Journal of Oral Biology and Craniofacial Research 15 (2025) 1862–1873

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Artificial Intelligence–driven spatial transcriptomics in OSCC: Mapping the tumor microenvironment and personalizing therapy

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ARTICLE INFO

Keywords:
Oral squamous cell carcinoma (OSCC)
Spatial transcriptomics
Artificial intelligence
Tumor microenvironment
Precision oncology

ABSTRACT

Spatial transcriptomics (ST) represents a transformative approach in cancer research, offering high-resolution insights into the spatial organization of gene expression within tissues, particularly relevant for the complex tumor microenvironment (TME) of oral squamous cell carcinoma (OSCC). Unlike conventional bulk RNA sequencing, which masks spatial heterogeneity, ST retains the architectural context of tumors, enabling the mapping of molecular gradients, tumor–stroma interactions, and immune cell localization. Various ST platforms—such as 10x Genomics Visium, Slide-seqV2, MERFISH, NanoString GeoMx DSP, CosMx SMI, and BGI.

Stereo-seq—each offers unique advantages in resolution, sample compatibility, and transcriptome depth. Their application in OSCC has led to the identification of spatially distinct gene signatures, aiding in the stratification of tumor subtypes and uncovering novel prognostic markers. Furthermore, the integration of ST with artificial intelligence (AI) and machine learning has enhanced its analytical capabilities, enabling automated feature extraction, spatial clustering, and predictive modeling of disease progression. Despite these advancements, limitations such as high computational demands, limited access to fresh-frozen tissues, and platform-specific biases persist. Nonetheless, the synergy between ST and AI heralds a new era in precision pathology, with the potential to revolutionize diagnosis, risk assessment, and personalized therapeutic strategies for OSCC.

1. Introduction to spatial transcriptomics and OSCC

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity, comprising over 90 % of head and neck cancers.[1,27–29] Despite advances in surgery, chemotherapy, and radiotherapy, long-term survival in advanced OSCC remains below 50 %. A major reason for treatment failure is the complex tumor microenvironment (TME) of OSCC, which includes not only malignant epithelial cells but also immune cells, fibroblasts, blood vessels, and other stromal components.[2,30]. These diverse cell types interact in space to influence tumor growth, invasion, metastasis, and therapy response. Traditional bulk genomic assays and even single-cell sequencing lose spatial context, making it difficult to understand how cellular neighborhoods and tissue architecture drive OSCC progression. In this context, spatial transcriptomics (ST) has emerged as an ideal approach to profile gene expression in situ within intact tissue sections.[3,31] By preserving spatial coordinates, ST allows mapping of where specific transcripts are expressed and which cell types co-localize, thereby providing a new window into TME organization and heterogeneity.[32,33]

Spatial transcriptomic technologies have undergone rapid evolution over the past few years. Approaches can be broadly classified into: (a) imaging-based in situ hybridization or sequencing,[10] which measure transcripts of selected genes at single-molecule resolution, and (b) spatial barcoding and sequencing, which capture transcripts from tissue positions using barcoded arrays for high-throughput sequencing.[4–6] Each platform balances resolution, throughput, and practicality (Table 1). For example, 10x Genomics' Visium is a widely used barcoding platform with unbiased whole-transcriptome coverage but

This article is part of a special issue entitled: AI matters published in Journal of Oral Biology and Craniofacial Research.

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https://doi.org/10.1016/j.jobcr.2025.10.015
Received 4 August 2025; Received in revised form 7 October 2025; Accepted 16 October 2025

captures spots ~55 μm in diameter (encompassing dozens of cells). In contrast, in situ methods like MERFISH (MERSCOPE) achieve single-cell or subcellular resolution, but only for a predefined panel of transcripts.[7,8] Newer techniques aim to improve this trade-off: Slide-seq and Slide-seqV2 use 10 μm barcoded beads for near single-cell resolution at whole-transcriptome scale, while Nanostring's GeoMx Digital Spatial Profiler (DSP) uses DNA-barcoded probes to quantify RNAs or proteins in user-selected regions of interest (ROIs) from formalin-fixed tissues. Recent platforms like 10x Xenium and.

Nanostring CosMx combines high multiplexity (measuring 1000s of genes) with single-cell resolution imaging on FFPE samples. These technological advances now enable spatial mapping of gene expression in OSCC tumors with unprecedented detail.[9,11]

This review is structured as a narrative synthesis. To ensure comprehensive coverage, we searched PubMed, Scopus, and Web of Science (2016–2025) using combinations of keywords related to *spatial transcriptomics* (e.g., "Visium", "Slide-seq", "MERFISH", "GeoMx", "CosMx", "Stereo-seq"), *artificial intelligence* (e.g., "machine learning", "deep learning", "graph neural networks"), and disease terms (*oral squamous cell carcinoma*, OSCC, *head and neck squamous cell carcinoma*, HNSCC). Additional references were identified by manual screening of citations in key papers and reviews.

By applying these ST technologies to OSCC, researchers can spatially chart the tumor and its microenvironment, identifying where different cell types localize, which genes are active in the invasive front versus the tumor core, and how therapy may reshape the spatial landscape. Crucially, spatial transcriptomics generates immense, complex datasets (often with thousands of spatial spots or single cells and thousands of genes), which require advanced computational analyses. This is where artificial intelligence (AI) and machine learning (ML) come into play. AI-driven analytics can unravel patterns from high-dimensional spatial data that are beyond human intuition, such as subtle gene expression gradients or multicellular interaction networks predictive of outcomes.[12] For a broad audience of biomedical researchers, clinicians, and data scientists, this review will first provide an overview of AI/ML techniques developed for spatial 'omics, then examine how these approaches are being used to map the OSCC TME and derive clinically relevant biomarkers. We discuss technical considerations (platforms, data integration strategies) in parallel with translational applications (tumor heterogeneity, biomarker discovery, therapy guidance). Key subtopics include.

- ➢ Introduction to spatial transcriptomics and OSCC (the current section),
- ➢ Overview of AI/ML techniques in spatial omics,
- ➢ Mapping the tumor microenvironment in OSCC,
- ➢ Clinical implications for therapy stratification and personalized treatment, and ¬ Current challenges and future perspectives.

Several recent reviews have discussed spatial transcriptomics in oncology and head and neck cancers [Choi et al., 2025; Nieszporek et al., 2025; Jin et al., 2024; Zahedi et al., 2024]. However, these works primarily focus on either broad tumor immune microenvironments or technical advances in spatial profiling, with limited coverage of artificial intelligence. In contrast, our review provides a focused synthesis of AI-driven spatial transcriptomics in OSCC, including a comparative analysis of models (Table 1), a chronological outline of landmark discoveries, and a forward-looking discussion on challenges and translational potential. This OSCC-centric lens and emphasis on AI methodology distinguish our contribution from existing literature.[34–37]

Through this structured narrative, we aim to demonstrate how AI-driven spatial transcriptomics is transforming our understanding of OSCC biology and opening new avenues for precision oncology in this disease.

2. Overview of AI and machine learning techniques in spatial omics

Spatial transcriptomic data present unique computational challenges: the datasets are inherently multimodal (combining gene expression with spatial coordinates and often histology images), high-dimensional, and noisy. Early spatial studies often employed standard single-cell RNA-seq analysis tools; however, these proved inadequate for capturing the spatial structure and cell interactions within tissues. To fully exploit spatial omics, specialized statistical and machine learning methods have been developed. Moreover, the integration of deep learning (DL) has shown great promise in handling the scale and complexity of spatial data, automatically learning features from raw inputs like tissue images and expression matrices. Fig. 1 provides a conceptual overview of how computational approaches are applied in spatial transcriptomics – from identifying spatial expression patterns and domains, to integrating single-cell and spatial data, to inferring cell–cell interactions and reconstructing spatial trajectories.

The workflow begins with spatially resolved gene expression profiling (top left) to capture gene expression patterns in their original tissue context. Single-cell RNA sequencing (top right) is performed in parallel using cell reagents and barcode printing to generate high-resolution cell-level profiles. The resulting datasets undergo spatial

Table 1
Representative spatial transcriptomics platforms and features.

