteomics and metabolomics, has revolutionized the understanding of the tumor microenvironment (TME) by preserving the spatial architecture of molecular landscapes. This review synthesizes key advances enabled by spatial omics in the HNSCC tumor microenvironment (TME), highlighting the spatial organization of malignant cell states, the functional architecture of immune cell populations (e.g., tertiary lymphoid structures versus immunosuppressive stromal niches), and the critical contributions of heterogeneous cancer-associated fibroblasts (CAFs) and aberrant tumor vasculature. Spatially resolved intercellular communication networks that mediate resistance to immunotherapy and targeted therapies are further examined. Finally, current methodological limitations are discussed, along with the transformative potential of integrating artificial intelligence with spatial omics data to enhance patient stratification and facilitate the development of personalized therapeutic strategies.

Keywords:
head and neck squamous cell carcinoma; tumor microenvironment; multi-omics technologies; cancer-associated fibroblasts; intercellular communication networks

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) comprises a group of malignant tumors originating from the oral cavity, pharynx, larynx, and other sites within the head and neck region, and is characterized by marked molecular heterogeneity and high invasiveness [1]. The disease is characterized by persistently high rates of locoregional recurrence or distant metastasis, and the long-term survival rate is still stagnant despite the adoption of multi-modal treatment [2–4]. An increasing body of evidence indicates that the tumor microenvironment (TME)—comprising the diverse cellular and non-cellular components surrounding the tumor—is a critical regulator of disease pathogenesis. Its composition and functional state

* **Corresponding Author:**
Xiaohu Hao, Department of Thoracic Surgery and Institute of Thoracic Oncology, West China Hospital, Sichuan University, Chengdu 610041, China, Western China Collaborative Innovation Center for Early Diagnosis and Multidisciplinary Therapy of Lung Cancer, Chengdu 610041, China, mcz878mcz@connect.hku.hk

directly influence tumor progression, immune evasion, and therapeutic response [5–7]. The HNSCC TME is a highly dynamic, multicellular ecosystem comprising malignant epithelial cells, heterogeneous immune cell populations, cancer-associated fibroblasts (CAFs), vascular networks, and diverse extracellular matrix (ECM) components. Together, these elements form a complex and interactive milieu that exhibits substantial intertumoral and intratumoral heterogeneity [8–10].

The past decade has witnessed a revolution in our understanding of TME heterogeneity with the advent of spatial omics technologies. These approaches include spatial transcriptomics [e.g., Xenium (10x Genomics), CosMx SMI and RNAscope], spatial proteomics [e.g., GeoMx DSP, imaging mass cytometry (IMC) and deep visual proteomics], multiplexed imaging platforms [e.g., PhenoCycler-Fusion (PCF; formerly CODEX) and multiplex immunofluorescence (mIF)] and emerging spatial metabolomics methods. Together, they have transformed the study of TME heterogeneity. By preserving tissue architecture while providing molecular information at near-single-cell resolution, these technologies enable comprehensive characterization of the spatial organization and functional states of cells within the tumor microenvironment [11–15]. As shown in Table 1, each technology class offers distinct advantages: transcriptomics platforms reveal gene expression patterns, proteomics methods characterize functional effector proteins, metabolomics imaging captures the distribution of small molecules, and emerging epigenomics technologies map regulatory landscapes. These advances enable the precise localization of cellular subtypes, including diverse malignant, immune, and stromal cell populations, and reveal how they are spatially organized within distinct functional microenvironments, such as immune-excluded niches, immune "cold" regions, and hypoxic regions [16–18]. Unlike traditional single-cell approaches, spatial omics provides critical insights into the spatial relationships and interactions among different cell subtypes, which are essential for understanding tumor progression, immune evasion and therapeutic resistance [19]. The integration of spatial and molecular data enables the reconstruction of intercellular communication networks within tumors, providing a comprehensive understanding of the dynamic tumor ecosystem.

Table 1: Overview of spatial multi-omics technologies.

Omics Layer	Core Technology/ Platforms	Molecules Detected	Spatial Resolution	Key Advantages	Major Challenges
Spatial Transcriptomics	Visium/Xenium (10x Genomics), CosMx SMI (NanoString), BGI Stereo-seq, MERFISH, RNAscope	Whole transcriptome or targeted RNAs (100 s–1000s)	Spot-level (~55 μm) to subcellular	Unbiased discovery of gene expression programs and cell states	Lacks protein information, cell phenotype often requires inference
Spatial Proteomics	IMC, PCF, GeoMx DSP, Deep visual proteomics, mIF, AKOYA PCF	Proteins (10 s–100 s)	Single-cell to subcellular	Direct detection of functional effectors, strong clinical pathology link	Limited by antibody availability, unable to discover novel targets
Spatial Metabolomics	MALDI-MSI, DESI-MSI	Metabolites, lipids, drugs (100 s)	~10–50 μm	Label-free detection of functional phenotypes	Difficult metabolite identification, incomplete databases
Spatial Epigenomics	Spatial-ATAC-seq	Chromatin accessibility, histone modifications	Currently low, improving	Reveals regulatory mechanisms of gene expression	Technically immature, fewer applications
Integrated Multi-Omics	CosMx, GeoMx	RNA and protein simultaneously	Platform-dependent	Direct correlation of different molecular layers in the same spatial context	Complex experimental design, high computational demand for integration

2026, Vol. 3, Article ID. 2026.0026
https://doi.org/10.69709/GenomC.2026.155654

In the context of HNSCC, spatial omics offers unprecedented opportunities to elucidate the spatial determinants of tumor heterogeneity, identify microenvironments associated with disease recurrence, and develop spatially informed biomarkers for clinical stratification. This review focuses on the application of spatial omics technologies to elucidate the HNSCC tumor microenvironment, with particular emphasis on: (i) mapping cellular heterogeneity within a spatial context; (ii) characterizing intercellular communication networks and the formation of distinct microenvironmental niches; and (iii) translating spatial information into personalized precision medicine strategies. By fostering the integration of multi-omics research, computational biology, and clinical oncology, spatial oncology is expected to advance precision medicine beyond conventional molecular profiling toward spatially guided therapeutic interventions.

2. An Overview of Spatial Omics Technologies

Spatial omics encompasses a rapidly evolving suite of technologies that enables the comprehensive analysis of molecular information, including RNA, protein, and metabolite abundance, while preserving the native spatial architecture of tissue sections [20–22]. According to their basic principles, these methods can be roughly divided into two categories: imaging-based methods and sequencing-based methods [23,24].

Imaging-based technologies, such as mIF, IMC, and PCF, employ antibody- or oligonucleotide-labeled probes to simultaneously visualize dozens to hundreds of proteins or RNA molecules at subcellular resolution [25–27]. These platforms excel at characterizing cell phenotypes and states with high spatial fidelity but are limited by predefined targets. In contrast, sequencing-based and image-based transcriptomic technologies, such as 10x Genomics Visium, Xenium, CosMx SMI, and Stereo-seq, either capture polyadenylated RNA from spatially barcoded tissue regions or directly image and quantify hundreds to thousands of RNA transcripts at single-cell resolution [28,29]. These methods offer greater unbiased discovery potential for novel gene expression patterns, but vary in spatial resolution and throughput [28,29].

As each platform possesses distinct strengths and limitations, the selection of an appropriate technology should be guided by the specific objectives of the study (Table 2). Technical platform selection typically involves trade-offs among spatial resolution, multiplexing capacity, sample throughput, and discovery potential. Integrating spatial datasets with complementary approaches, such as single-cell RNA sequencing, is essential for maximizing biological insight. Computational strategies, including deconvolution and data integration methods, facilitate the inference of cell-type composition and enable a deeper understanding of the system-level mechanisms governing cellular ecosystems [16,30]. By leveraging the complementary strengths of spatial omics platforms in resolution and multiplexing capacity, researchers can move beyond simple cataloging of cellular diversity. It provides unprecedented opportunities to precisely map the cellular composition and heterogeneity within HNSCC tumor microenvironments in their native tissue context, thereby actively decoding the spatial laws that govern these complex ecosystems.

Table 2: Comparison of major sequencing-based and imaging-based spatial omics platforms.

