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PERSPECTIVE: SPATIAL OMICS

Beyond the Lab and Into the Hospital: An Outlook on the Clinical Utility of Spatial Omics Technologies

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Abstract

Spatial omics technologies, including highly multiplexed histologic protein assays, nucleic acid abundance and/or sequence mapping, and spatial epigenetics assays, offer powerful tools for interrogating the complex biology of human tissues. These technologies have been broadly applied in basic and translational research, which presages deployment in clinical settings as well. In this article, we discuss spatial omics technologies with an emphasis on retrieval of disease-related information in single samples, with potential clinical applications in specialties such as oncology and immunology, and in the development of personalized treatment. Capable of localizing detailed molecular information within histologic structures, spatial omics technologies provide both cell-intrinsic information and microenvironmental interaction context. This will allow more precise diagnostic and prognostic classifications and more accurate predictions about treatment responses to be made. While technical and financial challenges to widespread deployment in clinical laboratories remain, spatial omics technologies are expected to dramatically expand actionable information obtained by human tissue sampling for pathologic analysis.

The emergence of spatial omics technologies, which enable highly multiplexed assays of protein abundance, gene expression, and/or chromatin state, while preserving information about their native locations in tissue, has provided fresh perspectives on normal biological processes, such as development, as well as on pathologic dysfunction.[1,2]

Spatial omics tools have numerous uses, including in-depth studies of cell type compositions, transcriptome mapping in diverse tissues, and determining microenvironmental influences among adjacent cells (e.g., paracrine signaling in embryonic development).[3] Currently, spatial omics technologies serve primarily as tools in investigative biology and in pathway discovery in human tissue experiments.

While the idea of spatially resolved, multiplexed characterization of tissues at cellular or subcellular level resolution is not new, in recent years the widely available platforms offering spatial omics technologies have expanded substantially. They include a variety of assays built on new technologies for examining local genetic expression.[4,5]

This paper focuses on platforms with cellular or near-cellular ($\sim$1–25 μm) resolution, as these are most readily deployed in examining lesions in human samples of clinically relevant size. In many of these platforms, such as High Definition Spatial Transcriptomics, Slide-seq, and Deterministic Barcoding in Tissue ("DBiT"), spatially resolved oligonucleotide barcoding is employed—a unique nucleic acid identifier ligated to molecules in particular tissue regions to identify gene and protein locale.[6–8]

Other available methods take advantage of fluorescence markers, attaching fluorophore tags to nucleic acid sequence probes or libraries of antibodies applied to tissue sections, which can then be subjected to multiple rounds of fluorescence imaging, such as in smFISH, MERFISH, MOSAICA, Xenium, and Phenocycler assays.[9–13]

These assays allow for the examination of transcription and translation-level features (thousands of expressed genes, or hundreds of proteins), at each of many tissue locations *in situ*; in parallel with the biotechnology developments that have made spatial omics technologies more accessible, new

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computational tools have emerged for analyzing the large, complex datasets resulting from these assays.[14,15] Several of these tools are open-source and highly accessible on commonly used platforms.[16]

Tissue bioanalytical assays in current clinical practice

Histopathologic assessment by anatomic pathologists, the main modality for examination of resected or biopsied tissue in the current clinical setting, is driven largely by expert interpretation of morphology in hematoxylin & eosin (H&E)-stained tissue sections. Nonetheless, there are well-recognized limitations on the discriminability of important cell types by H&E morphology (e.g., fibroblasts).[17]

Special histological stains and multiplexed protein technologies such as immunohistochemistry (IHC), immunofluorescence, and multiplexed ion beam imaging microscopy (MIBI), along with Fluorescence In-situ Hybridization (FISH) analysis and sequencing assays are often used as confirmatory analyses or additional diagnostic procedures. Protein expression assays by IHC and nucleic acid detection by FISH, while extremely useful in aiding expert diagnosis, are limited to low complexity (i.e., a single or few simultaneous targets).

In contrast, assays such as flow cytometry (which allows for high plex protein detection) and high plex next-generation sequencing (NGS) cannot map detected molecular features to localized areas of tissue, as they rely on dissociating tissue into suspensions of molecules, cells, or nuclei.

The clinical importance of multiplex tissue imaging is demonstrated by the current wide adoption of histology-based classification and patient-stratification techniques. In diffuse large B-cell lymphoma, for example, outcomes are markedly different for tumors arising from different cells of origin (the main subtypes being germinal center B-cell-like (GCB) with a more favorable prognosis and activated B-cell-like (ABC) with a less favorable prognosis).

While strict cell of origin typing requires gene expression profiling covering 100 gene loci, a number of condensed panels (i.e., multiplex assessment) of IHC markers have been devised that show good, but not perfect, concordance (77–87%), with gene expression profiling for GCB versus ABC subtypes.[18,19]

Similarly, microsatellite instability in colorectal cancer confers increased susceptibility to treatment with immunotherapy, but definite classification into microsatellite stable, low microsatellite unstable, and high microsatellite unstable requires testing by NGS or multiplex PCR, and thus a panel of four IHC markers (MLH1, PMS2, MLH2, and MSH6) has been developed, which, taken together, can act as a surrogate classifier.

Single-cell sequencing, which has yet to be widely adopted in clinical laboratories, can be used for identification of individual cells and their states via genetic markers. However, it is possible for different cell types to express some similar markers under the influence of the local microenvironment, such as uterine stroma and epithelium.[20]

Even when using complex analyses such as graph-based clustering, pseudotime analyses, or density analyses, to determine higher-order structures such as tumor subclone or immune infiltrate composition, dissociated single-cell methods do not provide complete contextual information about the relationships between important cell subpopulations and acellular features of tissue lesions.[21,22]

Advantages of spatial omics technologies

Methods currently in clinical use do not support potentially important analyses such as localized cell-to-cell interactions and classification of the local microenvironment of lesions. In the following sections, we discuss how spatial omics technologies have the potential to support more complex and nuanced diagnostics by augmenting existing assays with spatially resolved molecular data.

Spatial omics technologies allow for simultaneous examination of many molecular features among physically adjacent cells, enabling comparisons of these features to neighboring cell clusters and local tissue elements such as anatomic structure, extracellular lesions, and lesion architecture.