Platform (Year)	Approach (Coverage)	Spatial Resolution	Sample Compatibility	Key Strengths	Key Limitations
10x Visium (2019)	Barcoded spot array (whole transcriptome)	~55 μm spot (captures ~5–50 cells)	Fresh-frozen (full assay); FFPE (targeted panel version)	High throughput, unbiased gene coverage; widely adopted for tissue-wide mapping	Moderate resolution (multiple cells per spot); fresh tissue required for full transcriptome
Nanostring GeoMx DSP (2019)	ROI-based digital profiling (targeted panels)	ROI user-defined (~10–50 μm)	FFPE or fresh-frozen	Works on standard FFPE; RNA & protein profiling; tumor vs stroma ROI selection.	Not single-cell; limited to targeted panels (~100s of genes)
Slide-seq V2 (2021)	Barcoded bead array (whole transcriptome)	~10 μm bead (near single-cell)	Fresh-frozen	Very high spatial resolution (approaches single-cell); genome-wide	Lower sensitivity (fewer transcripts/spot); complex custom bead prep
MERFISH/ MERSCOPE (2021)	Multiplexed in situ FISH imaging (targeted genes)	Single-cell to subcellular (0.1–1 μm)	Fresh-frozen or fixed tissue	True single-cell resolution; RNA localization; highly multiplexed (500–1000 genes)	Preselected gene panels (not whole transcriptome); lengthy iterative imaging
BGI Stereo-seq (2021)	DNA nanoball array + sequencing (whole transcriptome)	0.5 μm spots (subcellular); cm-scale tissue area	Fresh-frozen	Ultra-high resolution and very large area coverage	Enormous data volume; lower per-spot sensitivity (needs binning); limited availability
Nanostring CosMx SMI (2022)	Single-molecule in situ hybridization (multiplexed)	Single-cell & subcellular (<10 μm)	FFPE or fresh-frozen	Detects 1000s of RNAs & proteins at true single-cell level; FFPE compatible	Targeted gene set; requires specialized imaging & complex analysis.

Table 1 - Each spatial transcriptomics platform involves trade-offs between resolution, gene throughput, and sample requirements. High-resolution methods often target selected genes, whereas array-based sequencing methods profile transcriptomes unbiasedly but at coarser resolution.

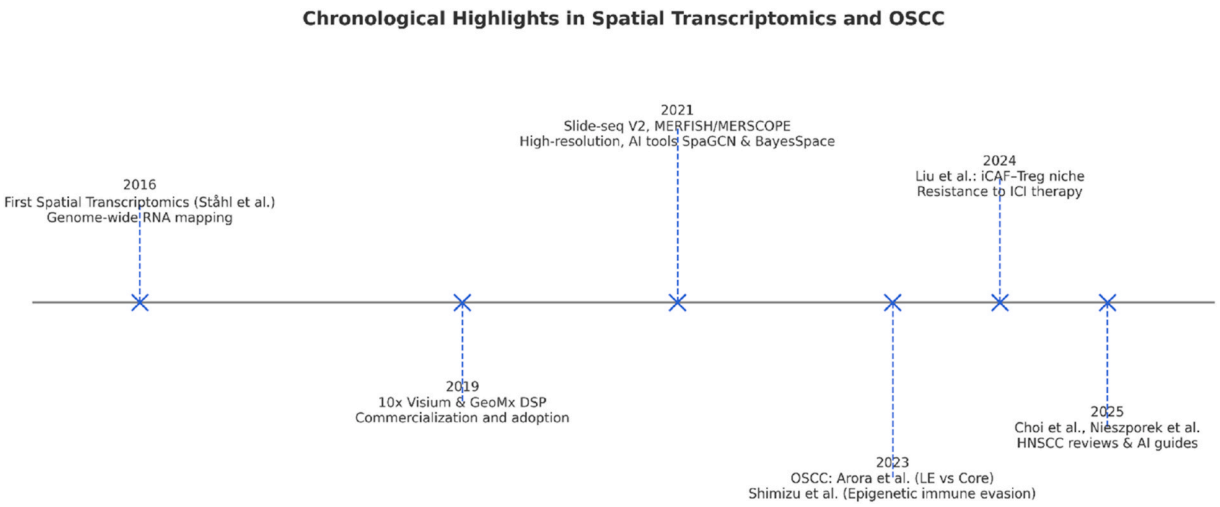


Fig. 1. Chronological highlights in spatial transcriptomics and OSCC.
Timeline of key milestones from the first spatial transcriptomics method in 2016 to OSCC-specific discoveries in 2023–2024 and integrative AI-driven approaches in 2025, highlighting the progression from technology development to clinical translation.

decomposition(middle) to assign cell-type–specific expression patterns to defined tissue locations. This is followed by spatial reconstruction (middle right), which maps gene expression back onto the tissue architecture. Cell–cell and gene–gene interactions (middle left) are then inferred to understand molecular communication within the tissue. Finally, the process includes ligation and sequencing (bottom) to obtain high-fidelity transcriptomic data for integrated analysis.

In general, machine learning methods for spatial transcriptomics can be grouped by the specific analysis tasks they address. Major tasks include: (1) Spatial domain identification – finding regions in the tissue with distinct expression programs; (2) Spot or pixel deconvolution – inferring the cell types or states present in a mixed capture spot; (3) Spatially variable gene detection – identifying genes with expression patterns that are non-random in space; (4) Cell–cell interaction inference – predicting how neighboring cells communicate via ligand–receptor pairs or other signals; and (5) Data integration – combining spatial data with other modalities (e.g., histology images, single-cell sequencing, proteomics) for a more comprehensive analysis. Table 2 summarizes some representative AI/ML techniques used in these areas, illustrating how they improve upon simpler methods.

For instance, Arora et al.[1] applied *graph convolutional networks (GCNs)* to spatial transcriptomic data to classify tumor-core versus leading-edge regions, integrating gene-expression matrices and spatial coordinates from 12 OSCC samples (≈20,000 spatial spots). Their GCN model (SpaGCN) achieved over 90 % concordance with histological annotation, revealing conserved invasive-edge transcriptional programs across patients and tumor types.[1] Likewise, Liu et al.[2] used a *Bayesian deconvolution framework (Cell2location)* combining single-cell RNA-seq

Table 2
AI/ML techniques for spatial transcriptomics analysis.

Task & Methodology	Purpose and Approach	Example Tools/Models (Year) and Notes
Histology image integration – Deep learning on tissue images	Combines morphological features from histology (e.g., H&E sections) with spatial gene expression to refine clustering or predict gene expression. CNNs extract image features that are fused with transcriptomic data.	ST-Net (2019): CNN model trained to predict spatial gene expression patterns directly from histology; useful for virtual staining and niche identification. stLearn (2020): Integrates Visium data with histology via pretrained CNN features, improving spatial coherence of clusters.
Spatial clustering/domain identification – Graph Neural Networks	Unsupervised identification of tissue domains based on gene expression and spatial proximity. Graph-based models learn region patterns, often integrating histology with expression for improved accuracy.	BayesSpace (2020): Bayesian model that refines 10x Visium data by detecting subspot clusters; applied to tumor/stroma boundaries in OSCC. SpaGCN (2021): GCN model combining expression, spatial coordinates, and histology; identifies anatomically meaningful tumor regions.
Cell type deconvolution – Bayesian and matrix factorization	Estimates cell type composition of each spatial spot using scRNA-seq references. Methods use non-negative matrix factorization or Bayesian regression to assign proportions or absolute counts.	SPOTlight (2020): NMF-based deconvolution aligning spots to scRNA-seq signatures; applied to immune/stromal mapping in OSCC. cell2location (2021): Bayesian model integrating scRNA-seq references; robustly estimates absolute cell counts; applied in HNSCC immune mapping.
Cell–cell interaction and ligand–receptor modeling	Analyzes spatial neighbors to infer cell–cell interactions and ligand–receptor signaling. Graph-based methods detect communication patterns and community structures in tissues.	NicheNet (2020): Predicts ligand–receptor activity; in OSCC, showed CAF–T cell signaling via CXCL12–CXCR4. Giotto (2021): Pipeline for spatial cell–cell network analysis; identifies immune clustering around tumor niches. Emerging GCN-based models predict cell fate influenced by neighbors.
Spatial trajectory and pseudo-time analysis	Infers spatial developmental trajectories or gradients (e.g., tumor core → invasive front). Uses pseudo-time algorithms adapted from scRNA-seq and integrates RNA velocity for future-state prediction.	Spatial pseudotime (2022): Adapted Monocle/Slingshot approaches to spatial data; maps tumor progression. RNA velocity with spatial: Applied by Arora et al. (2023) to OSCC ST data, revealing gradients of tumor cell differentiation from core to edge.

and 10x Visium datasets (~40,000 spatial spots) to predict fibroblast–-Treg immunosuppressive interactions, further validated through *NicheNet* ligand–receptor modeling.[2] Collectively, these AI-driven frameworks—spanning deep learning (GCN/CNN) and probabilistic regression approaches—demonstrate how spatial transcriptomics can be computationally modeled to uncover clinically relevant prognostic and therapeutic patterns in OSCC.