Sequencing-Based	10x Visium	10x Xenium	10x Visium HD	CosMx SMI	Stereo-seq
Resolution	Low (~55–100 μm spots)	Subcellular (~200 nm)	High (near single cell)	Subcellular	500 or 715 nm
Throughput	Whole transcriptome	Targeted panels (100 s of genes)	Whole transcriptome	1000+ RNAs, 64+ proteins	Genome-wide
Panel Type	Whole transcriptome	Fixed/custom RNA probes	Whole transcriptome	Targeted RNA/Protein	Whole transcriptome
Strengths	Broad transcriptome survey	High-res cell-level biology, rare cell types	High-res transcriptomics + full gene coverage	Ultra-high-plex, multi-omics	Extremely high resolution & large field of view, unbiased
Limitations	Not single-cell, requires deconvolution	Targeted panel, higher cost	Larger data size, higher cost	Complex platform	Requires specialized bioinformatics expertise

Table 2: *Cont.*

Sequencing-Based	**10x Visium**	**10x Xenium**	**10x Visium HD**	**CosMx SMI**	**Stereo-seq**
Ideal for	General tissue-level trends	Cell interactions, FFPE pathology, and defined gene sets	Cell-level spatial studies with full gene coverage	High-plex, single-cell resolution mapping of both RNA and protein targets	comprehensive, cell-level maps of large tissue areas,
Imaging-Based	**CODEX**	**NanoString GeoMx DSP**	**mIF**	**MERFISH**	**seqFISH/+**
Resolution	Subcellular	50–100 μm (Region of Interest)	Single-cell	Subcellular	Subcellular
Throughput	40–60 proteins	Whole Transcriptome/100+ proteins	6–10 markers	2D: 100–1000 genes 3D: 10,000 genes	10,000+ genes
Panel Type	Protein	RNA/Protein	Protein	Targeted RNA	Targeted RNA
Strengths	Extremely high resolution, cell phenotyping	Region-of-interest flexibility, high-plex	High clinical adoption, easy integration	Extremely high sensitivity and resolution for RNA	Highest multiplexing capacity for imaging RNAs
Limitations	Antibody-dependent, complex tissue processing	ROI selection can be subjective, not single-cell	Limited plex, channel crosstalk	Requires specialized imaging, complex probe design	Long imaging times, complex data analysis
Ideal for	High-dimensional, single-cell phenotyping of protein expression	Hypothesis-driven, high-plex spatial profiling of predefined tissue regions	Validating established cellular biomarkers and spatial relationships	Mapping the precise subcellular localization of hundreds to thousands of RNA transcripts.	Near-complete transcriptome imaging at subcellular resolution.

3. Cellular Composition and Spatial Heterogeneity in HNSCC TME: Insights from Spatial Omics

Building upon the advanced capabilities of spatial omics technologies discussed in the previous section, it is now possible to gain unprecedented insights into the intricate cellular composition and spatial heterogeneity of the HNSCC tumor microenvironment.

3.1. Malignant Cell Subtypes and Their Spatial Distribution

As revealed by these high-resolution approaches, HNSCC exhibits significant heterogeneity at the cellular level, encompassing both molecular and spatial dimensions [31]. For instance, malignant epithelial cells in HNSCC do not exist only as discrete subtypes but instead occupy a continuum of phenotypic states, particularly along the epithelial–mesenchymal transition (EMT) gradient [9,10]. Spatial omics has shown that dominant malignant cell populations often retain a core epithelial phenotype, characterized by strong intercellular adhesion and high proliferative activity, and are mainly located within the tumor core (TC) [32]. Conversely, cells co-expressing epithelial and mesenchymal markers and exhibiting partial epithelial–mesenchymal transition (pEMT) features are often enriched at the invasive tumor margins [33]. This precise spatial distribution, revealed by spatial technologies, suggests that pEMT directly promotes local tissue invasion and dissemination. Moreover, malignant cells with cancer stem cell–like characteristics—often associated with EMT programs and therapeutic resistance—are not randomly distributed across the tumor [34]. They tend to cluster in specific microenvironments, such as around blood vessels or in hypoxic regions, where spatial interactions with stromal cells support their maintenance [35,36]. Therefore, the spatial organization of HNSCC, spanning the proliferative core to the invasive edge enriched with pEMT and stem cell–like populations, reflects a functional tumor ecosystem that underlies tumor progression and therapeutic resistance.

2026, Vol. 3, Article ID. 2026.0026
https://doi.org/10.69709/GenomC.2026.155654

3.2. Immune Cell Populations and Spatial Organization

The spatial organization of the immune microenvironment is a fundamental regulatory determinant of immune and therapeutic responses in HNSCC, extending beyond the mere presence of immune cells. As highlighted by Fu et al., the formation of tertiary lymphoid structures (TLS) at the tumor-stroma interface is a key structural feature that promotes the coordinated T-cell and B-cell activation and is associated with improved patient prognosis [37]. Li et al. further clarified the functional importance of TLS, suggesting that specific CD4+ T-cell populations within mature TLS may act as core regulatory factors that activate and maintain T-cell and B-cell responses in tumors, thereby providing a potential mechanistic basis for their clinical benefit [38]. Nevertheless, such antitumor immunity is often counteracted by spatially organized immunosuppressive mechanisms. In HNSCC, immune escape often arises from spatial antagonism, whereby the function of CD8+ T cells is suppressed through regulatory interactions with cancer-associated fibroblasts [39,40]. This suppression is further exacerbated by an increased spatial ratio of regulatory T cells (Tregs) to CD8+ T cells, as well as the presence of CD56^dim NK cells and M2 polarized macrophages, all of which are enriched within specific microenvironmental niches, thereby impairing effective antitumor immune responses [5,41]. Importantly, this immune microenvironment is not static; instead, it undergoes substantial spatial remodeling during disease recurrence, which can significantly diminish therapeutic efficacy. Watermann et al. confirmed that recurrent HNSCC exhibits significant alterations in the tumor immune microenvironment (TIME), characterized by a marked decrease in CD8+ T cells and B lymphocytes, along with a relative increase in neutrophils and macrophages [42]. In summary, deciphering the spatial organization of immune interactions including the localization of immunosuppressive cells relative to cytotoxic immune activity and the physical barriers that restrict T cell infiltration is critical for developing spatially informed immunotherapies capable of reprogramming the immunosuppressive HNSCC microenvironment.

3.3. Stromal Components and Their Spatial Niches

In addition to the immune components, stromal components, particularly CAFs, are key architects of the HNSCC tumor microenvironment and play a crucial role in shaping its physical structure and functional state [43]. CAFs do not constitute a single homogeneous cell population; rather, they exhibit substantial heterogeneity, with distinct subtypes occupying specific spatial niches and driving different aspects of tumor biology [44]. In HNSCC, several key CAF subpopulations have been identified, including myofibroblastic CAFs (myCAFs), which are typically located within the tumor core and contribute to the formation of a dense extracellular matrix; inflammatory CAFs (iCAFs), which are generally found at the tumor periphery and secrete cytokines and growth factors that promote inflammation and angiogenesis; and antigen-presenting CAFs (apCAFs), which can directly interact with immune cells [45]. This article comprehensively outlines the spatial and functional heterogeneity of these CAF subpopulations, which is a key resource for understanding their unique contributions to the TME (Table 3).

Table 3: Key CAF subtypes and their functions in HNSCC.

CAF Subtype	Characteristic Markers	Spatial Localization	Key Functions	Implication for Therapy
myCAF	α-SMA, FAP	Tumor Core	Deposits dense ECM, forms a physical barrier	Impedes drug penetration, chemoresistance
iCAF	IL-6, IL-11, CXCL12	Peri-tumoral/ Stromal Regions	Secretes inflammatory cytokines, recruits immunosuppressive cells	Drives T-cell exhaustion, immunotherapy resistance
apCAF	MHC-II, CD74	Immune-Rich Niches	May present antigen to CD4+ T cells; context-dependent role	Complex, potentially immunosuppressive
POSTN^{+} CAF	Periostin (POSTN)	Invasive Front	Promotes EMT, creates a pro-metastatic niche	Associated with lymph node metastasis, poor prognosis

Table 3: *Cont.*

CAF Subtype	Characteristic Markers	Spatial Localization	Key Functions	Implication for Therapy
MHC-IhiGal9$^+$ CAF	MHC-I, Galectin-9	Immunosuppressive Niche	Sequesters and disables CD8+ T cells via Galectin-9	Contributes to non-response to immunotherapy
FRC-like CAF	CCL19, CCL21	Within TLS	Supports TLS structure and function, coordinates adaptive immunity	Associated with a response to immunotherapy and a favorable prognosis

Beyond these basic classifications, emerging studies in spatial cancer research have begun to elucidate the specific roles of distinct CAF subtypes in the progression of HNSCC. For instance, Liu et al. demonstrated, through integrated single-cell and spatial transcriptomic analyses, that POSTN-positive CAFs promote cancer cell metastasis via epithelial–mesenchymal transition (EMT), thereby facilitating lymph node metastasis (LNM) in oral squamous cell carcinoma (OSCC), the major subtype of HNSCC [16]. In another spatially resolved study, Li et al. identified *IFN-induced MHC-IhiGal9*$^+$CAFs, which form an immunosuppressive microenvironment that captures CD8+ T cells and weakens their cytotoxic function in the TME [40]. Wang et al. further demonstrated the clinical significance of specific CAF subgroups, showing that SFRP2^+ CAFs were associated with enhanced tumor development and poorer survival outcomes in patients with HNSCC [46]. Overall, these findings highlight the critical role of spatially distinct CAF subtypes in driving tumor invasion and immune evasion. Therefore, analyzing the spatial and functional specialization of CAF subgroups, including their coordinated microenvironments that regulate immunosuppression, extracellular matrix remodeling, and metastasis, is essential for developing new therapies that can effectively disrupt these pathological ecosystems in HNSCC.