By examining genetic markers typically used to identify cells with an added spatial component (i.e., the ability to register cellular locale with respect to underlying tissue histology), a single assay can result in significantly higher confidence regarding cell type, inter-cellular interactions, and the local microenvironment of the tissue.

For example, Lundmark et al. used spatial RNA sequencing to map immune cell response in inflamed periodontal tissues; immune cell gene expression was found localized around gum epithelium of periodontitis patients, the typical entrance point for immune populations into damaged tissues.[23] All this information can be useful in formulating diagnoses and assessing other predictive features of lesions, such as the likelihood that treatment may require damaging vulnerable nearby tissue (e.g., neural tissue).[24]

Finally, and most critically, some information clinically relevant to disease processes can only be obtained by examination of the way in which particular functional subsets of cells are positioned within an affected tissue. This occurs most frequently in the interactions between biological systems that operate under different constraints, such as: a tumor and the immune response to it; the immunologic milieu in one tissue compartment and that in another; or a developmentally abnormal population and its neighboring tissue (i.e., as in dysplasias or hamartomatous growths).[25]

Detailed characterization of these interfaces may provide important diagnostic information about overall disease trajectory.

Increased information retrieval from limited tissue samples

A significant challenge in diagnostic pathology is how to best utilize limited amounts of resected tissue, particularly small biopsies, in the era of advanced molecular testing. The extraction of as much useful information as possible from tissue samples is a major goal of diagnostic tissue sampling procedures. Morphologic, immunophenotypic, and genomic information are all routinely used in modern practice to establish diagnostic and ancillary biological parameters of lesions.[26,27]

Tissue sampling procedures for diagnosis carry risk. Even in limited sampling procedures such as transthoracic needle biopsies, 25% of procedures result in at least some pneumothorax, and liver and kidney biopsies carry a small risk of hemorrhage.[28,29] Many tissue samples are thus limited in size to reduce risk of harm to the patient.

However, the resulting small size of biopsy samples can present challenges for pathologic analysis, particularly when using techniques that require a larger amount of tissue, such as traditional RNA sequencing and proteomics.[30] Further, accurate diagnosis of small cellular clusters or infiltrates within larger reactive tissue regions remains a persistent challenge in anatomic pathology. NGS has created a powerful diagnostic tool in these settings, but in many cases, particularly those with limited tissue available, the correlation between molecular findings and histomorphology is imprecise.

In many cases, spatial omics technologies can produce data on thousands of expressed genes and their specific locations of expression in a single histological slide section from human samples.[8,10] This allows for the determination of the gene expression profiles of spatially distinct regions within tissue samples. On many platforms, histomorphology is preserved and co-registered with molecular data (Fig. 1).

Assignment of complex molecular features to small clusters of cells in their precise tissue context (e.g., clusters of suspicious cells in the subcapsular space of a lymph node) can provide crucial diagnostic clues. This also can be useful in detecting pathologic niches that anticipate malignant transformation or precede tissue remodeling and dysfunction, such as stress signatures in myocardium tissue, or fibrotic microenvironments in the kidney, which offer potential early therapeutic targets.[31,32]