Machine learning enables advanced analysis of spatial omics data, ranging from the unsupervised discovery of tissue regions to integration with imaging and the decoding of intercellular communication. For example, deep learning models like SpaGCN automatically learn informative features from combined histological and gene expression inputs to delineate spatial domains in a tissue. Likewise, incorporating single-cell references via probabilistic models (Cell2location, SPOTlight) improves cell type mapping in each region. These AI-driven methods provide a more holistic view of tissue organization than traditional analyses.[14–16]

Graph-based clustering methods such as *SpaGCN* and *BayesSpace* have advanced domain identification in spatial data. *SpaGCN* constructs an adjacency graph where each spatial spot is treated as a node, with edges representing neighborhood proximity, and integrates histology features with gene expression to learn domain-specific embeddings. *BayesSpace*, on the other hand, improves resolution by computationally subdividing 55 μm Visium spots into subspots and assigning them probabilistically to clusters, thereby refining tumor–stroma boundaries and enabling more precise mapping of invasive fronts.[12,14]

For cell type deconvolution, approaches such as *SPOTlight* and *cell2location* integrate spatial transcriptomic profiles with single-cell references. *SPOTlight* applies non-negative matrix factorization to align mixed spatial spots with scRNA-seq–defined signatures and outputs cell-type proportions. *cell2location* employs Bayesian regression to estimate absolute cell counts per spot, reducing dropout-related bias and enabling robust quantification of immune infiltration in HNSCC and OSCC tissues.[15,17]

Spatially variable gene (SVG) detection methods, such as *SpatialDE* and its derivatives, model gene expression variance as a function of spatial coordinates, distinguishing random fluctuations from genuine spatial enrichment. These tools have been widely used to uncover edge-specific EMT signatures, localized immune gradients, and other drivers of intratumoral heterogeneity.[16,17]

In the area of cell–cell communication, *NicheNet* integrates prior ligand–receptor knowledge with transcriptomic output to predict active signaling between neighboring cell types. In OSCC, this approach has confirmed that cancer-associated fibroblasts communicate with T cells through the CXCL12–CXCR4 axis, explaining immunosuppressive niches. More recent graph-based machine learning models extend this principle by automatically detecting recurrent interaction motifs in cell neighborhoods and predicting how these relationships influence cellular fate.[2,40]

Image–omics fusion is another major frontier. Tools such as *stLearn* extract histology features (e.g., H&E morphology) with pretrained convolutional neural networks and integrate them with spatial expression data, yielding anatomically coherent clusters. *ST-Net* goes a step further by predicting gene expression directly from histology, functioning as a virtual staining platform that bridges pathology with molecular readouts.[18]

Finally, emerging architectures, including graph neural networks, transformers, and multimodal integration models, are pushing the field beyond clustering and deconvolution. These frameworks are now being applied to combine gene expression, spatial relationships, and histological features in a unified space, supporting predictive modeling of prognosis, immune response, and therapeutic stratification in OSCC. Together, these AI-based methodologies represent a rapidly evolving toolkit that provides unprecedented resolution for dissecting OSCC subclonal niches, decoding immune–stromal interactions, and guiding precision oncology.

In practice, many spatial transcriptomics studies use a combination of these methods.[16,17] A typical workflow might first perform unsupervised clustering to find major spatial domains, then deconvolve each domain to identify constituent cell types, and finally analyze cell–cell interactions within and between domains (often correlating with histology). For OSCC specifically, machine learning has been pivotal in integrating multimodal data – for instance, combining spatial transcriptomes with *The Cancer Genome Atlas* (TCGA) bulk expression or with proteomic data to validate discovered biomarker.[18,19] As we move into the next sections on OSCC tumor microenvironment mapping, we will highlight concrete examples where AI-driven analysis of spatial data uncovered new insights.

This table summarizes major categories of AI/ML approaches applied to spatial transcriptomics, highlighting representative models, their key strengths and limitations, and example applications in oral squamous cell carcinoma (OSCC). These models encompass tasks such as spatial domain identification, cell type deconvolution, detection of spatially variable genes (SVGs), cell–cell communication inference, and image–omics fusion. Collectively, they demonstrate how computational frameworks can dissect tumor heterogeneity, immune–stromal interactions, and clinically relevant biomarkers in OSCC (Table 3) (see Table 4).

3. Mapping the tumor microenvironment in OSCC

One of the most powerful applications of spatial transcriptomics is creating detailed maps of the tumor microenvironment (TME) in cancers like OSCC.[1,3,31] OSCC tumors are known for significant heterogeneity – not just genetically among tumor cells, but also in the composition and spatial arrangement of immune and stromal cells across the tumor mass. By preserving spatial context, ST allows us to ask: which cell types neighbor each other? How do gene expression profiles differ between the tumor's interior and the invasive edges? Where are immunosuppressive versus immunostimulatory regions located? Answering these questions can illuminate mechanisms of tumor progression and resistance unique to OSCC's anatomy (e.g., invasive fronts in oral tissues). Several recent studies have leveraged spatial transcriptomics (often combined with single-cell sequencing and AI analyses) to dissect the OSCC TME, revealing distinct spatial architectures with biological and clinical significance.

Table 3
Comparative analysis of AI models used in spatial transcriptomics.

Task	Representative models	Strengths	Weaknesses	Example application in OSCC
Domain identification	SpaGCN, BayesSpace, SPACEL	Integrates spatial + histology; sub-spot refinement	Sensitive to batch effects	Edge vs core tumor domains (Arora et al., 2023)
Deconvolution	cell2location, SPOTlight	Absolute cell quantification	Requires scRNA-seq reference	Mapping CAF/Treg infiltration
SVG detection	SpatialDE, trendsceek	Detects spatially variable genes	High false positives	EMT-related markers at invasive fronts
Cell–cell communication	NicheNet, graph models	Ligand–receptor inference	Reliant on priors	CAF–Treg CXCL12–CXCR4 signaling
Image–omics fusion	stLearn, ST-Net	Combines H&E morphology with ST	Needs high-quality co-registration	Predicting gene programs from H&E slides

Table 4
– Selected spatial biomarkers and findings in OSCC.

Spatial Feature (Biomarker)	Description and Clinical Association	Source (Open-Access Reference)
Leading Edge (LE) Gene Signature	Genes upregulated in tumor cells at the invasive front (LE zone). Enriched for partial EMT, migration, and immunomodulatory pathways. Clinical: Strongly associated with worse survival and higher metastasis risk. Patients with high LE signature (and low core signature) show poorer outcomes across multiple cancers.	Arora et al., *Nat Commun.* 2023
Loss of MHC-I & High EZH2/HDAC in Tumor Core	Tumor core regions with low expression of MHC class I genes (HLA-A, HLA-B/C) and high expression of epigenetic repressors (EZH2, HDACs). Clinical: Signifies immune evasion and resistance to anti–PD-1 therapy in OSCC. Suggests potential benefit of adding epigenetic therapy (HDAC/EZH2 inhibitors) to restore antigen presentation.	Shimizu et al., *Biomolecules* 2023
iCAF–Treg Immunosuppressive Niche	Spatial co-occurrence of inflammatory CAFs (HIF1A, CXCL12) with Tregs (TGFB1, CXCR4) in hypermetabolic tumor areas. Clinical: Indicates an immunosuppressed TME, correlates with ICI resistance. Suggests benefit from combining CAF-targeting agents (e.g., HIF1α/CXCL12 inhibitors) with immunotherapy.	Liu et al., *Int J Oral Sci.* 2024
Tertiary Lymphoid Structures (TLS) Location	Organized B- and T-cell aggregates in tumor periphery/stroma. Clinical: Presence of TLS is linked with improved survival and better immunotherapy response. Dense peritumoral TLS clusters predict longer disease-free survival.	Oh et al., *Front Immunol.* 2025
Spatial "Cold" Tumor Islands (Immune Deserts)	Tumor regions (often interior or stroma-separated) lacking T-cell infiltration, but enriched in suppressive myeloid cells/CAFs. Clinical: Predicts poor response to ICIs; patients with immune deserts may need priming strategies (e.g., TGF-β inhibitors).	Mes et al., *Front Immunol.* 2025

Spatial biomarkers in OSCC range from gene expression signatures confined to specific tumor regions to the physical presence of immune structures. Their predictive power often exceeds that of bulk measurements because they capture the heterogeneity of the tumor's ecosystem. For instance, an OSCC with a strong LE invasive signature and immunosuppressive CAF pockets would be considered high-risk and a candidate for aggressive or targeted therapy. In contrast, individuals with organized TLS at the margins may have an ongoing anti-tumor immune response and a better prognosis.