3.4. Vascular and Lymphatic Endothelial Cells in the TME

In HNSCC, blood and lymphatic vessels are active regulators of the tumor microenvironment and play crucial roles in immunosuppression and metastasis [47,48]. Tumor-associated endothelial cells exhibit distinct functional phenotypes characterized by altered expression of adhesion molecules, growth factor receptors, and immunomodulatory ligands [49]. Through the upregulated secretion of factors such as vascular endothelial growth factor A (VEGFA) and CXCL12, they promote the development of vascular abnormalities and increased permeability, which contribute to hypoxia and establish both physical and chemokine-mediated barriers that restrict CD8+ T cell infiltration [50,51]. Evidence suggests that lymphatic endothelial cells may also contribute to the apoptosis of activated T cells by upregulating PD-L1 and presenting FAS ligand, thereby promoting tumor immune escape. At the same time, their strategic localization at the invasive tumor margin further facilitates tumor cell dissemination to regional lymph nodes [52,53]. Recent single-cell and spatial transcriptomic studies have further revealed substantial heterogeneity among endothelial cells, identifying specialized subpopulations with distinct transcriptional programs that support processes such as angiogenesis, immunomodulation, and therapeutic resistance [54]. In summary, these findings reposition endothelial cells from passive conduits to active regulators of tumor progression, highlighting the importance of vascular normalization and targeted inhibition of endothelial immune checkpoints as potential treatment strategies for HNSCC.

The studies discussed in this section collectively demonstrate that the HNSCC tumor microenvironment is not a random mixture of cells but a highly organized ecosystem composed of specialized functional niches. To synthesize these spatial relationships, a conceptual model is proposed that maps key cellular components to their distinct topological environment (Figure 1). The figure maps key cellular components, including malignant cells, cancer stem cells, T cells, B cells, macrophages, cancer-associated fibroblasts, and associated vasculature, to their distinct topological environments within the HNSCC TME. This comprehensive spatial framework, which integrates the diverse elements discussed in Sections 3.1–3.4, lays the necessary foundation for understanding the intercellular communication network discussed in the next section.

2026, Vol. 3, Article ID. 2026.0026
https://doi.org/10.69709/GenomC.2026.155654

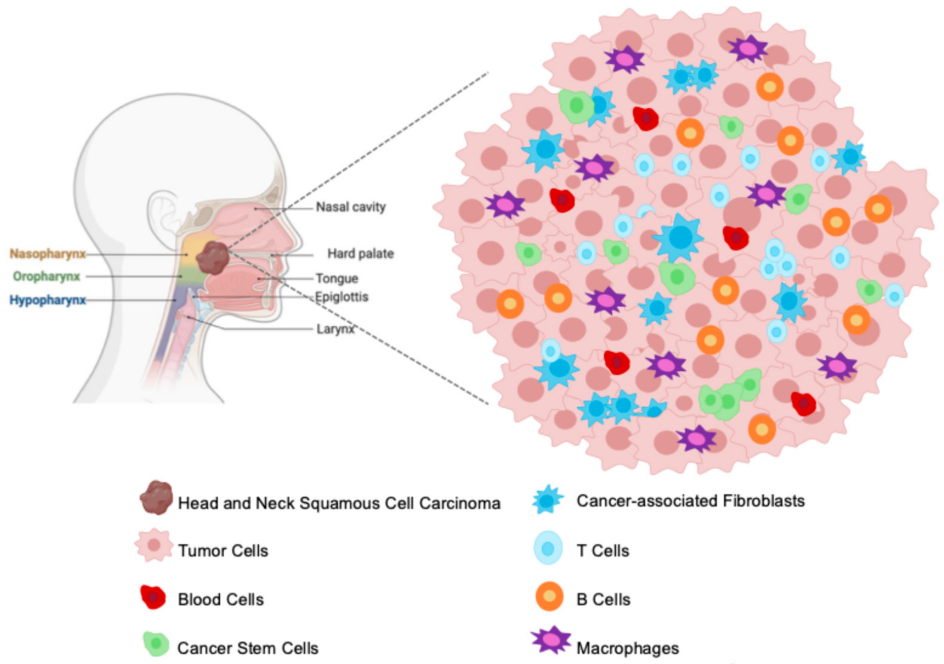

Figure 1: Spatial architecture of the HNSCC TME. This schematic provides a conceptual overview of the HNSCC TME and illustrates the spatial localization and interactions of key cellular components. The left panel shows the common anatomical sites where HNSCC tumors typically arise in the human head and neck region (e.g., nasopharynx, oropharynx, hypopharynx, tongue, larynx), with the dark brown mass representing a macroscopic HNSCC tumor. The right panel provides a conceptual schematic of the HNSCC TME at a microscopic level, showcasing the diverse cellular components that interact within this complex ecosystem. It depicts Tumor Cells (pink, spiky outline) as the predominant malignant population, intermingled with other critical cell types including Cancer Stem Cells (green), Blood Cells (red), Cancer-associated Fibroblasts (light blue stars), T Cells (light blue circles), B Cells (orange circles), and Macrophages (purple stars). This schematic emphasizes cellular diversity and the mixed distribution of components within the HNSCC TME.

4. Spatially Resolved Cell–Cell Crosstalk in HNSCC

4.1. The Spatially Organized Immune Landscape: Beyond Cold and Hot

Current spatial studies of HNSCC have refined the classic "hot" and "cold" immune classification, revealing that immune phenotype depends not only on immune-cell density but also on coordinated spatial interactions between immune cells and specific stromal components [55,56]. The "hot" immune phenotype, often represented by HPV-positive tumors, is characterized by the presence of structured TLS. Importantly, these microenvironments are enriched in CCL19-positive fibroblasts, which are spatially associated with CD4-positive T cells and B cells, thereby supporting coordinated antitumor immune responses and contributing to improved immunotherapy efficacy [56]. In contrast, the "cold" immune phenotype, more common in HPV-negative tumors, is characterized by spatial exclusion of cytotoxic T cells. This immunosuppressive environment is primarily composed of iCAF, myCAF, and proto-CAF populations, the latter characterized by low expression of canonical myCAF and iCAF marker genes. They are spatially associated with inflammatory monocytes and help form a matrix barrier, thus hindering the infiltration and function of effective T cells [56]. Therefore, the precise topology of these different CAF subtypes is the fundamental determinant of the HNSCC immune microenvironment and its clinical impact.

4.2. Stromal-Immune Interactions: Orchestrating Suppression and Exclusion

In HNSCC, CAFs actively shape the immunosuppressive microenvironment through spatially defined mechanisms. Specific CAF subtypes play specialized roles: *POSTN*^+ CAFs at the invasive margin form physical ECM barriers that exclude CD8^+ T cells from the tumor nest; *MHC-I^hi Gal9*^+ CAFs form functional immune traps through ligand–receptor interactions that isolate and suppress T-cell activity; and CXCL13^+ CAFs promote B-cell recruitment and antibody production while also contributing to the activation and subsequent exhaustion of *CXCL13*^+ CD8^+ T cells within tumor-cell aggregates in nasopharyngeal car-

cinoma, a subtype of HNSCC [16,40,57]. In addition to physical exclusion, apCAFs may promote immunosuppressive microenvironments by altering local T-cell composition, potentially favoring CD4^+ T-cell dominance over CD8^+ T-cell activity, a pattern associated with tumor progression [58]. Simultaneously, iCAFs secrete soluble factors such as *CXCL12* and *TGF-β*, establishing chemokine gradients that recruit regulatory T cells and promote T-cell exhaustion in peri-tumoral regions [59]. Through these spatially defined interactions, CAFs emerge as central regulators of the immunosuppressive stroma in HNSCC. Spatially resolved identification of these distinct CAF populations and their interactions with immune cells is critical. For instance, if spatial data reveal an abundance of $POSTN^+$ CAFs that physically exclude T cells, this finding directly suggests a therapeutic strategy combining immune checkpoint inhibitors with CAF targeting agents, such as fibroblast activation protein inhibitors, or extracellular matrix remodeling drugs to overcome the physical barrier and enhance T cell infiltration and function.

4.3. Vascular and Hypoxic Niches: Hubs for Immune Evasion and Therapy Resistance

Abnormal vasculature and hypoxia in HNSCC form distinct spatial microenvironments that contribute to immune escape and therapeutic resistance [60]. Abnormal, leaky blood vessels driven by high VEGFA signaling not only contribute to a hypoxic and acidic microenvironment that directly impairs T cell function but also fail to support effective T cell infiltration, thereby forming a physical barrier that restricts immune cell entry [61]. In these hypoxic perivascular microenvironments, tumor and stromal cells upregulate immunosuppressive metabolites and immune-checkpoint molecules, further suppressing local T-cell activity and promoting niches that support the survival of drug-resistant, stem-cell-like cancer cells [41,50]. Spatial profiling technologies are instrumental in precisely mapping these hypoxic niches and abnormal vascular structures. Identifying such regions within a tumor can directly inform treatment decisions, suggesting combination therapies that include anti-angiogenic agents or hypoxia-modifying drugs alongside immunotherapies to improve oxygenation, normalize vasculature, and enhance immune cell access and function.

4.4. Reconstruction of Global Communication Networks from Spatial Data

The key advantage of spatial biology is its ability to directly reconstruct and quantify intercellular communication networks within intact tissue architecture [62]. By analyzing the co-localization of ligand and receptor mRNAs or proteins, key signaling pathways can be mapped to specific cellular regions [63–65]. For instance, spatial transcriptomics has revealed that in glucose-deficient regions of HNSCC, cancer cell-derived CXCL8 interacts with macrophages to establish a feedforward loop that promotes antioxidant production and confers resistance to nutrient-starvation therapies (anlotinib) [63]. In HPV-negative HNSCC, systematic profiling of ligand-receptor interactions has identified prognostic signaling networks involving ECM and immune regulation. Integration of these networks with histopathological features enables improved risk stratification [64]. In addition, spatial analysis has revealed a mutually exclusive expression pattern between the immune checkpoint B7-H4 (VTCN1) and PD-L1. B7-H4-positive tumor regions show marked exclusion of CD8+ T cells, highlighting its potential as a therapeutic target in immune-cold tumors [65]. Integrating these spatially resolved interactions into global network models not only identifies dominant signaling circuits driving tumor progression but also highlights novel targets for disrupting the pathological ecosystem of HNSCC.