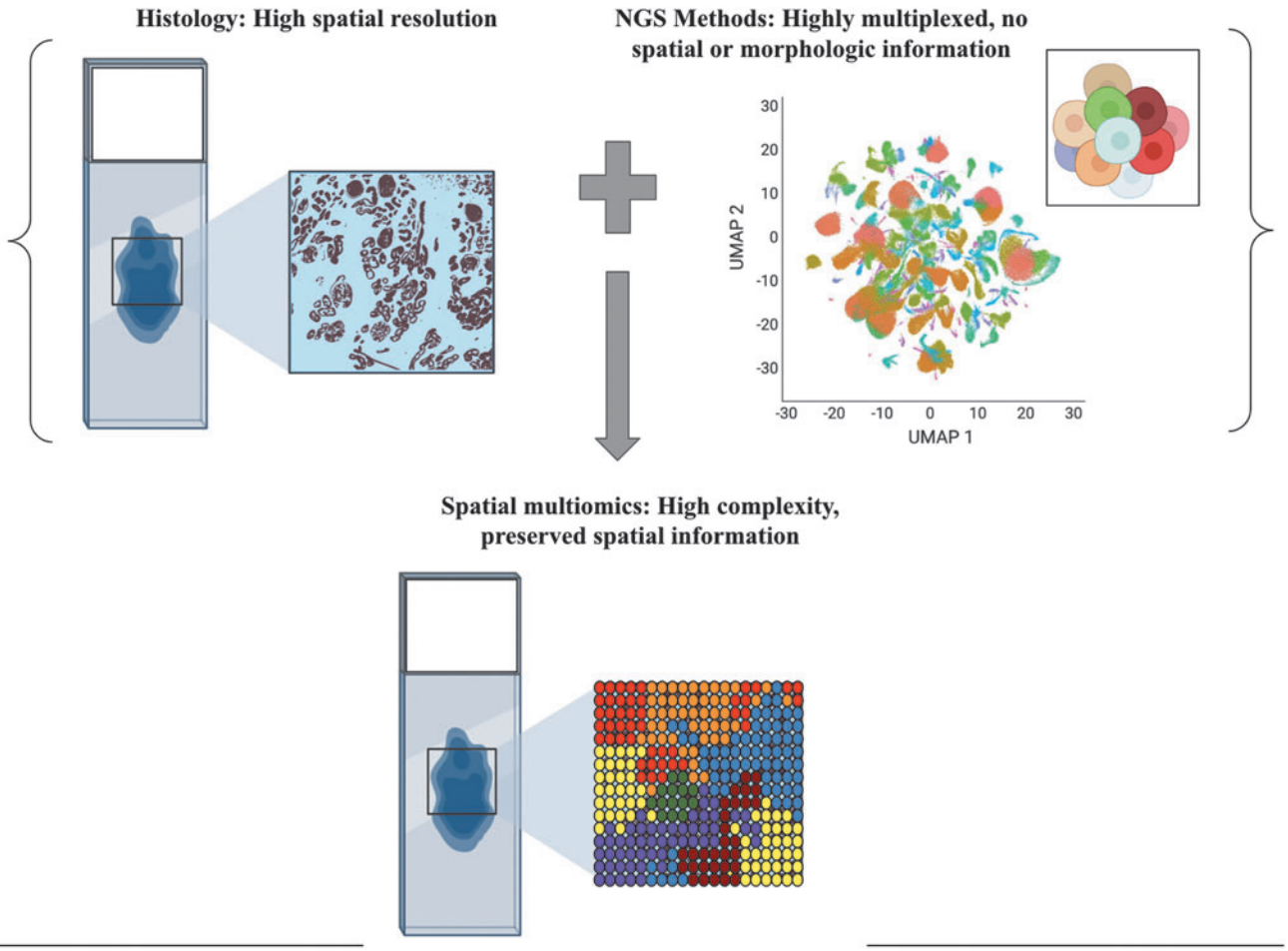

FIG. 1. A comparison of histology and NGS methods to spatial omic technologies.
Histomorphology, the primary data type for anatomic pathology diagnosis over the last century and a half, has high spatial resolution and fidelity with the configuration of tissue *in vivo*. Immunohistochemistry has added a valuable layer of molecular data, but this is low complexity. In contrast, NGS-based methods, including single-cell sequencing, offer highly complex data at the cost of dissociation of tissue with the resulting loss of structure. Spatial omics technologies attempt to retain histomorphology through the process of assaying large numbers of targets, resulting in high-complexity data registered to the underlying tissue and cellular structure. NGS, next-generation sequencing.

In the case of tumors, co-localizing mutations and other molecular features with morphologically defined cell populations and other histologic features provides a highly informative map of tumor invasion and stromal interaction.

Another advantage of spatial omics technologies is that they enable the analysis of highly multiplexed gene expression within intact tissue samples (see Fig. 2). This eliminates the need for tissue dissociation and cell sorting, which can be time-consuming and may introduce biases or artifacts in the data, evidenced by the levels of experimental variability in single-cell assays.[33]

Spatial omics technologies also enable gene expression analysis across multiple spatial scales—at the level of a single cell in some cases, classifications of small areas by cell type occupancy, at the level of microanatomic structure (i.e., epithelial layers), or the tissue as a whole. Analyses at all these levels simultaneously are not currently available in clinical assays and may be particularly useful in gathering important information from limited amounts of resected tissue.

In addition, the interpretation of some pathological findings can be subjective and often varies between different observers, as suggested by studies such as Messerli et al.,[34] which found

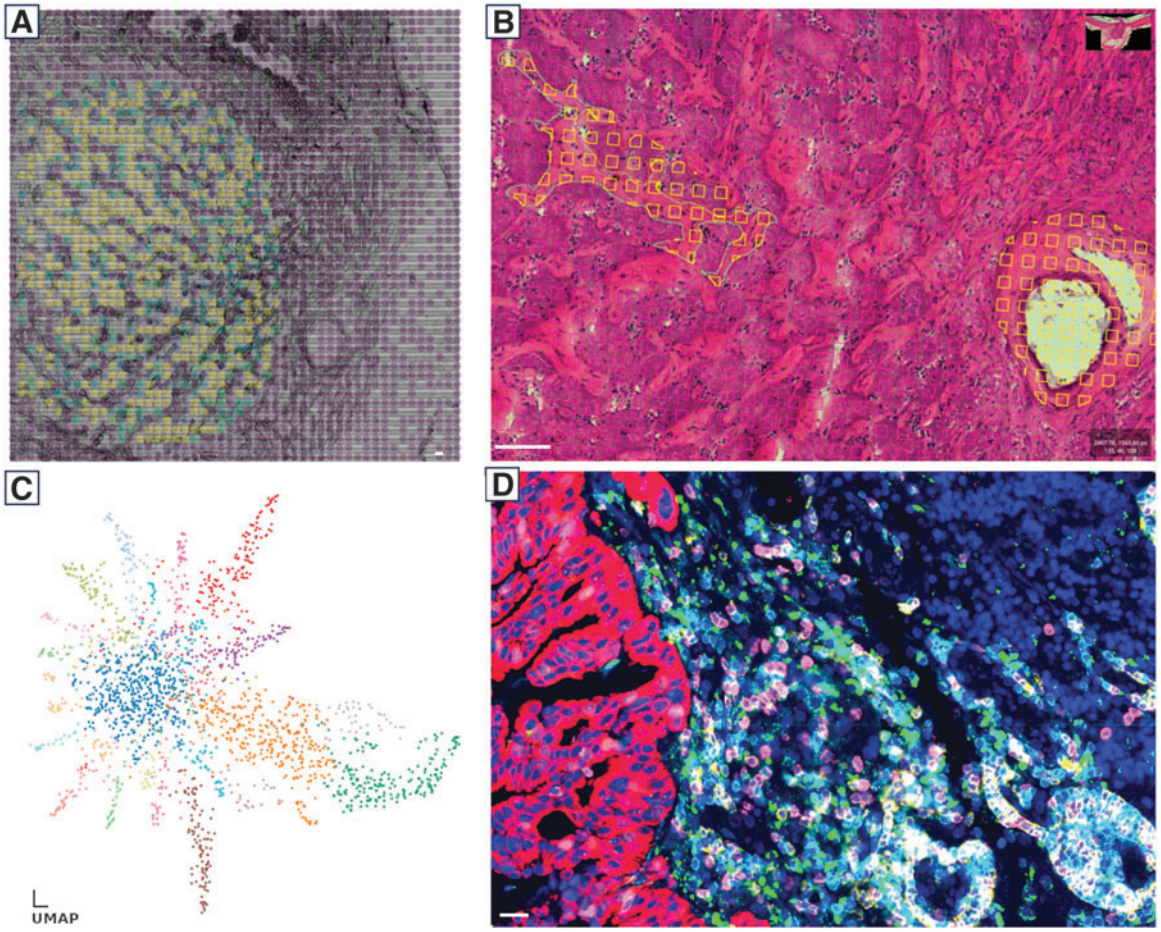

FIG. 2. Examples of complex spatially resolved information in human tissues as revealed by spatial omics technologies.

(A) Human optic nerve head with individual transcriptomic data loci, categorized by underlying tissue niche (yellow = nerve; green = septae; purple = other).

(B) Virtual dissection of distinct tissue structures present in transcriptomic foci, including nerve, septae, and blood vessels using expert annotation of co-registered histologic image (H&E stain).

(C) UMAP projection of transcriptomic data from optic nerve head (each point represents a single tissue locus), with Louvain cluster identities of expression profiles in different colors.