3.1. Tumor core vs leading edge: conserved architectures and heterogeneity

A striking example of spatial TME mapping in OSCC comes from Arora et al. (2023),[1] who performed an integrative single-cell and spatial transcriptomic analysis of human OSCC tumors. They specifically compared the tumor core (TC) to the leading edge (LE) – the invasive margin of the tumor. Unsupervised spatial clustering of malignant cell gene expression in 12 OSCC samples identified two major spatial domains corresponding to TC and LE, with a third "transitory" cluster sharing features of both. The leading edge regions had unique transcriptional programs: OSCC cells at the edge showed a partial epithelial–mesenchymal transition (p-EMT) gene signature associated with invasion. In contrast, core tumor cells expressed more differentiation markers. Importantly, these spatially-defined gene signatures were linked to patient outcomes – the LE gene signature was associated with worse clinical prognosis. In contrast, the core-enriched signature indicated improved survival across multiple cancer types. In other words, certain genes upregulated at the invasive front (LE) signified aggressive behavior and shorter survival, whereas core-specific genes correlated with better outcomes.[1] This suggests that OSCC tumors harbor at least two functionally distinct subregions: a peripheral invasive niche that is more aggressive and a central core that is less so.

Notably, Arora et al.[1] found the LE signature to be conserved across cancers (e.g., similar gene patterns at invasive fronts in other carcinoma types), indicating common mechanisms of tumor invasion. Using machine learning models, they demonstrated that LE regions exhibit shared features across different patients and even different tumor types, whereas TC regions are more tumor-specific. This conserved edge program included immunosuppressive factors and EMT-related genes, which may enable the tumor to invade surrounding tissue. The study also employed RNA velocity and spatial trajectory analysis to infer developmental relationships, revealing that tumor cells in OSCC could progress from a core-like state to an edge-like state, acquiring invasive characteristics as they migrate outward. By mapping these spatial dynamics, researchers identified potential therapeutic targets "bridging" the core and edge – for example, druggable pathways active at the interface that might be disrupted to prevent invasion. Using an *in silico* model,[1] Arora et al. predicted drugs that specifically affect the spatial signaling between TC and LE compartments (the "information flow" from core to edge). This underscores how spatial transcriptomics, guided by AI analysis, not only charts OSCC heterogeneity but also identifies potential interventions, such as targeting the invasive edge program, which could improve outcomes for patients with aggressive tumors.

3.2. Spatial immune landscapes: immune hotspots and cold niches

The immune contexture of OSCC – which immune cells are present and where – is a critical determinant of response to immunotherapy and overall prognosis. Spatial transcriptomics has unveiled that immune cells are *not uniformly distributed* within OSCC tumors. Instead, there may be immune-rich "hot" regions and immune-sparse "cold" regions corresponding to different functional states. A recurring observation is that CD8 T cells (cytotoxic T lymphocytes) tend to concentrate at the tumor invasive margins, whereas immunosuppressive cells accumulate deeper in the tumor mass.[38] In HNSCC, including OSCC, Frontiers researchers described that the invasion front (the interface between tumor and normal tissue) is often enriched in CD8 T cells, which can attack tumor cells, while the inner tumor and leading edge (the most advanced front) harbor more regulatory T cells (Tregs) and M2-polarized macrophages that suppress immune activity. These spatial patterns create an "immune-excluded" phenotype in parts of the tumor – even if T cells are present at the border, they may not effectively penetrate into tumor nests due to physical and molecular barriers.[19,20] Spatial transcriptomic mapping confirms such compartmentalization: for example, a high-resolution atlas of one OSCC tumor showed dense clusters of exhausted CD8 T cells and macrophages in subregions, co-localized with high expression of checkpoint molecules like PD-1/PD-L1 in those niches.[38]

By mapping immune cell gene signatures spatially, one can identify immunologically "hot" spots vs "cold" spots within the same tumor. "Hot" spots might feature active cytotoxic cells and interferon signaling, whereas "cold" spots might show upregulation of suppressive cytokines (e.g., TGF-β, IL-10) and exclusion of effector cells. Indeed, a spatial study of an OSCC case before and after anti-PD-1 immunotherapy found that pre-treatment, the tumor had active immune pathways (antigen

presentation, interferon-γ signaling) predominantly in certain regions. Still, after the tumor developed resistance, those immune-activated regions disappeared and were replaced by epigenetic and metabolic reprogramming pathways.[39] This suggests that the tumor evolved spatially to shut down local immune responses, possibly by recruiting suppressive cells or altering antigen presentation, which could be observed as new spatial patterns post-therapy. For instance, spatial analysis revealed that after therapy resistance, OSCC regions had markedly decreased expression of MHC class I genes (needed for T cells to recognize cancer) and increased expression of histone-modifying enzymes (e.g., HDACs, PRC2 complex) that can globally dampen antigen presentation. Such insights, only possible by comparing spatial profiles before vs after treatment, highlight how the immune landscape can be dynamically remodeled.[19,20]

Furthermore, spatial mapping can identify structures like tertiary lymphoid structures (TLS), which are lymph node-like aggregates of B cells, T cells, and dendritic cells within the tumor stroma. TLS often signifies an ongoing anti-tumor immune response.[22] In OSCC and other HNSCC, patients whose tumors contain TLS or dense B-cell clusters tend to have better outcomes. Using spatial transcriptomics or spatial proteomics to locate TLS in tissue can strengthen their value as biomarkers. For example, a study integrating spatial data in HNSCC found that regions with high B-cell density (characterized by germinal center-like structures) correlated with improved progression-free and overall survival.[23] These spatial immune "hotspots" could serve as predictive markers for who might respond to immunotherapy. On the other hand, identification of "cold" regions devoid of T cells but filled with suppressive fibroblasts or macrophages might explain primary resistance to immunotherapy in some OSCC cases.[24]

3.3. *Cancer-associated fibroblasts and metabolic niches*

Apart from immune cells, cancer-associated fibroblasts (CAFs) are key stromal components in OSCC that can modulate the TME. Spatial transcriptomics has provided new evidence for functional heterogeneity of CAFs across different tumor regions. A 2024 study by Liu et al. focused on how metabolic characteristics of different regions in OSCC influence the immune microenvironment,[2] particularly via fibroblast subtypes. By combining 10x Visium spatial transcriptomics with single-cell RNA-seq data and computational deconvolution, the authors distinguished hypermetabolic vs hypometabolic regions within OSCC tumors. Hypermetabolic regions (areas with high glycolysis and hypoxia scores) were mostly in the tumor epithelial zones, whereas surrounding stromal areas were less metabolically active. Strikingly, the hypermetabolic tumor regions were enriched not only in tumor cells but also showed increased CD4+T-cell infiltration and elevated TGF-β expression, suggesting an immunosuppressive milieu forming there.

Liu et al.[2] uncovered a specific cellular crosstalk axis: in hypermetabolic tumor regions, tumor cells produce excess lactate (a byproduct of glycolysis, i.e., the Warburg effect), which nearby fibroblasts uptake. This lactate uptake triggers those fibroblasts to differentiate into an inflammatory subtype (iCAFs) characterized by high HIF1α activity and secretion of the chemokine CXCL12. The spatial data showed that CXCL12 was highly expressed in fibroblasts, precisely in the lactate-rich tumor areas. CXCL12, in turn, attracts regulatory T cells (Tregs) to those regions (Tregs bear the CXCL12 receptor CXCR4, and the authors confirmed Tregs with the highest CXCR4 were localized in those zones). The recruited Tregs secrete TGF-β, compounding the immunosuppressive environment and blunting cytotoxic T-cell function. In summary, spatial transcriptomics delineated a metabolic-feedback loop: hypermetabolic tumor → lactate → iCAF activation (HIF1α^{+} fibroblasts) → CXCL12 → Treg recruitment → TGF-β → immune suppression. This axis was supported by spatial co-localization of lactate-regulated genes and the cell-type markers (e.g., spatial co-occurrence of high LDHA or HIF1α spots with fibroblast CXCL12 and nearby FOXP3 Tregs.