5. Spatial Features Linked to Therapy Response and Resistance in HNSCC

5.1. Immunotherapy

Immune checkpoint inhibitors, particularly PD-1 and PD-L1 blockade, have significantly reshaped the treatment landscape for a subset of patients with HNSCC; however, overall response rates remain limited [66–68]. Spatial omics helps clarify why the efficacy of immune checkpoint inhibitors (ICIs) depends not only on the presence of CD8^+ T cells but also on the spatial organization of multiple immune and stromal cell types within the TME [69]. This refined understanding provided by spatial data directly impacts patient stratification and treatment planning. For instance, the spatial identification of mature, functional TLS within tumors, enriched in CD4+ T cells, memory B cells, and plasma cells, serves as a robust positive spatial predictive biomarker of improved response to immune checkpoint inhibitors [70]. Patients whose tumors exhibit such organized TLS, detectable through spatial profiling, could be prioritized for immunotherapy or considered for de-escalation strategies if a robust response is observed. In contrast, spatial features associated with resistance to immune checkpoint inhibitors often include CD8^+ T cells located close to immuno-

2026, Vol. 3, Article ID. 2026.0026
https://doi.org/10.69709/GenomC.2026.155654

suppressive components, such as regulatory T cells in perivascular microenvironments or M2-like macrophages in stromal regions [71]. In these cases, spatial analysis directly informs the need for combination strategies, such as adding agents that deplete suppressive cells (e.g., anti-CCL22 to target Tregs) or disrupt physical barriers (e.g., targeting specific CAFs), to reprogram the TME and improve ICI effectiveness. This spatially informed approach moves beyond simple PD-L1 expression or CD8 T cell density, enabling more precise identification of patients likely to benefit from immune checkpoint inhibitors and guiding the design of rational combination therapies for non-responders. In addition, B cells and plasma cells located outside the TLS structure may also acquire tumor-promoting properties, which further illustrates how the spatial environment determines immune function [57]. In summary, the response of HNSCC to immune checkpoint inhibitors reflects a balance between effective immune activation within tertiary lymphoid structures and functional immune suppression within the stromal or vascular microenvironment, and this balance is spatially regulated.

5.2. Targeted Therapies

TP53 is one of the most frequently mutated genes in HNSCC, with mutation rates of approximately 70%, although this prevalence varies significantly depending on factors such as HPV status and anatomical subsite [72,73]. In addition, the *EGFR* gene is widely altered, with up to 80–90% of HNSCCs exhibiting either overexpression or mutations [74]. Other important alterations include *FAT1, PIK3CA, CDKN2A,* and *NOTCH1*, which collectively highlight the therapeutic potential of molecularly informed treatment strategies [75–78]. Despite these molecular alterations, the clinical efficacy of targeted therapies remains suboptimal, highlighting a critical translational gap between genomic profiling and therapeutic success [79,80]. Spatial technologies are revealing that therapeutic response depends not only on mutational status but also on the spatial organization of target-gene expression and protective tumor-microenvironmental ecosystems [32,39]. For instance, spatial transcriptomics analysis of early-onset tongue cancer uncovers both significantly enriched MAPK and JAK-STAT signaling pathways and a distinctive TME characterized by increased plasma cell gene signatures and TLS featuring plasma cell and lymphocyte aggregations, particularly at the invasive front, thereby suggesting the need for customized targeted interventions for these specific molecular subtypes [81]. Similarly, studies of tumor budding have identified *NSD1* mutations as negatively correlated with tumor budding and revealed key roles for CAV1 and MMP14. The spatial expression gradients of these proteins from the tumor core to budding regions highlight progressively enhanced invasive processes, which may represent potential therapeutic targets [82].

Further spatial dissection of the tumor leading edge consistently identifies upregulated collagen deposition, CD99 expression, and non-canonical WNT signaling pathways that drive invasion, whereas the tumor core is characterized by Angiopoietin-like protein (ANGPTL) and POSTN-associated signaling modules. This compartment-specific organization suggests a targeted therapeutic strategy that encompasses both the tumor nest and the surrounding stroma [83]. In precancerous lesions, spatial regulation of VEGF signaling, in conjunction with immunosuppressive mononuclear cells, contributes to the formation of a microenvironment that promotes malignant transformation, suggesting a potential target for therapeutic intervention [84]. Notably, integrated multi-omics analysis links POSTN-mediated extracellular-matrix remodeling and CAF-secreted TGF-β with epithelial EMT and LNM in OSCC, suggesting that the POSTN–TGF-β axis may be a potential target for disrupting the metastatic cascade [16]. In summary, these spatially resolved insights extend beyond static mutational profiles to reveal the spatial distribution of actionable signaling pathways and tumor–matrix interactions, thereby providing a framework for the development of mechanism-based combination therapies and context-specific targeted treatments for HNSCC.

5.3. Translational Outlook: Spatial Biomarkers for Clinical Stratification

The clinical translation of spatial genomic research requires distilling complex spatial data into actionable biomarkers to guide patient selection, individualized treatment, and real-time monitoring. Characteristics such as cell subtype, intercellular interaction, ligand-receptor signaling, and spatial co-localization patterns of tumor-associated CAFs represent potential spatial biomarker candidates [85,86]. Specifically, spatial biomarkers derived from immune escape mechanisms, such as the spatial density and precise localization of specific CAF subtypes (e.g., $POSTN^+$ CAFs physically excluding T cells), the presence and maturity of TLS (indicating immune competence), or the spatial proximity of $CD8^+$ T cells to immunosuppressive myeloid cells (indicating resistance), can serve as powerful tools for patient stratification. These biomarkers can predict response to ICIs, identify patients requiring combination therapies, and guide the selection of appropriate therapeutic

partners to overcome specific spatially driven immune escape mechanisms.

Combined with digital pathology and computational image analysis, these features can be automatically and quantitatively assessed in conventional histological specimens, thereby enabling scalable integration into clinical workflows [87–89]. For example, deep learning models that extract spatial features such as tumor infiltrating lymphocyte density, tumor microenvironment heterogeneity, and granulocyte enrichment at the invasive margin from standard hematoxylin and eosin-stained slides have demonstrated strong predictive performance for overall survival benefit from the PI3K inhibitor buparlisib in recurrent and metastatic HNSCC, even outperforming conventional CD3 immunohistochemistry [89]. Digital pathology platforms combined with computational analysis are being utilized to standardize challenging PD-L1 combined positive scoring by objectively quantifying staining intensity and distribution, thereby reducing inter-observer variability and technical discordance between different assay platforms [90]. In addition, a computational pipeline applied to digital pathology slides has successfully identified prognostically relevant spatial markers, such as the spatial distribution pattern of FOXP3 across tumor and stromal compartments, providing a cost-effective strategy for spatial biomarker discovery [91]. In the context of immunotherapy, an AI-driven single-cell spatial biomarker quantifying specific cell-cell interactions within the tumor microenvironment has proven superior to PD-L1 expression alone in predicting progression-free survival and objective response to immune checkpoint inhibitors in advanced non-small cell lung cancer, with potential applicability to HNSCC [87]. These examples collectively highlight the transformative potential of integrating computational pathology with spatial biology to generate robust, automated, and clinically applicable biomarkers. Crucially, validation of these spatial biomarkers in prospective, multicenter clinical trials represents a necessary next step to translate these experimental findings into improved clinical outcomes for patients with HNSCC.

Future multi-center clinical trials should include spatial biomarker endpoints to verify their predictive and prognostic efficacy, evaluate their repeatability on different platforms, and determine their cost-effectiveness. In conclusion, integrating spatial biology into clinical decision-making will bridge the gap between molecular profiling and microenvironment-based therapeutic intervention, providing a new paradigm for individualized treatment of HNSCC.

6. Limitations

While spatial omics approaches provide unprecedented insights into the localized molecular complexity of disease, they are associated with several methodological and translational limitations that warrant careful consideration. A significant challenge lies in reproducibility and protocol standardization, as the diverse array of platforms and complex experimental workflows can lead to variability across studies and laboratories, hindering robust cross-study comparisons. Sample preparation remains critical; for example, the use of formalin fixed paraffin embedded (FFPE) tissues, although widely available, can introduce technical biases due to RNA degradation and fragmentation, potentially affecting transcript capture efficiency and overall data quality. Conversely, fresh-frozen samples, while providing superior RNA integrity, present challenges related to tissue handling and preservation of morphological structure. Furthermore, current spatial transcriptomics technologies often operate at a resolution that is multi-cellular rather than true single-cell, which can obscure the precise attribution of gene expression to individual cell types within a heterogeneous TME. Technical biases can also arise from platform-specific capture efficiencies, probe design, and batch effects. Finally, the interpretation of these complex, high-dimensional spatial datasets require sophisticated bioinformatics tools and expertise, posing a significant challenge for many research groups. From a translational perspective, high cost, relatively low throughput for large patient cohorts, and the lack of standardized diagnostic pipelines mean that integrating spatial omics findings into routine clinical practice remains challenging. Addressing these methodological gaps will be pivotal for transitioning spatial omics from a discovery tool to a routine clinical diagnostic platform.