(D) Complex, heterogeneous immune cell infiltrate in the tumor microenvironment of non-small cell lung cancer metastasis to the cerebellum, visualized with a subset of the 48 markers assayed using highly multiplexed immunofluorescence.[9] All scale bars = 25 μm.

H&E, hematoxylin & eosin; UMAP, uniform manifold approximation and projection.

high diagnostic variability for associated tumors in gestational trophoblastic neoplasia.[28] This is particularly true in tissues with multiple cell types, where pathologists may have differing opinions on the significance of certain findings or the presence of abnormal cells.

Even after the use of common ancillary diagnostic techniques including IHC, diagnostic discrepancies occur.[34] The availability of as much data as possible on the cellular architecture of the tissue in question and the unique features of each cell type present can mitigate interobserver variability. Spatial omics technologies are, therefore, expected to play a role in "decluttering" an otherwise diagnostically challenging piece of tissue, improving cohesion and accuracy between observers.

Spatially resolved highly multiplexed molecular data can also be integrated with other types of data, such as classical IHC, to provide a more comprehensive understanding of tissue biology that may be useful in diagnosing and treating conditions on an individual basis. Therefore, spatial omics technologies substantially amplify the information available from limited amounts of tissue and maintain the spatial context of gene expression patterns within the tissue microenvironment.

Modern histologic practice and integration with spatial omics technologies

The promise of spatially resolved highly multiplexed information to provide insight into human disease processes has re-emphasized the importance of tissue context to deliver true insights into disease biology. The data generated by these technologies comprise a skeleton; the assays themselves provide the relative locations of the analyzed molecules (and thus proximities to each other).

These molecules exist in and around cells that are situated in tissue architecture, creating the networks, physiological units, microenvironments, barriers, and organs that keep us alive and whose dysfunction corresponds to disease.

This context, which is essentially the traditional histologic description of health and disease, has been the subject of intense study since the 17th century, providing an immense body of knowledge of the changes in cellular identities, cytomorphology, and microenvironment that accompany disease initiation and progression. Deployment of spatial omics technologies productively to understand molecular pathways implicated in disease requires application to tissue in which these structural elements can be identified and related to disease phenotype.

Thus, close integration with histology is of the utmost importance when designing and analyzing spatial "-omics" experiments. This requires histopathologic surveying and assessment of the tissues under study, and the identification of key areas to which an appropriate spatial omics assay may be applied to yield insights into pathology.

The advent of large-scale digitization of histologic slides by whole-slide imaging in the clinical practice provides the substrate for direct integration with spatial omics data: high-resolution digital representation of intact tissue stained by traditional methods.[35] Leveraging this will require improvement in the reliability and accessibility of algorithms to do two things: (1) Accurately align the highly multiplexed data generated by a spatial "-omics" experiment with images of the tissue from which it was derived (i.e., image registration), and (2) Capture expert and/or automated identification of important locations and features in tissue and integrate them into the analysis of the molecular data.

Deep-learning algorithms for histopathological image analysis and computer-assisted diagnosis (CAD) are being increasingly deployed.[36] Deep-learning techniques can segment and quantify vast numbers of tissue features, adding quantitative descriptions beyond the limitations of human observers. Algorithms such as the weakly supervised survival convolution neural network (WSS-CNN) have also been tested in clinical trials by correlating metadata extract from images of H&E stained tissues with survival data, allowing for increased efficiency, consistency across larger data sets, and more accurate prognosis.[37]

The use of deep machine-learning algorithms in improving basic staining techniques such as H&E itself is likely to be an upcoming and useful field for diagnostic pathology. Computer-generated staining on images of unstained tissues can be particularly useful for "applying" multiple stains to a single section of tissue. H&E stains can now be performed by deep-learning algorithms, for example in the formulation of virtual stains on unstained and previously stained imaged tissue for prostate cancer tumor diagnosis.[38]

Registration of such richly quantified histomorphology to spatial omics data via computational techniques thus holds the promise of extracting a vast amount of clinically relevant data from tissue samples.

Deconvolving information from integrated biological systems

All tissues within the human body are composed of different cell types, and they participate in and are affected by the function of multiple physiologic systems. The presence of multiple cell types, particularly from distinct biological systems, can complicate the characterization of diseased tissues and confound the classification of possible abnormal cells. This can lead to incomplete or inaccurate diagnoses, particularly when various cells within diseased tissue express similar genetic markers.

The immune system is an excellent example of a biological system with high complexity and dynamic spatial arrangement in tissues. Immune cells themselves can have varying roles in tumor biology, with some promoting tumor growth (e.g., some tumor-associated macrophages and myeloid-derived suppressor cells) and others inhibiting it (i.e., CD8+ and CD4+ T cells).

Even within a given cell lineage, there is a growing appreciation for functional diversity and plasticity. For example, cytotoxic CD8+ T cells are an essential component of the adaptive immune response against viral infections and cancers, whereas a subset of CD8+ T cells may also play an essential role in regulating immune responses.[39]

Emerging evidence also has also revealed that certain lineages can differ from traditional immune cell phenotypes; for example, although T cells are often seen as beneficial components

of the immune system combating infection and disease, they have been shown to cause pathogenic inflammatory storms in COVID-19 patients, and infiltrating regulatory T cells can suppress anti-tumor immune activity.[40,41] This makes determining their precise tissue location especially important.[42]

The interactions among immune cells themselves and between immune cells and the constituent tissues of other organs are essential to the maintenance of homeostasis and in the pathogenesis and trajectory of myriad diseases. While basic histology and IHC can help to identify and partially classify immune infiltrates in tissue, practical limitations (e.g., the inability to isolate sufficient immune cells from a tissue sample for flow cytometry, or the challenging logistics of using multiple assays that require different preparation and fixation of tissue such as flow cytometry and IHC) may introduce inaccuracy and bias into clinical findings.

Spatial omics technologies offer a solution to this dilemma, by providing highly multiplexed genetic information of both immune infiltrates and their surroundings, localized to a specific tissue subregion in a small tissue sample (e.g., Fig. 2D).

Congenital structural disorders with disruptions to the organization of typical tissue structures can also be assessed more fully using spatial omics technologies. The process of tissue patterning involves the interaction of multiple germ layers and cell types that have to interact to generate proper architecture and function. Focal cortical dysplasia (FCD), for example, is a common cause of epilepsy characterized by abnormal development and migration of cortical neurons.