What makes this finding clinically important is that it identifies

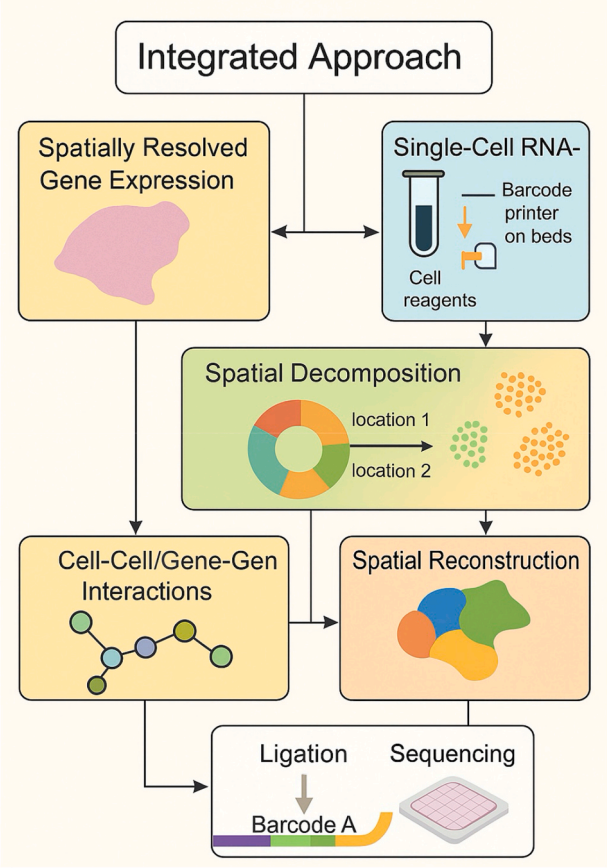
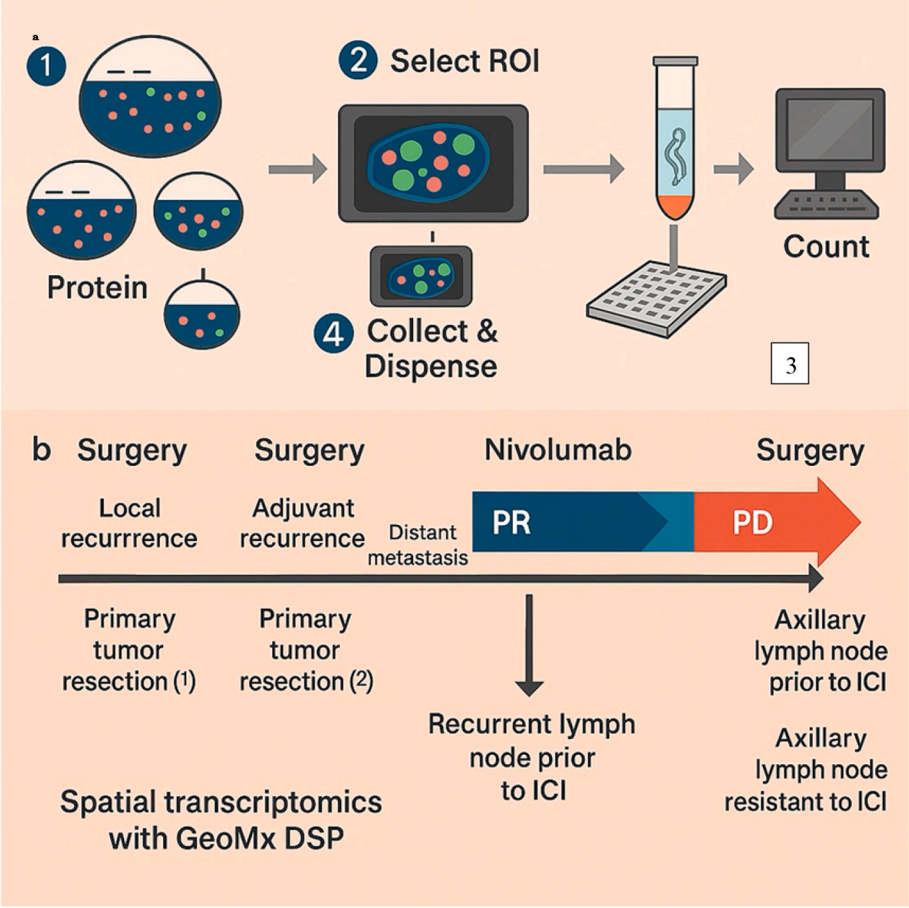


Fig. 2. Workflow of the integrated approach that combines spatially resolved gene expression profiling with single-cell RNA sequencing to enable comprehensive spatial transcriptomic analysis.

potential therapeutic targets within spatially confined niches of OSCC. For instance, targeting HIF1α in CAFs or CXCL12–CXCR4 signaling could disrupt the formation of Treg-rich suppressive pockets, thereby boosting immune attack on the tumor. Indeed, drugs like CXCR4 inhibitors or TGF-β blockers might be especially effective in tumors where this lactate-CAF-Treg axis is prevalent. Notably, these spatial niches might not have been evident without integrating multi-omics: the study combined spatial transcriptomics (to map gene expression and cell types in situ) with single-cell sequencing (to define cell subclusters and their gene programs) and *in vitro* experiments (treating CAFs with lactate) to validate the mechanism.[40] This underscores how AI-driven data integration is key – algorithms like NicheNet and cell–cell communication models helped predict and prioritize which ligand–receptor interactions (e.g., CXCL12–CXCR4) were enriched in the hypermetabolic niches. Computational metabolic scoring pinpointed which regions were "hot" in terms of metabolism. Together, these methods mapped a "metabolic immunosuppressive niche" in OSCC that links tumor metabolism with immune evasion.

In addition to CAFs and immune cells, spatial studies in OSCC and related cancers have identified other TME components with location-specific roles – for example, pockets of myeloid cells (monocytes/macrophages) in proximity to certain tumor cell clusters driving T-cell exhaustion, or endothelial cells lining blood vessels that create immune-privileged zones. The spatial regulation of immune surveillance vs escape is a recurring theme. Some regions of a tumor actively engage immune cells (forming TLS or immunostimulatory niches), while others physically or chemically exclude them. Spatial transcriptomics, especially when coupled with AI clustering and pattern recognition, can separate these regions and allow independent analysis of their gene



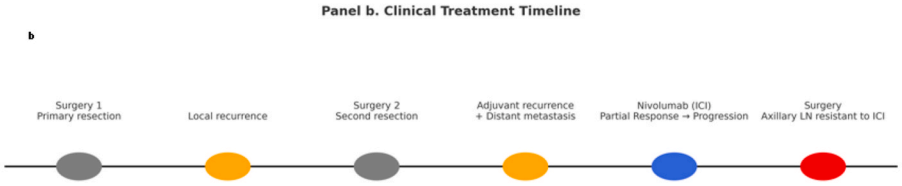


Fig. 2.1. Workflow for spatial transcriptomics analysis and clinical treatment timeline.
(a) Schematic representation of the GeoMx Digital Spatial Profiler (DSP) workflow. Step 1: Tissue preparation with protein staining. Step 2: Selection of the region of interest (ROI) for analysis. Step 3: Molecular profiling with extraction and barcoding of biomolecules. Step 4: Collection and dispensing of captured material for downstream quantification. The extracted signals are counted using an automated system.
(b) Clinical timeline showing the patient's treatment course. The sequence begins with primary tumor resection (Surgery 1), followed by local recurrence and a second resection (Surgery 2). The patient subsequently developed adjuvant recurrence and distant metastasis, after which nivolumab (immune checkpoint inhibitor, ICI) therapy was initiated. This initially achieved a partial response (PR), but the disease later progressed (PD). Spatial transcriptomics was performed on recurrent lymph node tissue before ICI therapy. Finally, axillary lymph node metastases resistant to ICI were surgically resected.

expression profiles.[25] For instance, one might find that an immune-excluded tumor core region has high expression of EZH2 (a suppressive epigenetic regulator) and low MHC-I, as reported in an immunotherapy-resistant OSCC case. In contrast, the periphery of the same tumor expresses chemokines attracting T cells. Each region could be treated differently – an epigenetic drug for the core and an immune checkpoint inhibitor for the periphery, highlighting a future of spatially informed combination therapies.[26]

Fig. 2 illustrates one approach to spatial transcriptomics in OSCC that facilitated these discoveries. It illustrates the workflow of GeoMx DSP, which was utilized in an OSCC study examining immunotherapy resistance. Tissue sections are stained for morphology, specific regions of the tumor and TME are selected for profiling, and RNA probes with UV-cleavable barcodes capture transcripts in those ROIs for sequencing By comparing ROIs from before and after therapy, researchers pinpointed the spatially localized changes (e.g., loss of antigen presentation signals in the tumor area after resistance). This ability to separately profile "tumor core" vs "tumor margin" vs "stromal band" ROIs is critical in OSCC, where a bulk average could mask these differences.[12,13]

In summary, AI-driven spatial transcriptomics is providing a detailed atlas of the OSCC microenvironment: distinguishing aggressive invasive edges, delineating immune cell niches, and identifying metabolically specialized regions that drive immune suppression. These spatial insights are not merely descriptive – as the next section will discuss, they carry direct implications for prognostication and therapy selection in OSCC.

4. Clinical implications: therapy stratification, prognosis, and personalized therapy

The ultimate goal of profiling the OSCC tumor microenvironment with advanced tools is to improve patient outcomes through personalized medicine. By understanding the spatial biology of an individual's tumor, clinicians could potentially predict which therapies are most likely to succeed, stratify patients into risk categories, and even design novel treatment combinations that target the patient's specific TME makeup. The integration of AI analytics ensures that subtle but clinically relevant patterns in spatial data (which might be missed by eye) are recognized and matched to outcomes. Several emerging clinical implications of AI-driven spatial transcriptomics in OSCC are evident.