7. Conclusion and Perspective

Spatial omics has transitioned HNSCC research from bulk-level averages to a high-resolution, context-dependent understanding of tumor biology. By mapping the precise coordinates of cellular interactions, these technologies have identified novel biomarkers and structural features, such as the invasive budding front and mature tertiary lymphoid structures, that hold significant prognostic value. However, the clinical translation of spatial oncology remains hindered by high costs, a lack of protocol standardization, and the immense complexity of data integration.

Looking ahead, the integration of spatial multi-omics with artificial intelligence and deep learning will be pivotal. AI-driven platforms capable of synthesizing

multiplexed spatial data with routine clinical pathology could democratize access to these advanced insights. Future efforts should prioritize validating spatial signatures in prospective clinical trials to establish their utility for guiding immunotherapy and targeted treatment strategies. Ultimately, bridging the gap between spatial discovery and clinical implementation will be essential to achieving precision medicine for HNSCC patients.

List of Abbreviations

ANGPTL	Angiopoietin-like Protein
apCAFs	Antigen-Presenting CAFs
CAF	Cancer-Associated Fibroblast
CAV1	Caveolin-1
CD4+	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
ECM	Extracellular Matrix
EMT	Epithelial-Mesenchymal Transition
FFPE	Formalin-Fixed Paraffin-Embedded
FOXP3	Forkhead Box P3
HNSCC	Head and Neck Squamous Cell Carcinoma
iCAFs	Inflammatory CAFs
ICIs	Immune Checkpoint Inhibitors
IMC	Imaging Mass Cytometry
LNM	Lymph Node Metastasis
MAPK	Mitogen-Activated Protein Kinase
mIF	Multiplex Immunofluorescence
MMP14	Matrix Metalloproteinase 14
myCAFs	Myofibroblastic CAFs
NSD1	Nuclear Receptor Binding SET Domain Protein 1
OSCC	Oral Squamous Cell Carcinoma
PCF	PhenoCycler-Fusion
PD-1	Programmed Cell Death-1
PD-L1	Programmed Cell Death Ligand 1
pEMT	Partial EMT
PI3K	Phosphoinositide 3-Kinase
TC	Tumor Core
TIME	Tumor Immune Microenvironment
TLS	Tertiary Lymphoid Structures
TME	Tumor Microenvironment
Tregs	Regulatory T cells
VEGFA	Vascular Endothelial Growth Factor A
VTCN1	V-set Domain Containing T Cell Activation Inhibitor

Author Contributions

Conceptualization: Y.L.; Visualization: Y.L.; Data curation: X.Y. and C.M.; Writing—original draft preparation: Y.L.; Writing—review and editing: Y.L. and X.H.; Supervision: X.H. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

The study did not receive any external funding and was conducted using only institutional resources.

Acknowledgments

Declared none.

AI Declaration

The AI language model ChatGPT (OpenAI) was used solely to enhance the language, grammar, readability, and overall clarity of this review manuscript. Its use was limited to linguistic refinement and did not involve the generation of scientific content, literature analysis, interpretation of findings, formulation of conclusions, or preparation of tables and figures. All scientific content, interpretations, and conclusions presented in this manuscript were developed by the authors, who take full responsibility for the accuracy, integrity, and content of the manuscript.

References

[1] Johnson, D.E.; Burtness, B.; Leemans, C.R.; Lui, V.W.Y.; Bauman, J.E.; Grandis, J.R. Head and Neck Squamous Cell Carcinoma. *Nat. Rev. Dis. Primers* **2020**, *6*, 92. [CrossRef]

[2] Rettig, E.M.; D'Souza, G. Epidemiology of Head and Neck Cancer. *Surg. Oncol. Clin. N. Am.* **2015**, *24*, 379–396. [CrossRef] [PubMed]

[3] Goel, B.; Tiwari, A.K.; Pandey, R.K.; Singh, A.P.; Kumar, S.; Sinha, A.; Jain, S.K.; Khattri, A. Therapeutic Approaches for the Treatment of Head and Neck Squamous Cell Carcinoma-An Update on Clinical Trials. *Transl. Oncol.* **2022**, *21*, 101426. [CrossRef]

[4] Van den bossche, V.; Zaryouh, H.; Vara-Messler, M.; Vignau, J.; Machiels, J.-P.; Wouters, A.; Schmitz, S.; Corbet, C. Microenvironment-Driven Intratumoral Heterogeneity in Head and Neck Cancers: Clinical Challenges and Opportunities for Precision Medicine. *Drug Resist. Updates* **2022**, *60*, 100806. [CrossRef]

[5] Elmusrati, A.; Wang, J.; Wang, C.-Y. Tumor Microenvironment and Immune Evasion in Head and Neck Squamous Cell Carcinoma. *Int. J. Oral. Sci.* **2021**, *13*, 24. [CrossRef]

[6] Wang, G.; Zhang, M.; Cheng, M.; Wang, X.; Li, K.; Chen, J.; Chen, Z.; Chen, S.; Chen, J.; Xiong, G.; et al. Tumor Microenvironment in Head and Neck Squamous Cell Carcinoma: Functions and Regula-

tory Mechanisms. *Cancer Lett.* **2021**, *507*, 55–69. [CrossRef]

[7] Gong, Y.; Bao, L.; Xu, T.; Yi, X.; Chen, J.; Wang, S.; Pan, Z.; Huang, P.; Ge, M. The Tumor Ecosystem in Head and Neck Squamous Cell Carcinoma and Advances in Ecotherapy. *Mol. Cancer* **2023**, *22*, 68. [CrossRef]

[8] Liu, Z.L.; Meng, X.Y.; Bao, R.J.; Shen, M.Y.; Sun, J.J.; Chen, W.D.; Liu, F.; He, Y. Single Cell Deciphering of Progression Trajectories of the Tumor Ecosystem in Head and Neck Cancer. *Nat. Commun.* **2024**, *15*, 2595. [CrossRef] [PubMed]

[9] Punovuori, K.; Bertillot, F.; Miroshnikova, Y.A.; Binner, M.I.; Myllymäki, S.-M.; Follain, G.; Kruse, K.; Routila, J.; Huusko, T.; Pellinen, T.; et al. Multiparameter imaging Reveals Clinically Relevant Cancer Cell-Stroma Interaction Dynamics in Head and Neck Cancer. *Cell* **2024**, *187*, 7267–7284.e7220. [CrossRef] [PubMed]

[10] Puram, S.V.; Tirosh, I.; Parikh, A.S.; Patel, A.P.; Yizhak, K.; Gillespie, S.; Rodman, C.; Luo, C.L.; Mroz, E.A.; Emerick, K.S.; et al. Single-Cell Transcriptomic Analysis of Primary and Metastatic Tumor Ecosystems in Head and Neck Cancer. *Cell* **2017**, *171*, 1611–1624.e24. [CrossRef]

[11] Marco Salas, S.; Kuemmerle, L.B.; Mattsson-Langseth, C.; Tismeyer, S.; Avenel, C.; Hu, T.; Rehman, H.; Grillo, M.; Czarnewski, P.; Helgadottir, S.; et al. Optimizing Xenium In Situ Data Utility by Quality Assessment and Best-Practice Analysis Workflows. *Nat. Methods* **2025**, *22*, 813–823. [CrossRef]

[12] Chen, A.; Liao, S.; Cheng, M.; Ma, K.; Wu, L.; Lai, Y.; Qiu, X.; Yang, J.; Xu, J.; Hao, S.; et al. Spatiotemporal Transcriptomic Atlas of Mouse Organogenesis Using DNA Nanoball-Patterned Arrays. *Cell* **2022**, *185*, 1777–1792.e21. [CrossRef] [PubMed]

[13] Bollhagen, A.; Whipman, J.; Coelho, R.; Heinzelmann-Schwarz, V.; Jacob, F.; Bodenmiller, B. High-resolution imaging mass cytometry to map subcellular structures. *Nat. Methods* **2025**, *22*, 2601–2608. [CrossRef]

[14] Mund, A.; Coscia, F.; Kriston, A.; Hollandi, R.; Kovács, F.; Brunner, A.-D.; Migh, E.; Schweizer, L.; Santos, A.; Bzorek, M.; et al. Deep Visual Proteomics Defines Single-Cell Identity and Heterogeneity. *Nat. Biotechnol.* **2022**, *40*, 1231–1240. [CrossRef]

[15] Wang, F.; Flanagan, J.; Su, N.; Wang, L.C.; Bui, S.; Nielson, A.; Wu, X.; Vo, H.T.; Ma, X.J.; Luo, Y. RNAscope: A Novel In Situ RNA Analysis Platform for Formalin-Fixed, Paraffin-Embedded Tissues. *J. Mol. Diagn.* **2012**, *14*, 22–29. [CrossRef]

[16] Liu, Y.; Yang, Z.; Pu, J.J.; Zhong, J.; Khoo, U.S.; Su, Y.X.; Zhang, G. Proteogenomic Characterisation of Primary Oral Cancer Unveils Extracellular Matrix Remodelling and immunosuppressive Microenvironment Linked to Lymph Node Metastasis. *Clin. Transl. Med.* **2025**, *15*, e70261. [CrossRef] [PubMed]