Features include the formation of heterotopic neurons, balloon cells, and dysmorphic neurons, which are often arranged in a disorganized and heterogeneous manner within the cortical tissue.[43,44] It can be challenging to definitively identify and localize the epileptogenic zone within affected brain tissue.[45] The heterogeneity of FCD tissue can also lead to variability in seizure semiology and treatment response, further complicating the management of this condition.

By using spatial omics technologies to characterize resected epileptogenic foci in conditions such as FCD, highly detailed molecular details about these neurons and their surroundings may yield a more definitive diagnosis and improved classification of the complex architecture and interrelationships in FCD tissue in resected samples.

Personalized tumor diagnostics. Highly individualized cancer treatment, in which clinicians tailor therapies to the individual molecular and genetic characteristics of each patient's tumor, has shown promise in improving treatment outcomes and reducing side effects in a range of cancer types, including breast, lung, and colorectal cancer.[46–50] A functional approach to personalized cancer treatment is targeting specific aggressive clone populations within a tumor.

Certain subclones of a tumor can be particularly resistant to certain therapies, driving tumor growth and leading to metastasis.[51] By identifying these aggressive clones through genomic sequencing and molecular profiling techniques, clinicians can develop targeted therapies that selectively kill these populations. While subclonal mutation analysis is possible with NGS of dissociated tumor tissue, the distinct arrangement and microenvironmental interactions particular to tumor subclones cannot be determined.

These features can be discerned by utilizing spatial omics technology. In glioblastoma, for example, spatially resolved multiomics analysis via 10X Visium has revealed spatially distinct transcriptional programs among subclones that are strongly influenced by features of the local microenvironment.[52] Under local conditions of metabolic or immune stress, subclones show higher propensity to migrate toward healthy parts of the brain.

Assaying these parameters in a single patient's resection may provide valuable information for prognosis and treatment selection. Associated molecular signatures could then be tracked over time using bulk sequencing or cell-free tumor DNA analysis to monitor the clinical trajectory or response to treatment.

Deploying spatial omics technology testing on patient tumor samples offers the possibility of identifying where particularly vulnerable elements of the tumor microenvironment (TME) (such as lymphatic vessels) lie in relation to aggressive clones. With continued advances in personalized medicine and precision oncology, the identification and targeting of aggressive clone populations via spatial omics is a promising strategy for improving cancer treatment and associated patient outcomes.

In part, the challenge in characterizing tumor heterogeneity and the degree of synergy between cancerous clones stems from private mutations and clonal evolution.[53,54] Yet, determining regions of tissue at higher risk for aggressive clone development may provide important guidance for the choice of management.

The ability to localize aggressive clones and their transcripts in spatial proximity to each other with spatial omics can help classify these clones and determine high-risk tissue areas for the aggressive development of cancer, for example, assessing higher levels of transcription within certain clones displaying increased proliferation rates.

The TME also plays a critical role in the development, progression, and response to the treatment of many types of cancer.[55,56] Tumors are composed of many different cell types, including cancer cells, immune cells, stromal cells, and others.[57] Each of these cell types can interact with each other and with the extracellular matrix in complex ways, leading to a highly heterogeneous microenvironment.

The genomic and phenotypic heterogeneity of many tumors and the resulting complexity in interactions with the stromal environment and other cells creates a challenge in formulating precise predictions about overall tumor behavior.[58] For example, it can be challenging to determine how changes in the extracellular matrix are affecting invasive tumor cell behavior.[59]

In addition, the TME can be highly dynamic, with changes in cellular composition, cytokine, and chemokine production, and extracellular matrix remodeling occurring over time and in response to various stimuli.[60] This dynamic nature of the TME presents a challenge in selecting appropriate treatments, as therapies that target precise features of the TME may only be effective at certain phases of tumor development as functional trajectories diverge among subclones.

For example, a study by Radtke et al., 2022 used "Iterative Bleaching Extends multipleXity" (IBEX) to reveal high-B-cell heterogeneity in TME niches surrounding Follicular Lymphoma lesions, which integrated with single-cell data revealed selective gene expression for early relapses such as programmed cell death protein 1 (PD-1), interferon α, and interferon β, thus improving clinical outlook via predictive capability.[61]

In modeling the evolutionary trajectory of tumor clones and how they interact with each other and the surrounding tissue, this information provides a possible avenue for developing personalized treatment options for patients. This higher-level diagnostic information may help determine cancer evolution and mechanisms of resistance with high specificity for a patient and help develop a more precise treatment plan.

Immuno-oncology. The immune system plays a crucial role in tumor development and progression. In many tumor types, immune cells infiltrate the TME to influence or eliminate harmful cancer cells.[62–66] The function of these immune infiltrates can be enhanced by therapy (e.g., immune checkpoint inhibition, cytokine therapies, or transfection of immune cells to drive tumor antigen recognition).[67–71]

Assessment of baseline and evolving anti-tumor immune response is critical for selecting and managing immunomodulatory therapies. Currently, the classification of tumor-immune interactions in clinical oncology is primarily low-dimensional in nature (as in PD-1/programmed death-ligand 1 [PD-L1] expression testing by IHC, scored as positive or negative).[72] However, the profile of infiltrating immune cells and their interaction with tumor biology is much more complex.

There are numerous functional subsets of effector and regulatory immune cells, and complex interactions among them, such as the formation of tertiary lymphoid structures, and dynamic relationships with tumor and stromal tissue.[73] Completely different cell types may express similar morphological and molecular characteristics, and some otherwise similar cell types may appear heterogeneous.[74,75]

Accurate identification of specific cell types and states requires testing for expression of multiple markers beyond the capability of simple IHC, most commonly tested instead using flow cytometry.[18,19] In such dissociated preparations, however, their direct relationship with neoplastic cells cannot be determined. These functional subsets are also subject to shifting microenvironmental influences, which can be assessed using spatial omics.

Namely, the high-plex immunohistological probe Multiple Iterative Labeling by Antibody Neodeposition (MILAN) has revealed that CD8+ T-cell activation states can vary depending on the microenvironment niche circa particular cancer types, from high activation leading to tumor resection (i.e., melanoma and lung cancer) to tumor-impartial activation.[76] Establishing the states of the immune milieu present in particular niches of the TME, and their relationship with subclonal populations requires both the spatial/morphologic and multiplex molecular data generated by spatial omics technologies.