4.1. Prognostic biomarkers from spatial signatures

Spatial transcriptomic analyses have yielded new candidate biomarkers that correlate with disease outcome more strongly than traditional bulk markers. For example, as described earlier, the *leading edge gene signature* identified by Arora et al.[1]. is prognostic in OSCC. Instead of single markers, this is a spatially-defined gene set – high expression of a cluster of invasion-associated genes at the tumor margin – which predicted poorer survival across multiple cohorts. This suggests that assessing an OSCC biopsy for the presence and intensity of an "invasive edge program" (e.g., via imaging or multiplex assays for a subset of those genes) could help identify high-risk patients who might benefit from more aggressive or alternative therapies. Similarly, the spatial immune context matters: one study found that patients with HPV-negative HNSCC whose tumors had T-cell-inflamed margins (high CD8 expression at the interface) but immunosuppressive cores had intermediate outcomes, whereas those lacking T cells, even at the margins, had the worst outcomes.[19,20] A practical implication is that spatially resolved immune scores (for instance, an "edge CD8/Treg ratio") might outperform conventional immunoscore that ignores location. Indeed, the presence of TLS or B-cell clusters in the tumor periphery has been linked to improved survival in OSCC – a patient whose spatial map shows robust TLS formation might have a better prognosis and possibly better response to immunotherapy.[22,23] Table 3 lists some key spatially resolved biomarkers emerging in OSCC research.[1]

4.2. Therapy stratification and selection

The heterogeneity revealed by spatial transcriptomics suggests that not all OSCC patients will respond equally to a given therapy, and AI can help stratify patients by matching tumor spatial profiles to likely effective treatments. For example, patients whose OSCC tumors show an "immune hot" phenotype (lots of $CD8^+$ T cells infiltrating, high interferon signature spatially) might be prime candidates for immune checkpoint inhibitor (ICI) therapy like anti-PD-1, as their tumor microenvironment is already conducive to T-cell activity.[19,20]

Conversely, patients with "immune cold" tumors (few T cells in any region, or T cells stuck only at the rim without infiltrating) might need immunotherapy combinations – perhaps adding a TGFβ blocker or VEGF inhibitor to remodel the stroma and allow T-cell entry.[22] Spatial analysis can identify such features: one study noted that OSCC tumors resistant to ICIs often had T cells confined to the stromal septa and an absence of them in tumor cell nests. These would be stratified as "excluded" phenotype and enrolled in trials of ICI plus stroma-targeting agents.[23]

Another angle is targeted therapy: OSCC doesn't have many common targetable mutations (aside from EGFR, which is variably expressed), but spatial transcriptomics can reveal new vulnerabilities. For instance, the discovery of high CXCL12–CXCR4 activity in certain OSCC spatial niches suggests using CXCR4 inhibitors (like AMD3100) to disrupt Treg recruitment.Patients whose spatial data indicate a potential pathway could be selected for trials of CXCR4 inhibitors combined with immunotherapy.[2,40] Similarly, an OSCC with high EZH2 in the tumor core (spatially) could be a candidate for adding an EZH2 inhibitor; interestingly, EZH2 upregulation spatially correlates with MHC-I downregulation, so an EZH2 inhibitor might restore antigen presentation and synergize with immunotherapy.[2] AI tools can help here by connecting spatial features to drug databases – for example, by using algorithms to find which gene expression modules (in specific regions) map onto known drug targets or pathways. Arora et al.[1] did an *in silico* screen using their spatial data to find compounds that would disrupt the signaling between the OSCC core and edge compartments. They identified candidate drugs that theoretically could preferentially affect the leading edge cells (which drove invasion) without as much toxicity to core cells. While such predictions require experimental validation, they represent a future where AI-driven spatial analyses directly inform drug choice.

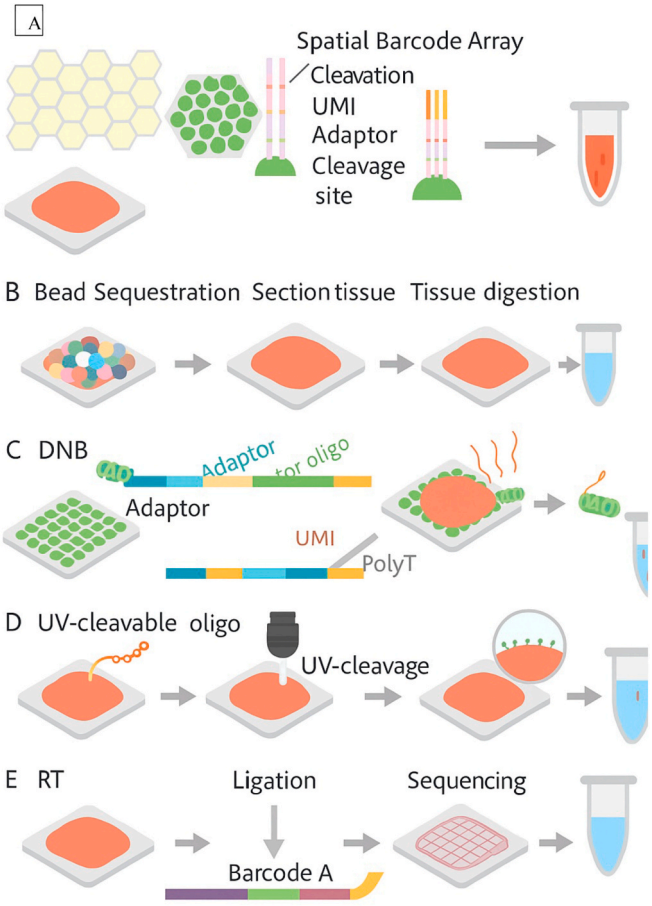


Fig. 3. Schematic overview of spatial transcriptomics workflows.
(A) Spatial barcode array method: A tissue section is placed on an array containing spatial barcodes, unique molecular identifiers (UMIs), adaptors, and cleavage sites. Messenger RNA (mRNA) released from the tissue binds to the barcodes for spatially resolved capture.
(B) Bead sequestration method: Barcoded beads are deposited on a slide, followed by tissue sectioning and digestion. The released mRNA hybridizes to bead-bound oligonucleotides.
(C) DNA nanoball (DNB) capture method: DNB arrays with adaptor and capture oligonucleotides hybridize to mRNA via polyT tails. UMIs and adaptors are incorporated during capture for subsequent sequencing.
(D) UV-cleavable oligo method: Oligonucleotides attached to tissue sections are released upon ultraviolet (UV) light exposure, enabling spatially resolved RNA capture.
(E) Reverse transcription (RT) and library preparation: Captured mRNA is reverse-transcribed, ligated with barcodes, and sequenced, preserving spatial information.

4.3. Personalized combination therapies

Perhaps the most exciting implication is designing *personalized combination therapies* based on an individual tumor's spatial makeup. Since OSCC TMEs are so heterogeneous, a monotherapy might only hit part of the tumor's "ecosystem." Spatial transcriptomics could allow a clinician to mix and match therapies to cover all bases. For a hypothetical example: an OSCC patient's spatial profile shows (a) a strongly immunosuppressive, fibroblast-rich region with high TGF-β (suggesting poor immune penetration), and (b) an area of rapidly proliferating tumor with an active EGFR pathway.[25,26] A personalized plan might combine an anti-EGFR agent (cetuximab) to slow tumor cell growth with a TGF-β inhibitor to modulate the stroma, plus possibly an ICI if any T cells are present to be reinvigorated. Another patient's tumor might exhibit an HPV-negative immune-desert phenotype with focal TLS; they may benefit from therapies that expand TLS-driven responses (e.g., agonists for CD40 to activate dendritic cells within the TLS).[23,24] The key is that spatial data provides actionable information about multiple targets – AI can parse this high-dimensional data and suggest combinations likely to be effective. Indeed, approaches such as Bayesian modeling or network analysis can be applied to spatial data to predict how blocking one pathway might ripple through the tumor microenvironment.[38,40]

It's worth noting that traditional pathology has always recognized spatial patterns (like "inflamed margins" or "perineural invasion"), but AI-enhanced spatial transcriptomics quantifies these patterns in an unbiased way and links them to molecular pathways.[24] This could also bridge into the clinic via digital pathology: once spatial transcriptomic studies identify which patterns matter (e.g., "T cells at margin, none in center" or "TLS present"), computer vision algorithms could be trained on routine histology to detect those patterns as a proxy. Some early work outside OSCC has shown that deep learning on H&E images can predict gene expression profiles and even outcomes. For OSCC, one can imagine an AI pathology tool that, informed by spatial transcriptomic ground truth, automatically scores a tumor as "immune-high invasive front, CAF-rich core," which then maps to a specific treatment plan.[38]