[17] Oliveira, M.F.d.; Romero, J.P.; Chung, M.; Williams, S.R.; Gottscho, A.D.; Gupta, A.; Pilipauskas, S.E.; Mohabbat, S.; Raman, N.; Sukovich, D.J.; et al. High-Definition Spatial Transcriptomic Profiling of Immune Cell Populations in Colorectal Cancer. *Nat. Genet.* **2025**, *57*, 1512–1523. [CrossRef]

[18] Choi, J.I.; Cho, E.J.; Yang, M.J.; Noh, H.J.; Park, S.H.; Kim, S.; Kim, Y.S.; Sung, C.O.; Lee, D. Hypoxic Microenvironment Determines the Phenotypic Plasticity and Spatial Distribution of Cancer-Associated Fibroblasts. *Clin. Transl. Med.* **2023**, *13*, e1438. [CrossRef]

[19] Zhu, J.; Wang, Y.; Chang, W.Y.; Malewska, A.; Napolitano, F.; Gahan, J.C.; Unni, N.; Zhao, M.; Yuan, R.; Wu, F.; et al. Mapping Cellular Interactions from Spatially Resolved Transcriptomics Data. *Nat. Methods* **2024**, *21*, 1830–1842. [CrossRef] [PubMed]

[20] Williams, C.G.; Lee, H.J.; Asatsuma, T.; Vento-Tormo, R.; Haque, A. An Introduction to Spatial Transcriptomics for Biomedical Research. *Genome Med.* **2022**, *14*, 68. [CrossRef]

[21] Lundberg, E.; Borner, G.H.H. Spatial Proteomics: A Powerful Discovery Tool for Cell Biology. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 285–302. [CrossRef] [PubMed]

[22] Alexandrov, T. Spatial Metabolomics: From a Niche Field Towards a Driver of Innovation. *Nat. Metab.* **2023**, *5*, 1443–1445. [CrossRef]

[23] Ozirmak Lermi, N.; Molina Ayala, M.; Hernandez, S.; Lu, W.; Khan, K.; Serrano, A.; Lubo, I.; Hamana, L.; Tomczak, K.; Barnes, S.; et al. Comparison of Imaging Based Single-Cell Resolution Spatial Transcriptomics Profiling Platforms Using Formalin-Fixed Paraffin-Embedded Tumor Samples. *Nat. Commun.* **2025**, *16*, 8499. [CrossRef]

[24] You, Y.; Fu, Y.; Li, L.; Zhang, Z.; Jia, S.; Lu, S.; Ren, W.; Liu, Y.; Xu, Y.; Liu, X.; et al. Systematic Comparison of Sequencing-Based Spatial Transcriptomic Methods. *Nat. Methods* **2024**, *21*, 1743–1754. [CrossRef]

[25] Gerdes, M.J.; Sevinsky, C.J.; Sood, A.; Adak, S.; Bello, M.O.; Bordwell, A.; Can, A.; Corwin, A.; Dinn, S.; Filkins, R.J.; et al. Highly Multiplexed Single-Cell Analysis of Formalin-Fixed, Paraffin-Embedded Cancer Tissue. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 11982–11987. [CrossRef]

[26] Kuett, L.; Catena, R.; Özcan, A.; Plüss, A.; Ali, H.R.; Sa'd, M.A.; Alon, S.; Aparicio, S.; Battistoni, G.; Balasubramanian, S.; et al. Three-Dimensional Imaging Mass Cytometry for Highly Multiplexed Molecular and Cellular Mapping of Tissues and the Tumor Microenvironment. *Nat. Cancer* **2022**, *3*, 122–133. [CrossRef] [PubMed]

[27] Black, S.; Phillips, D.; Hickey, J.W.; Kennedy-Darling, J.; Venkataraaman, V.G.; Samusik, N.; Goltsev, Y.; Schürch, C.M.; Nolan, G.P. CODEX Multiplexed Tissue Imaging with DNA-Conjugated Antibodies. *Nat. Protoc.* **2021**, *16*, 3802–3835. [CrossRef]

[28] Ren, J.; Luo, S.; Shi, H.; Wang, X. Spatial Omics Advances for In Situ RNA biology. *Mol. Cell* **2024**, *84*, 3737–3757. [CrossRef]

[29] Liu, L.; Chen, A.; Li, Y.; Mulder, J.; Heyn, H.; Xu, X. Spatiotemporal Omics for Biology and Medicine. *Cell* **2024**, *187*, 4488–4519. [CrossRef] [PubMed]

[30] Longo, S.K.; Guo, M.G.; Ji, A.L.; Khavari, P.A. Integrating Single-Cell and Spatial Transcriptomics to Elucidate Intercellular Tissue Dynamics. *Nat. Rev. Genet.* **2021**, *22*, 627–644. [CrossRef] [PubMed]

[31] Chai, Y.; Zhang, J.; Shao, W.; Zhang, Z. Single-Cell Insights into HNSCC Tumor Heterogeneity and Programmed Cell Death Pathways. *Transl. Oncol.* **2025**, *54*, 102341. [CrossRef]

[32] Arora, R.; Cao, C.; Kumar, M.; Sinha, S.; Chanda, A.; McNeil, R.; Samuel, D.; Arora, R.K.; Matthews, T.W.; Chandarana, S.; et al. Spatial Transcriptomics Reveals Distinct and Conserved Tumor Core and Edge Architectures That Predict Survival and Targeted Therapy Response. *Nat. Commun.* **2023**, *14*, 5029. [CrossRef] [PubMed]

[33] Aggarwal, V.; Montoya, C.A.; Donnenberg, V.S.; Sant, S. Interplay Between Tumor Microenvironment and Partial EMT as the Driver of Tumor Progression. *iScience* **2021**, *24*, 102113. [CrossRef] [PubMed]

[34] Dakal, T.C.; Bhushan, R.; Xu, C.; Gadi, B.R.; Cameotra, S.S.; Yadav, V.; Maciaczyk, J.; Schmidt-Wolf, I.G.H.; Kumar, A.; Sharma, A. Intricate Relationship Between Cancer Stemness, Metastasis, and Drug Resistance. *MedComm* **2024**, *5*, e710. [CrossRef]

[35] Liu, Q.; Guo, Z.; Li, G.; Zhang, Y.; Liu, X.; Li, B.; Wang, J.; Li, X. Cancer Stem Cells and Their Niche in Cancer Progression and Therapy. *Cancer Cell Int.* **2023**, *23*, 305. [CrossRef]

[36] Ritchie, K.E.; Nör, J.E. Perivascular Stem Cell Niche in Head and Neck Cancer. *Cancer Lett.* **2013**, *338*, 41–46. [CrossRef]

[37] Fu, T.; Dai, L.-J.; Wu, S.-Y.; Xiao, Y.; Ma, D.; Jiang, Y.-Z.; Shao, Z.-M. Spatial Architecture of the Immune Microenvironment Orchestrates Tumor Immunity and Therapeutic Response. *J. Hematol. Oncol.* **2021**, *14*, 98. [CrossRef] [PubMed]

[38] Li, H.; Zhang, M.-J.; Zhang, B.; Lin, W.-P.; Li, S.-J.; Xiong, D.; Wang, Q.; Wang, W.-D.; Yang, Q.-C.; Huang, C.-F.; et al. Mature Tertiary Lymphoid Structures Evoke Intra-Tumoral T and B Cell Responses via Progenitor Exhausted CD4+ T Cells in Head and Neck Cancer. *Nat. Commun.* **2025**, *16*, 4228. [CrossRef]

[39] Nieszporek, A.; Wierzbicka, M.; Khan, A.; Jeziorny, M.; Kraiński, P.; Cybinska, J.; Gazinska, P. Spatial Profiling Technologies for Research and Clinical Application in Head and Neck Squamous Cell Cancers. *Curr. Res. Biotechnol.* **2025**, *10*, 100321. [CrossRef]

[40] Li, C.; Guo, H.; Zhai, P.; Yan, M.; Liu, C.; Wang, X.; Shi, C.; Li, J.; Tong, T.; Zhang, Z.; et al. Spatial and Single-Cell Transcriptomics Reveal a Cancer-Associated Fibroblast Subset in HNSCC That Restricts Infiltration and Antitumor Activity of CD8+ T Cells. *Cancer Res.* **2024**, *84*, 258–275. [CrossRef]

[41] Mandal, R.; Şenbabaoğlu, Y.; Desrichard, A.; Havel, J.J.; Dalin, M.G.; Riaz, N.; Lee, K.-W.; Ganly, I.; Hakimi, A.A.; Chan, T.A.; et al. The Head and Neck Cancer Immune Landscape and its Immunotherapeutic Implications. *JCI Insight* **2016**, *1*(17), e89829. [CrossRef]

[42] Watermann, C.; Pasternack, H.; Idel, C.; Ribbat-Idel, J.; Brägelmann, J.; Kuppler, P.; Offermann, A.; Jonigk, D.; Kühnel, M.P.; Schröck, A.; et al. Recurrent HNSCC Harbor an Immunosuppressive Tumor Immune Microenvironment Suggesting Successful Tumor Immune Evasion. *Clin. Cancer Res.* **2021**, *27*, 632–644. [CrossRef]

[43] Li, X.; González-Maroto, C.; Tavassoli, M. Crosstalk Between CAFs and Tumour Cells in Head and Neck Cancer. *Cell Death Discov.* **2024**, *10*, 303. [CrossRef]