Detection of specific immune cell infiltrations associated with cancer, and closely connected spatial distributions of genetic markers and proteins, is a strong functional advantage of utilizing spatial omics. Immunohistochemical assays of immune checkpoint inhibitors such as PD-L1 are predictive for tumor response to checkpoint inhibition, and others are being investigated for this purpose (e.g., CTLA4).

Spatial associations between tumor-infiltrating lymphocytes and tumor and microenvironment structure promise to improve these predictions. In diffuse large B-cell lymphoma, for example, TMEs are present with distinct immunologic properties (dendritic cell-enriched, macrophage-enriched, and immune-deficient), as revealed by MIBI, and topological Imaging Mass Cytometry assays have helped to identify subregions of immune expression, such as PD-L1, PD-1, and TIM-3 tumor cell positivity, that associate with poor response to immune-checkpoint inhibitors.[77,78]

Specific higher-order organizational features, as revealed by MERFISH, of native immune responses to non-small cell lung carcinoma, are associated with immunotherapy response, such as PD-L1 inhibitor therapy.[79] There is also predictive value in the composition of immune cell infiltrates into tissue regions yet to be involved by tumor, which can help classify and forecast cancer progression.[78]

Detection of specific immune responses that have been linked to clinical outcomes, including T-cell/macrophage colocalization characteristic of a type 1 interferon response and T-cell/B-cell colocations suggestive of tertiary lymphoid structure formation, has been demonstrated in HER-2 expressing breast carcinoma using Ståhl et al.'s barcode-based assay.[80,81]

Immune landscapes of metastatic brain tumors can also help to classify the immunologic reaction to the tumor, and thereby guide the selection of immuno-oncologic therapy. Immunohistochemical techniques have revealed T-cell and macrophage infiltrate and extracellular microenvironment differences that promote an immunosuppressive environment around carcinomas metastatic to the brain.[82]

The specific mediators of this effect in different patients and tumor types represent personalized therapeutic targets. Spatial omics technologies have also helped determine how the immune system interacts with tumor cells in squamous cell carcinoma. As demonstrated by highly multiplexed spatial fluorescent assays in Tanaka et al., pro-inflammatory cells may recruit Th1 and IFNψ+ CD8 T-cells, whereas immunosuppressive tumor cells tend to associate with macrophage recruitment.[83]

Accurately assaying the composition and activation state of inflammatory infiltrates is a crucial part of therapy selection in modern oncology. Spatial omics technologies, thus, have the potential to be highly impactful in the pathologic workup for a wide variety of cancers.

Autoimmune diseases. Misdirection of immune system attack against normally functioning tissues of the body can lead to debilitating symptoms. However, diagnosing and classifying these diseases can be challenging, as they often present with overlapping symptoms and diagnostic testing results. Autoimmune diseases are primarily classified based on clinical phenotype, genetic background (i.e., HLA groups), and serology (i.e., which antibodies circulate in the serum, including rheumatoid factor, anti-DNA, ANCA, and myositis-specific autoantibodies).

However, the direct immunologic effects on tissue, while readily observed, are often quite challenging to classify on biopsy samples. Further, the pathogenesis of autoimmune diseases is often occult, with unknown specific antigens against which dysregulated immune attacks are mounted, and unknown molecular mediators driving the sustained immune activation.

This confounds disease classification on the basis of laboratory testing. Spatial transcriptomic assays, however, are a promising resolution to this dilemma—providing highly multiplexed information that can help classify both immune populations and their relationships to surrounding lesional (and normal) tissue, to provide more detailed classification.

Taking as an example autoimmune disease involving the skin, including systemic lupus erythematosus, blistering diseases in the pemphigus family, and psoriasis, spatial omics technologies can provide for the analysis of gene expression and protein accumulation patterns in lymphoid infiltrates present in specific areas of the skin, providing a detailed map of proinflammatory and regulatory functions.

Pemphigus diseases, including pemphigus vulgaris, pemphigus foliaceus, and paraneoplastic pemphigus, are all characterized by blisters resulting from immunoglobulin (IgG) antibody-mediated keratinocyte acantholysis and may be difficult to differentiate from both each other and other blistering disorders such as Grover disease on biopsy.[84]

While each of these major subtypes of pemphigus are characterized by the presence of a particular antibody (i.e., pemphigus vulgaris is classified as circulating IgG antibodies against desmoglein-3, whereas pemphigus foliaceous circulates antibodies against desmoglein-1), there is evidence that these conditions can occur simultaneously in a single patient, and even change over time, making diagnoses more complex.[84,85]

However, using spatial omics, the precise characterization of the state of immune infiltrates would quickly narrow down the differential diagnosis, and add valuable information on pemphigus heterogeneity and evolution within a singular individual.

In inflammatory diseases of the central nervous system, there is a significant level of overlap between histologic findings in conditions with substantially different etiologies and patterns of evolution (e.g., infectious, autoimmune, and toxic etiologies). While clinical settings, disease course, radiographic features, and serologic testing are often sufficient to narrow differential diagnoses to a serviceable degree for treatment selection, failure to establish a specific etiology or even disease category occurs in many of these cases.

Spatial omics technologies can help overcome these challenges by providing a detailed picture of the molecular changes occurring within affected tissues. The specific composition of immune subpopulations located in specific niches of brain tissue (e.g., perivascular space, leptomeninges, neuropil) offers important diagnostic clues into disease type and mechanism.[4,5,86–88]

Further, cytokine expression patterns by neurons, glia, and endothelial cells diverge among different infectious agents, and from the patterns observed in autoimmune reactions.[89] These factors may all be exploited in spatial omics technology assays to enhance the classification of immune-mediated reactions in diagnostic samples.