Lastly, spatial analyses help in monitoring therapy response. By biopsying an OSCC during therapy and doing spatial profiling, one could see if previously "cold" regions are turning "hot" (a good sign, therapy is working by recruiting T cells) or if the tumor is evolving new escape niches (e.g., upregulating alternative checkpoints like TIM-3 or LAG-3 in specific areas).[2] This level of monitoring, although experimental now, could guide mid-course corrections in treatment. For example, if an on-treatment biopsy shows worrying spatial changes – say an increase in *iCAF signature* despite immunotherapy, suggesting the tumor is mounting stromal resistance – a clinician might add a CAF-targeting drug at that point. AI can detect these changes more quickly and reliably than manual examination by comparing the patient's baseline and follow-up spatial data and highlighting any significant new patterns.[40]

To concretize these ideas, Fig. 3 shows a compilation of spatial transcriptomics techniques and how they contribute to personalized oncology (see Fig. 2.1). Each panel represents a different approach to index the tumor's spatial heterogeneity – from unbiased sequencing capturing whole regions, to high-resolution mapping of cell neighborhoods, to multimodal mapping of RNAs and proteins in situ. Together, they allow us to inventory all the "habitats" within a tumor and devise strategies to eliminate each.

4.4. Clinical translation and ongoing trials

While much of the above is still in the research phase, the translation to clinical practice is underway. Some centers are beginning to perform spatial transcriptomic analysis on diagnostic biopsies of patients in clinical trials to see if spatial features predict response (for example, in trials of immunotherapy for head and neck cancer, retrospective spatial analysis might identify which patients had T-cell-inflamed margins correlating with response).[22] Over time, this could lead to spatial

Table 5
– Challenges in AI-Driven spatial transcriptomics and emerging solutions.

Major Challenge	Description and Impact on OSCC Research	Emerging Solutions and Future Directions
Resolution vs Throughput Trade-off	No single platform yet captures *all* genes at single-cell resolution in whole OSCC tumors. 10x Visium has an unbiased transcriptome but mixes cells in a spot; FISH-based methods resolve single cells but cover a limited genes. This can obscure signals (e.g., immune cells diluted in a multi-cell spot) and leave gaps in data (undetected genes).	New high-res methods (Slide-seqV2, HDST, Stereo-seq) are closing the gap by increasing resolution and sensitivity. Integrative algorithms (e.g., using scRNA-seq to deconvolute spots) recover single-cell information. Future spatial assays may combine sequencing and imaging (joint RNA–protein mapping) to get both breadth and single-cell detail.
Sample Prep and Cost	Spatial profiling requires intact RNA and morphology, which is challenging for OSCC biopsies that are often FFPE. Until recently, many techniques needed fresh tissue, limiting clinical applicability. Additionally, experiments are costly and labor-intensive (requiring special equipment and long imaging times), which hinders large patient studies.	FFPE-compatible platforms (GeoMx, CosMx, Xenium) now enable spatial profiling on routine OSCC pathology samples. Protocol improvements (automation of sectioning, optimized permeabilization) are increasing reliability. Costs are expected to drop as technology matures and targeted panels (focusing on key OSCC genes) become available for clinical use. AI may aid by *virtualizing* some assays – e.g., predicting spatial expression from cheaper histology, thereby reducing the need for full experiments.
Computational Complexity and Scale	OSCC spatial datasets are large (spots × genes), with inherent noise (many zero values). Analyzing and storing these data requires significant computational resources and expertise in statistics, which can be a barrier for clinical labs. AI models risk overfitting due to the relatively small size of training datasets and heterogeneity between experiments.	Advanced algorithms specifically designed for spatial data are emerging (e.g., graph-based smoothing, zero-inflation models to handle dropouts). Efforts to build large pooled datasets and spatial atlases of OSCC will provide training data for robust AI models. Cloud computing and specialized software (some open source) are increasingly available to labs for handling big data. The community is also developing standard pipelines that conceal complexity behind user-friendly interfaces, enabling broader adoption.
Interpretability of AI Models	Complex ML/DL models can be "black boxes," making it hard to explain *why* a region was classified as high-risk or *which* features drove a prediction. Clinicians may be wary of acting on AI results without biological clarity.	Explainable AI approaches are being integrated – e.g., SHAP or LIME algorithms to highlight top contributing genes or histology features to an AI decision. In research, combined analysis (AI + human expert pathology) is used to validate that AI-detected spatial patterns make sense (e.g., the model identifies "immune cold island" – confirmed by the pathologist). Over time, interpretable signatures (like a defined 5-gene panel in the margin) can be distilled from

(*continued on next page*)

Table 5 (*continued*)

Major Challenge	Description and Impact on OSCC Research	Emerging Solutions and Future Directions
Data Integration and Sharing	Merging multimodal data (transcriptomics, proteomics, imaging) for a holistic view is nontrivial – different scales and formats must be aligned. Also, the lack of centralized databases means that limited comparative studies are available; each lab's OSCC spatial data may be siloed.	AI models and adopted as biomarker tests. Integrative analysis frameworks (such as joint embedding of spatial transcriptomics and imaging data) are under development, often leveraging AI to find common structure. For example, algorithms can align H&E images with spatial gene expression maps to annotate regions. Meanwhile, consortia like the Human Tumor Atlas is beginning to compile spatial datasets. The push for data sharing in journals is leading to an increase in spatial data being made available in public repositories. Eventually, a physician could query a database for "OSCC with a similar spatial immune pattern" to guide treatment, once enough cases are amassed.

Looking forward, the convergence of improving technology, smarter algorithms, and collaborative data sharing will likely overcome current hurdles. As spatial transcriptomics becomes more accessible and AI models more transparent, the vision of routine spatial profiling of OSCC – to guide truly personalized therapy – comes closer to reality. In the next 5–10 years, we anticipate clinical trials stratifying OSCC patients based on spatial immune context (hot vs. cold), pathology reports incorporating spatial gene/protein signatures for risk assessment, and perhaps even spatially targeted interventions (such as localized immunotherapy injections) informed by these detailed maps.

profiling becoming part of pathology workflows for OSCC, especially if new, more accessible spatial methods (like multiplex immunofluorescence or targeted RNA panels) can serve as proxies.[34,35] AI will be crucial to automate the interpretation of these complex datasets into a reportable form, such as "Patient's tumor has high-risk spatial signature X; consider adding Y drug."[25,26]

It's also worth considering the long-term vision: combining spatial transcriptomics with other spatial "omics" (proteomics, metabolomics) and clinical imaging (like functional MRI or PET scans) under an AI framework to get a truly comprehensive view of the tumor.[27,28] Already, spatial proteomic technologies (like Imaging Mass Cytometry or CosMx for protein) can map dozens of proteins in OSCC tissue, complementing transcript data. AI can integrate these layers (for example, using deep learning to fuse mRNA and protein maps) to identify robust features.[29,30] One could imagine a future tumor board where, for each OSCC patient, a multimodal spatial tumor map is presented, and an AI system suggests a personalized therapy regimen based on patterns learned from thousands of prior patients.

In conclusion to this section, AI-driven spatial transcriptomics is poised to shift the clinical management of OSCC from a one-size-fits-all approach to one that is spatially informed and personalized. Patients stand to benefit through more accurate prognosis, better selection of therapies (avoiding ineffective treatments), and novel combination strategies that specifically dismantle their tumor's ecosystem. The next section will discuss the challenges that must be overcome to realize this potential and the future directions of this rapidly evolving field.

5. Current challenges and future directions

The integration of artificial intelligence with spatial transcriptomics in OSCC research holds great promise, but several key challenges remain. These challenges encompass technological limitations, computational hurdles, and practical issues related to clinical adoption. In this final section, we outline these obstacles and discuss emerging solutions and future directions, as summarized in Table 5.