[44] Luo, H.; Xia, X.; Huang, L.-B.; An, H.; Cao, M.; Kim, G.D.; Chen, H.-N.; Zhang, W.-H.; Shu, Y.; Kong, X.; et al. Pan-Cancer Single-Cell Analysis Reveals the Heterogeneity and Plasticity of Cancer-Associated Fibroblasts in the Tumor Microenvironment. *Nat. Commun.* **2022**, *13*, 6619. [CrossRef]

[45] Hu, C.; Zhang, Y.; Wu, C.; Huang, Q. Heterogeneity of Cancer-Associated Fibroblasts in Head and Neck Squamous Cell Carcinoma: Opportunities and Challenges. *Cell Death Discov.* **2023**, *9*, 124. [CrossRef]

[46] Wang, Q.; Zhao, Y.; Tan, G.; Ai, J. Single Cell Analysis Revealed SFRP2 cancer Associated Fibroblasts Drive Tumorigenesis in Head and Neck Squamous Cell Carcinoma. *npj Precis. Oncol.* **2024**, *8*, 228. [CrossRef] [PubMed]

[47] Michikawa, C.; Uzawa, N.; Kayamori, K.; Sonoda, I.; Ohyama, Y.; Okada, N.; Yamaguchi, A.; Amagasa, T. Clinical Significance of Lymphatic and Blood Vessel Invasion in Oral Tongue Squamous Cell Carcinomas. *Oral. Oncol.* **2012**, *48*, 320–324. [CrossRef] [PubMed]

[48] Maturana-Ramírez, A.; Espinoza, I.; Reyes, M.; Aitken, J.P.; Aguayo, F.; Hartel, S.; Rojas-Alcayaga, G. Higher Blood Vessel Density in Comparison to the Lymphatic Vessels in Oral Squamous Cell Carcinoma. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 13677–13686. [PubMed Central]

[49] Krishnamurthy, S.; Dong, Z.; Vodopyanov, D.; Imai, A.; Helman, J.I.; Prince, M.E.; Wicha, M.S.; Nör, J.E. Endothelial Cell-Initiated Signaling Promotes the Survival and Self-Renewal of Cancer Stem Cells. *Cancer Res.* **2010**, *70*, 9969–9978. [CrossRef]

[50] Zhu, C.; Gu, L.; Liu, Z.; Li, J.; Yao, M.; Fang, C. Correlation Between Vascular Endothelial Growth Factor Pathway and Immune Microenvironment in Head and Neck Squamous Cell Carcinoma. *BMC Cancer* **2021**, *21*, 836. [CrossRef] [PubMed]

[51] Bhat, A.A.; Yousuf, P.; Wani, N.A.; Rizwan, A.; Chauhan, S.S.; Siddiqi, M.A.; Bedognetti, D.; El-Rifai, W.; Frenneaux, M.P.; Batra, S.K.; et al. Tumor Microenvironment: An Evil Nexus Promoting Aggressive Head and Neck Squamous Cell Carcinoma and Avenue for Targeted Therapy. *Signal Transduct. Target. Ther.* **2021**, *6*, 12. [CrossRef]

[52] Ji, H.; Hu, C.; Yang, X.; Liu, Y.; Ji, G.; Ge, S.; Wang, X.; Wang, M. Lymph Node Metastasis in Cancer Progression: Molecular Mechanisms, Clinical Significance and Therapeutic Interventions. *Signal Transduct. Target. Ther.* **2023**, *8*, 367. [CrossRef]

[53] Zhu, J.; Petit, P.F.; Van den Eynde, B.J. Apoptosis of Tumor-Infiltrating T Lymphocytes: A New Immune Checkpoint Mechanism. *Cancer Immunol. Immunother.* **2019**, *68*, 835–847. [CrossRef]

[54] Kim, S.; Kee, H.J.; Kim, D.; Jang, J.; Jeong, H.-o.; Sim, N.S.; Selig, M.; Ihlow, J.; Penter, L.; Hwang, T.; et al. Multiregional Single-Cell Transcriptomics Reveals an Association Between Partial EMT and Immunosuppressive States in Oral Squamous Cell Carcinoma. *iScience* **2025**, *28*, 112988. [CrossRef]

[55] William, W.N.; Zhao, X.; Bianchi, J.J.; Lin, H.Y.; Cheng, P.; Lee, J.J.; Carter, H.; Alexandrov, L.B.; Abraham, J.P.; Spetzler, D.B.; et al. Immune Evasion in HPV−Head and Neck Precancer–Cancer Transition Is Driven by an Aneuploid Switch Involving Chromosome 9p Loss. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2022655118. [CrossRef]

[56] Jenkins, B.H.; Tracy, I.; Rodrigues, M.F.S.D.; Smith, M.J.L.; Martinez, B.R.; Edmond, M.; Mahadevan, S.; Rao, A.; Zong, H.; Liu, K.; et al. Single Cell and Spatial Analysis of Immune-Hot and immune-Cold Tumours Identifies Fibroblast Subtypes Associated with Distinct Immunological Niches and Positive Immunotherapy Response. *Mol. Cancer* **2025**, *24*, 3. [CrossRef]

[57] Liu, Y.; Ye, S.-Y.; He, S.; Chi, D.-M.; Wang, X.-Z.; Wen, Y.-F.; Ma, D.; Nie, R.-C.; Xiang, P.; Zhou, Y.; et al. Single-Cell and Spatial Transcriptome Analyses Reveal Tertiary Lymphoid Structures Linked to Tumour Progression and Immunotherapy Response in Nasopharyngeal Carcinoma. *Nat. Commun.* **2024**, *15*, 7713. [CrossRef]

[58] Ren, F.; Meng, L.; Zheng, S.; Cui, J.; Song, S.; Li, X.; Wang, D.; Li, X.; Liu, Q.; Bu, W.; et al. Myeloid Cell-Derived apCAFs Promote HNSCC Progression by Regulating Proportion of CD4(+) and CD8(+) T Cells. *J. Exp. Clin. Cancer Res.* **2025**, *44*, 33. [CrossRef]

[59] Liu, Z.; Zhang, Z.; Zhang, Y.; Zhou, W.; Zhang, X.; Peng, C.; Ji, T.; Zou, X.; Zhang, Z.; Ren, Z. Spatial Transcriptomics Reveals That Metabolic Characteristics Define the Tumor Immunosuppression Microenvironment via iCAF Transformation in Oral Squamous Cell Carcinoma. *Int. J. Oral. Sci.* **2024**, *16*, 9. [CrossRef]

[60] Peng, C.; Ye, H.; li, Z.; Duan, X.; Yang, W.; Yi, Z. Multi-Omics Characterization of a Scoring System to Quantify Hypoxia Patterns in Patients with Head and Neck Squamous Cell Carcinoma. *J. Transl. Med.* **2023**, *21*, 15. [CrossRef]

[61] Mulligan, J.K.; Day, T.A.; Gillespie, M.B.; Rosenzweig, S.A.; Young, M.R. Secretion of Vascular Endothelial Growth Factor by Oral Squamous Cell Carcinoma Cells Skews Endothelial Cells to Suppress T-Cell Functions. *Hum. Immunol.* **2009**, *70*, 375–382. [CrossRef]

[62] Causer, A.; Tan, X.; Lu, X.; Moseley, P.; Teoh, S.M.; Molotkov, N.; McGrath, M.; Kim, T.; Simpson, P.T.; Perry, C.; et al. Deep Spatial-Omics Analysis of Head & Neck Carcinomas Provides Alternative Therapeutic Targets and Rationale for Treatment Failure. *npj Precis. Oncol.* **2023**, *7*, 89. [CrossRef]

[63] Hu, X.; Ji, Y.; Zhang, M.; Li, Z.; Pan, X.; Zhang, Z.; Wang, X. Targeting CXCL8 Signaling Sensitizes HNSCC to Anlotinib by Reducing Tumor-Associated Macrophage-Derived CLU. *J. Exp. Clin. Cancer Res.* **2025**, *44*, 39. [CrossRef] [PubMed]

[64] Feng, B.; Zhao, D.; Zhang, Z.; Jia, R.; Schuler, P.J.; Hess, J. Ligand-Receptor Interactions Combined with Histopathology for Improved Prognostic Modeling in HPV-Negative Head and Neck Squamous Cell Carcinoma. *npj Precis. Oncol.* **2025**, *9*, 57. [CrossRef]

[65] Noda, Y.; Yagi, M.; Tsuta, K. Spatial Transcriptome Analysis of B7-H4 in Head and Neck Squamous Cell Carcinoma: A Novel Therapeutic Target for Anti-Immune Checkpoint Inhibitors. *Head. Neck Pathol.* **2025**, *19*, 78. [CrossRef] [PubMed]

[66] Lyford-Pike, S.; Peng, S.; Young, G.D.; Taube, J.M.; Westra, W.H.; Akpeng, B.; Bruno, T.C.; Richmon, J.D.; Wang, H.; Bishop, J.A.; et al. Evidence for a Role of the PD-1:PD-L1 Pathway in Immune Resistance of HPV-Associated Head and Neck Squamous Cell Carcinoma. *Cancer Res.* **2013**, *73*, 1733–1741. [CrossRef] [PubMed]