The complexity, mobility, and dynamic nature of immune system components make their assessment of target organs challenging in the clinical setting. This uncertainty underlies much

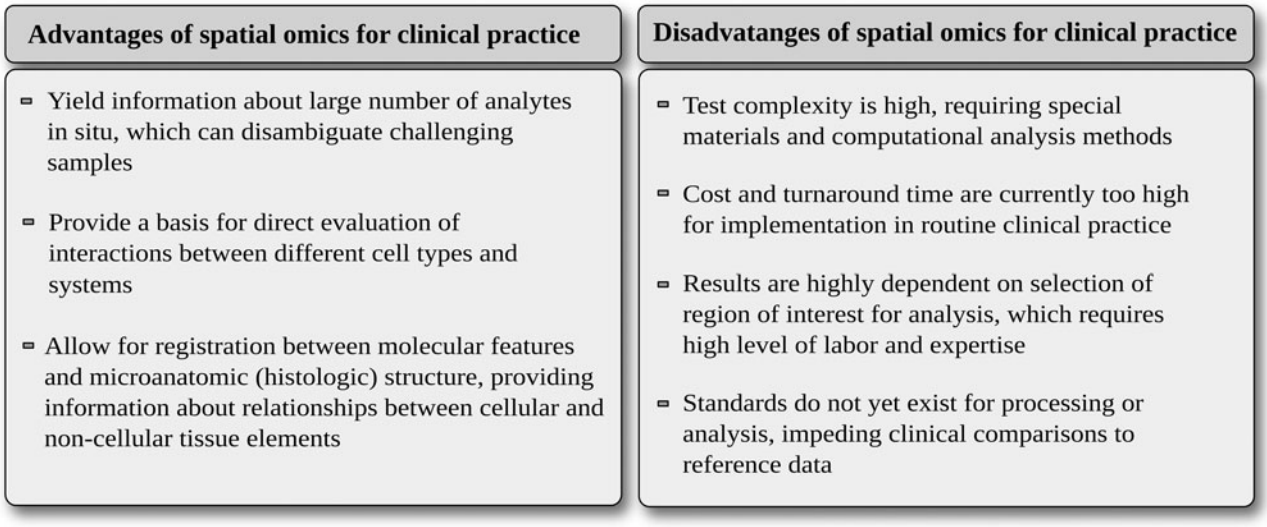

FIG. 3. Advantages and disadvantages of spatial transcriptomics for practical implementation in clinical practice.
While spatial omics technologies offer the possibility of revealing a degree of clinically useful information from diagnostic tissue sections beyond that of routinely used assays, there remain a number of practical challenges to their adoption as part of the toolset for pathologic workup in routine clinical practice.

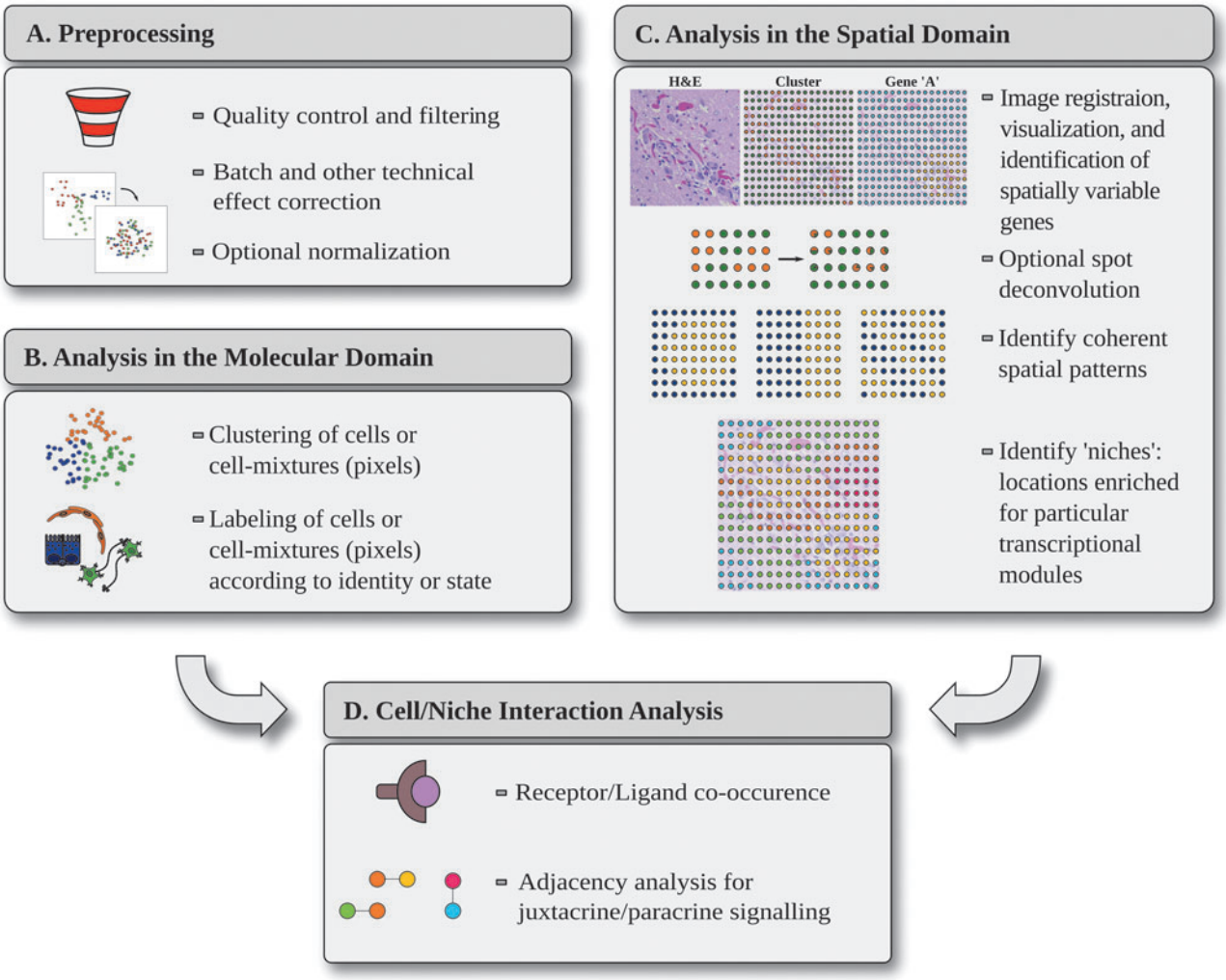

FIG. 4. Diagram of a typical workflow in analysis of spatial omics data sets.

(A) Preprocessing steps adapted from single-cell analysis are applied to filter out data from low-quality loci in tissue (due to technical or biological factors) and normalize across or integrate data from multiple batches.