Technological and Data Limitations: Despite rapid progress, current spatial transcriptomics still faces trade-offs in resolution, throughput, and sensitivity. Many methods either capture only a subset of genes (imaging-based) or cannot resolve single cells (sequencing-based).[9,11,28] For instance, 10x Visium's spot size (~55 μm) means signals from multiple neighboring cells are averaged, blurring cell-specific information – this is particularly problematic for studying small but important cells like lymphocytes in OSCC.[9] On the other hand, high-resolution methods like MERFISH is limited to hundreds of genes and might miss critical pathways not in the probe set.[28]

Additionally, the number of mRNA molecules captured per cell/spot is only a fraction of the total, leading to many dropouts (zero values) that complicate analysis.[25] These issues can result in incomplete or noisy images of the tumor, challenging the AI algorithms that are trying to find patterns. A related limitation is that most platforms focus on poly-A RNA (messenger RNA), thus excluding non-coding RNAs that may also be relevant in cancer.[26]

Emerging solutions: Ongoing technological development is addressing these issues. New chemistries (like 10x "Xenium" and others) We aim for higher sensitivity to detect more genes per cell. Improved resolution is being achieved by techniques like Slide-seqV2, HDST, and Stereoseq, as discussed, and by combining data from paired single-cell RNA-seq to computationally deconvolve spots (already, methods like cell2location help infer single-cell data from multi-cell spots[40]). There is also interest in capturing other biomolecules spatially: for example, spatially resolved *proteomics* or mapping metabolites. Nanostring's DSP and 10x's upcoming platforms allow simultaneous RNA and protein mapping,[20] which could be very useful (e.g., confirming that a gene's mRNA and its protein, such as PD-L1, are co-localized). In terms of non-coding RNAs, future protocols may integrate capture of lncRNAs or miRNAs, giving a more comprehensive view.

Practical and Sample-Related Challenges: Sample preparation for spatial transcriptomics can be complex and is often tissue-specific. OSCC samples, especially small biopsies, might be hard to process – they need careful optimization of section thickness, tissue permeabilization, etc., to preserve RNA and morphology. FFPE samples (the standard in pathology) were long incompatible with many spatial methods (which favored fresh-frozen), although now technologies like DSP, CosMx, and others support FFPE.[15,21] Cost is another issue: spatial transcriptomic experiments are currently expensive (often several times the Cost of bulk or single-cell sequencing), which could limit routine clinical use. Processing times and the need for specialized equipment also pose barriers for rapid turnaround.

Emerging solutions: Automation and cost reduction are focus areas. Some companies are releasing kits for easier sample processing and multiplexing samples on one slide to reduce the Cost per sample.[41] The development of high-throughput, lower-cost spatial assays (perhaps targeted panels for known OSCC genes) could bring spatial profiling into clinical labs.[35] For FFPE, continued improvements in probe design and chemistry (such as using oligonucleotide tags as in DSP and CosMx) are making it feasible to do spatial analysis on archived OSCC tumor banks – an invaluable resource for retrospective studies correlating spatial features with outcomes.[27,28] Additionally, alternative approaches, such as inputting spatial data from routine histology using AI, might provide cheaper surrogates in the clinic (though they will need robust validation against real spatial transcriptomics).[41]

Computational and AI Challenges: On the data analysis side, the vast volume and high dimensionality of spatial transcriptomic data require

substantial computational power and advanced algorithms.[25] OSCC datasets with >20,000 spots and thousands of genes can easily exceed the memory of standard computers. AI models, especially deep learning, require large training datasets to avoid overfitting.[26] However, spatial transcriptomic datasets are still limited in number and often vary between experiments (different platforms, different gene panels, etc.). This can make it hard to develop generalized AI models. Moreover, many current AI models (e.g., deep neural networks) are *black boxes*, producing predictions (such as cluster assignments or prognosis scores) that are not easily interpretable biologically. In a clinical setting, such a lack of interpretability could hinder adoption, as clinicians need to trust and understand the basis of a model's recommendation.[35]

Emerging solutions: The field is moving toward establishing standardized pipelines and data integration methods. New algorithms are being designed to handle sparsity and noise, such as using graph-based smoothing to borrow strength from neighboring areas, or zero-inflated models that acknowledge the dropout nature of data. Efforts like creating spatial atlases of normal and cancer tissues will provide larger reference datasets for training AI models.[38] In OSCC, one could envision a reference spatial atlas comprising many tumors, against which a new patient's data could be compared (similar to how TCGA bulk data is used now, but with spatial granularity). To address the interpretability issue, researchers are developing explainable AI techniques – for example, methods that identify which genes or spatial features were most important in an AI model's decision. In a prognostic model, this might mean highlighting that "high CXCL13^+ B-cell cluster in margin" was a key contributor to the favorable prediction, aligning with biological reasoning (CXCL13^+ B cells indicating TLS presence). Such explanations will build confidence in the models.[40]

Data integration and Sharing:

Another challenge is the integration of spatial data with other types (genomic, clinical) and the sharing of these large datasets. Multi-omics data integration is still nontrivial – for instance, combining spatial transcriptomics with single-cell RNA-seq and with imaging data requires complex alignment and normalization.[25,26] Creating user-friendly platforms for clinicians to visualize and explore spatial data is also in its infancy. Additionally, standardized databases for spatial omics are needed: unlike bulk gene expression (where one can query a gene in many datasets), spatial databases would let one query "where is gene X most expressed in the tissue?" or "show me OSCC tumors with similar spatial immune architecture".[35]

Emerging solutions: Initiatives are underway to build spatial omics databases and tools. For example, the NIH Human Tumor Atlas Network (HTAN) includes spatially-resolved data; there are also open-source platforms like "spatialLIBD" for brain spatial data, which could be extended to cancers. Standard formats (such as the SpatialExperiment Bioconductor class) are being adopted, making data more shareable. With such resources, AI models trained on one dataset can be validated on other datasets, thereby improving their robustness.[26,35]

Looking ahead, the future perspectives for AI-driven spatial transcriptomics in OSCC are bright. We anticipate: (1) Higher-resolution and multimodal spatial atlases of OSCC that map transcripts, proteins, and even cell–cell contacts, creating a rich picture of tumor ecology. (2) Personalized spatial diagnostics: where a routine biopsy is profiled (perhaps with a targeted spatial method or inferred by AI from histology) to determine if a patient's tumor has certain spatial hallmarks (like an "immune exclusion" score), which then guide therapy. (3) Spatially-guided therapies: eventually, one could deliver treatments to specific tumor regions (imagine intra-tumoral injections of a drug to the invasive edge guided by imaging, if that edge is the problem area). This is speculative but not far-fetched – analogous to how radiation can be focused on tumor margins if known to be high-risk. (4) Real-time spatial monitoring: With the improving speed of assays, one could analyze resected tumor margins during surgery to see if an immune infiltrate is present (indicating whether more tissue needs to be removed or if leaving some might help, as it contains immune cells).

Finally, combining spatial data with longitudinal patient data and outcomes will enable AI to continually learn and refine which spatial features truly matter for cure versus recurrence. For OSCC, where local recurrence is common and often deadly, spatial markers of residual disease (like tumor cells at the surgical margin) or early immune response could be game-changers in postoperative management.

Despite rapid progress, the application of spatial transcriptomics in OSCC faces several significant challenges. One of the foremost limitations is Cost, as platforms such as Visium and Visium HD incur per-sample library preparation expenses of approximately USD 6,800–7,900 (excluding sequencing), which restricts accessibility in resource-limited settings. Standardization also remains an issue, with the absence of universally accepted pipelines and reporting frameworks undermining reproducibility across studies. Another critical barrier is data integration, since combining histology, spatial transcriptomics, and single-cell sequencing requires sophisticated computational frameworks and benchmark datasets. The interpretability of black-box deep learning models presents additional difficulty, limiting clinical adoption unless coupled with explainable AI approaches.[35,41] Finally, clinical translation will depend on the development of workflows that are cost-effective, scalable, and validated in prospective cohorts. Looking ahead, promising directions include the implementation of panel-based targeted spatial assays, the use of virtual gene inference from histology images, and the advancement of explainable graph models specifically tailored to OSCC prognosis and therapeutic stratification.

6. Conclusion

In conclusion, artificial intelligence–driven spatial transcriptomics represents a powerful new frontier for understanding and treating oral squamous cell carcinoma. By uniting cutting-edge sequencing, imaging, and computational analytics, we can move beyond averaging a tumor's properties and instead embrace its spatial complexity. In doing so, we reveal that an OSCC tumor is not a uniform mass, but rather an ecosystem of regions, each with distinct vulnerabilities that can be exploited with tailored therapies. The journey from bench to bedside is underway: current challenges are being actively addressed by technological innovation and methodological advances. The future where precision oncology for OSCC leverages spatial information – determining the right drug for the right patient, based on the right tumor region – is on the horizon. This paradigm shift, driven by the synergy of spatial omics and AI, holds the promise of enhancing outcomes and improving the quality of life for patients with OSCC worldwide.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Given his/her/their role as, had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor." If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

AI: Artificial Intelligence; ST: Spatial Transcriptomics; OSCC: Oral Squamous Cell Carcinoma; HNSCC: Head and Neck Squamous Cell Carcinoma; TME: Tumor Microenvironment; CAF: Cancer-Associated Fibroblast; iCAF: Inflammatory CAF; ROI: Region of Interest; TLS: Tertiary Lymphoid Structure; SVG: Spatially Variable Gene.

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