[67] Whiteside, T.L. Head and Neck Carcinoma Immunotherapy: Facts and Hopes. *Clin. Cancer Res.* **2018**, *24*, 6–13. [CrossRef]

[68] Naei, V.Y.; Tubelleza, R.; Monkman, J.; Sadeghirad, H.; Donovan, M.L.; Blick, T.; Wicher, A.; Bodbin, S.; Viratham, A.; Stad, R.; et al. Spatial Interaction Mapping of PD-1/PD-L1 in Head and Neck Cancer Reveals the Role of Macrophage-Tumour Barriers Associated with Immunotherapy Response. *J. Transl. Med.* **2025**, *23*, 177. [CrossRef]

[69] Berrell, N.; Monkman, J.; Donovan, M.; Blick, T.; O'Byrne, K.; Ladwa, R.; Tan, C.W.; Kulasinghe, A. Spatial Resolution of the Head and Neck Cancer Tumor Microenvironment to Identify Tumor and Stromal Features Associated with Therapy Response. *Immunol. Cell Biol.* **2024**, *102*, 830–846. [CrossRef]

[70] Xu, S.; Han, C.; Zhou, J.; Yang, D.; Dong, H.; Zhang, Y.; Zhao, T.; Tian, Y.; Wu, Y. Distinct Maturity and Spatial Distribution of Tertiary Lymphoid Structures in Head and Neck Squamous Cell Carcinoma: Implications for Tumor Immunity and Clinical Outcomes. *Cancer Immunol. Immunother.* **2025**, *74*, 107. [CrossRef]

[71] Sun, Y.; Yinwang, E.; Wang, S.; Wang, Z.; Wang, F.; Xue, Y.; Zhang, W.; Zhao, S.; Mou, H.; Chen, S.; et al. Phenotypic and Spatial Heterogeneity of CD8+ Tumour Infiltrating Lymphocytes. *Mol. Cancer* **2024**, *23*, 193. [CrossRef]

[72] Zhou, G.; Liu, Z.; Myers, J.N. TP53 Mutations in Head and Neck Squamous Cell Carcinoma and Their Impact on Disease Progression and Treatment Response. *J. Cell Biochem.* **2016**, *117*, 2682–2692. [CrossRef]

[73] Zhang, Y.; Cui, Y.; Hao, C.; Li, Y.; He, X.; Li, W.; Yu, H. Development of the TP53 Mutation Associated Hypopharyngeal Squamous Cell Carcinoma Prognostic Model Through Bulk Multi-Omics Sequencing and Single-Cell Sequencing. *Braz. J. Otorhinolaryngol.* **2025**, *91*, 101499. [CrossRef]

[74] Nair, S.; Bonner, J.A.; Bredel, M. EGFR Mutations in Head and Neck Squamous Cell Carcinoma. *Int. J. Mol. Sci.* **2022**, *23*, 3818. [CrossRef] [PubMed]

[75] The Cancer Genome Atlas Network. Comprehensive Genomic Characterization of Head and Neck Squamous Cell Carcinomas. *Nature* **2015**, *517*, 576–582. [CrossRef] [PubMed]

[76] Kalyankrishna, S.; Grandis, J.R. Epidermal Growth Factor Receptor Biology in Head and Neck Cancer. *J. Clin. Oncol.* **2006**, *24*, 2666–2672. [CrossRef]

[77] Loganathan, S.K.; Schleicher, K.; Malik, A.; Quevedo, R.; Langille, E.; Teng, K.; Oh, R.H.; Rathod, B.; Tsai, R.; Samavarchi-Tehrani, P.; et al. Rare Driver Mutations in Head and Neck Squamous Cell Carcinomas Converge on NOTCH Signaling. *Science* **2020**, *367*, 1264–1269. [CrossRef] [PubMed]

[78] Muijlwijk, T.; Nauta, I.H.; van der Lee, A.; Grünewald, K.J.T.; Brink, A.; Ganzevles, S.H.; Baatenburg de Jong, R.J.; Atanesyan, L.; Savola, S.; van de Wiel, M.A.; et al. Hallmarks of a Genomically Distinct Subclass of Head and Neck Cancer. *Nat. Commun.* **2024**, *15*, 9060. [CrossRef]

[79] Otero-Rosales, M.; Álvarez-González, M.; Pazos, I.; de Luxán-Delgado, B.; Del Marro, S.; Pozo-Agundo, E.; Rodríguez-Santamaría, M.; López-Fernández, A.; Corte-Torres, D.; Granda-Díaz, R.; et al. CDK7-Targeted Therapy Effectively Disrupts Cell Cycle Progression and Oncogenic Signaling in Head and Neck Cancer. *Signal Transduct. Target. Ther.* **2025**, *10*, 363. [CrossRef]

[80] Long, Z.; Grandis, J.R.; Johnson, D.E. Emerging Tyrosine Kinase Inhibitors for Head and Neck Cancer. *Expert. Opin. Emerg. Drugs* **2022**, *27*, 333–344. [CrossRef]

[81] Patysheva, M.R.; Kolegova, E.S.; Khozyainova, A.A.; Prostakishina, E.A.; Korobeynikov, V.Y.; Menyailo, M.E.; Iamshchikov, P.S.; Loos, D.M.; Kovalev, O.I.; Zavyalova, M.V.; et al. Revealing Molecular Mechanisms of Early-Onset Tongue Cancer by Spatial Transcriptomics. *Sci. Rep.* **2024**, *14*, 26255. [CrossRef]

[82] Ourailidis, I.; Stögbauer, F.; Zhou, Y.; Beck, S.; Romanovsky, E.; Eckert, S.; Wollenberg, B.; Wirth, M.; Steiger, K.; Kuster, B.; et al. Multi-Omics Analysis to Uncover the Molecular Basis of Tumor Budding in Head and Neck Squamous Cell Carcinoma. *npj Precis. Oncol.* **2025**, *9*, 73. [CrossRef]

[83] Arora, R.; Cao, C.; Kumar, M.; Chanda, A.; Samuel, D.; Matthews, W.; Chandarana, S.; Hart, R.; Dort, J.C.; Hyrcza, M.; et al. Spatial Transcriptomics Unravels Novel Signaling Patterns at the Leading Edge of Oral Squamous Cell Carcinoma. *J. Clin. Oncol.* **2022**, *40*, e18043. [CrossRef]

[84] Sun, L.; Kang, X.; Wang, C.; Wang, R.; Yang, G.; Jiang, W.; Wu, Q.; Wang, Y.; Wu, Y.; Gao, J.; et al. Single-Cell and Spatial Dissection of Precancerous Lesions Underlying the Initiation Process of Oral Squamous Cell Carcinoma. *Cell Discov.* **2023**, *9*, 28. [CrossRef]

[85] Williams, H.L.; Frei, A.L.; Koessler, T.; Berger, M.D.; Dawson, H.; Michielin, O.; Zlobec, I. The Current Landscape of Spatial Biomarkers for Prediction of Response to Immune Checkpoint Inhibition. *npj Precis. Oncol.* **2024**, *8*, 178. [CrossRef]

[86] Gong, D.; Arbesfeld-Qiu, J.M.; Perrault, E.; Bae, J.W.; Hwang, W.L. Spatial Oncology: Translating Contextual Biology to the Clinic. *Cancer Cell* **2024**, *42*, 1653–1675. [CrossRef]

[87] Eweje, F.; Li, Z.; Li, Y.; Bergstrom, C.P.; Kim, T.; Olguin, F.; Willens, S.H.; Gopaulchan, M.; Nirschl, J.; Neal, J.W.; et al. Digital Pathology–Based AI Spatial Biomarker to Predict Outcomes for Immune Checkpoint Inhibitors in Advanced Non-Small Cell Lung Cancer. *J. Clin. Oncol.* **2025**, *43*, 8569. [CrossRef]

[88] Mi, H.; Sivagnanam, S.; Ho, W.J.; Zhang, S.; Bergman, D.; Deshpande, A.; Baras, A.S.; Jaffee, E.M.; Coussens, L.M.; Fertig, E.J.; et al. Computational Methods and Biomarker Discovery Strategies for Spatial Proteomics: A Review In Immuno-Oncology. *Brief. Bioinform.* **2024**, *25*, bbae421. [CrossRef] [PubMed]

[89] Desilets, A.; Le, M.T.; Lucas, J.; Matcovitch-Natan, O.; Bart, A.; Laniado, A.; Azulay, M.; Markovits, E.; Kerner, J.K.; Gutwillig, A.; et al. Spatial Biomarker-Driven Deep Learning Model via Digital Pathology Predicts Response to PI3K Inhibitor Buparlisib in Head and Neck Squamous Cell Carcinoma. *medRxiv* **2025**. [CrossRef]

[90] Canini, V.; Eccher, A.; d'Amati, G.; Fusco, N.; Maffini, F.; Lepanto, D.; Martini, M.; Cazzaniga, G.; Paliogiannis, P.; Lobrano, R.; et al. Digital Pathology Applications for PD-L1 Scoring in Head and Neck Squamous Cell Carcinoma: A Challenging Series. *J. Clin. Med.* **2024**, *13*, 1240. [CrossRef] [PubMed]

[91] Li, Y.; Zhong, F.; Liu, L. Computational Identification of FOXP3-Associated Spatial Prognostic Markers in HCC via Digital Pathology. *Comput. Methods Programs Biomed.* **2026**, *273*, 109119. [CrossRef]