(B) Typically data are processed in the molecular domain first, without consideration of spatial locations. A large variety of algorithms, also adapted from single-cell analysis, can be employed to assign cluster identities or other features (such as inferred location along a developmental trajectory) to each distinct tissue location. These locations may represent groups of cells and extracellular material, single cells, or subcellular compartments, depending on the spatial resolution of the assay. Agglomerative or community-detection clustering methods, which do not require assumptions about the shapes of clusters, have been used to successfully separate biologically relevant clusters. Interpretation of the clusters requires application of labels, usually informed by expression levels in each cluster of identifiable marker genes associated with known cell lineages or states. Transcriptional modules, that is, patterns of inter-related gene expression, are identified among the various clusters.

(C) Armed with molecular descriptors of each tissue locus, analysis in the spatial domain can proceed, usually beginning with co-registration (i.e., alignment) between the measured tissue loci and a histologic image of the tissue. Then, basic visualizations of tissue loci and their features (e.g., cluster identity or expression level of specific genes) can be generated. Downstream spatial analysis usually begins by testing for spatially variable genes (i.e., genes that are not distributed evenly throughout the tissue), and excluding evenly distributed genes from further analysis. This may trigger reclustering the molecular domain. In some cases, the contributions of multiple cells to the observed expression in a single locus can be computationally dissected through spot deconvolution, improving resolution for some features. Then, spatial patterns such as edges, gradients, or closed spaces are identified among genes, clusters, or other molecularly defined features, which defines niches. These niches can then be assessed for their association with underlying tissue structures.

(D) Finally, analysis in the molecular domain (e.g., receptor-ligand interaction inference) and spatial domain (e.g., cell adjacency or niche proximity assessment) can be combined to infer intercellular signaling pathways implicated in the underlying biological processes.

of the challenge in interpreting tissue samples for immune-mediated disease. Spatial omics technology offers an intriguing solution to this problem, giving detailed information on both the "what" and "where" of immune responses.

Limitations

Spatial omics technology is a powerful tool for analyzing the distribution of mutational signatures, epigenetic modifications, and gene and protein expression within tissue samples. Although these technologies have the potential to revolutionize our understanding of disease and expand the diagnostic information available in biopsies from individual patients, there are several challenges to their implementation in routine clinical use (see Fig. 3).

One of the main limitations of spatial omics technology is the cost and complexity of the technology. These methods require specialized equipment and expertise, which can be expensive and time-consuming to acquire and maintain. This can make it challenging to implement these technologies in a clinical setting, where resources and funding for both physical and training resources may be limited. Widespread implementation will likely require a decrease in sequencing and reagent costs and improved automation.

Selection of appropriate regions of interest from all available tissue in which to deploy spatial omics assays (which typically have working areas limited to 1 cm^2 or less) remains dependent on traditional histologic methods for screening, a step that can have dramatic effects on sensitivity. In addition, there are less discrete units (loci) available for analysis in spatial data sets as compared with single-cell data sets, and therefore the information provided by a singular locus in a spatial data-set is in some ways more influential on the overall conclusions than studies that use methods such as single-cell RNA-seq.

Consequently, limitations on statistical power may restrict the amount of reliable information obtained by the assay, particularly for heterogeneous tissues in which functional subsets and populations of particular cell types can be small. For example, in spatial omics data sets, a little over a thousand RNA transcripts or hundreds of proteins are typically the most that are measurable from each cell. Using several samples may help to increase this statistical power, for example comparing cell-types near similar structures (e.g., a particular nerve head, or vessel), but this introduces additional cost.

A general limitation, common to many RNA sequencing methods, is bias in the complement of transcripts that are amplified and thus detectable by these assays. Read length can affect recoverability. Biological considerations can also introduce bias; for example in cells that hyper-produce a few transcripts (e.g., plasma cells producing IgG antibody proteins), other gene products that are more informative about cell state but are expressed at a lower level may not be amplified adequately.

In addition, the interpretation of spatial omics technology data can be challenging, particularly in the context of clinical decision making. The complexity of these datasets can make it difficult to identify the most relevant information and integrate it into clinical decision-making processes.

This may require specialized expertise in bioinformatics and data analysis, which may not be available to all clinicians and may take a significant amount of time. Further, there is currently a lack of standardized protocols and data analysis pipelines for spatial omics technologies; for a general outline of the analysis workflow, see Figure 4 as well as a recent methodologic review by Dries et al.[90]

The complexity of spatial omics technology protocols and lack of standardized analysis may impede the validation and verification required by regulatory agencies before reporting patient results.[91] As specific uses of spatial omics technologies are prospectively evaluated as companion diagnostics in randomized clinical trials, reliable parameters for clinical use will be established.

Currently, the most practical path to the clinic appears to be in using spatial omics technologies as a discovery tool for the underlying biology of multisystem interaction in disease, uncovering specific markers of these interactions that can then be implemented with more focused and basic techniques. However, as the technologies and analyses mature, clinical standards are put in place, and training becomes more widely available, these assays are expected to form a reliable platform for diagnosis.

Conclusion

The integration of spatial omics tools into clinical care has the potential to modernize the diagnosis of and facilitate better treatments for numerous diseases. Existing methods do not provide high-complexity information relative to native tissue environments on a cellular or micro-structural level within the tissue, which can lead to confounding assumptions. Spatial omics technologies, on the other hand, allow for simultaneous analysis of large numbers of molecular features within tissue sections while preserving their spatial information, adding a much-needed perspective about the cellular microenvironment and inter-cellular interactions in lesional tissue.

Beyond providing more detailed information, and a comprehensive understanding of the molecular landscape of disease at the tissue level, spatial omics technologies are expected to allow for the identification of novel biomarkers, the prediction of disease progression, and the development of personalized therapeutic strategies for conditions such as aggressive cancers.

As computational and technological advancements continue to improve the sensitivity and resolution of spatial omics, this technology will become increasingly informative and applicable to a wide range of clinical settings, making it a promising tool with potential for enhancing clinical care and improving patient outcomes.

Authors' Contributions

D.M.P.: Conceptualization, manuscript—original draft, editing, and review, development of Figure 1, and data acquisition for Figure 2A–C. B.Y.L.: Manuscript—editing, and review, data acquisition for Figure 2D. I.Z.: Manuscript—editing, and review. MD.: Conceptualization, manuscript—editing, and review, funding acquisition, development of figures.

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