

Spatial Characteristics of the Immune Microenvironment in Brain Metastases Provide Critical Insights for Developing Predictive Markers for Anti-PD-1 Therapy

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Abstract

Brain metastases (BrM) exhibit variable intracranial responses to immune checkpoint blockade (ICB), and reliable biomarkers of benefit remain uncertain, possibly reflecting the distinctive brain microenvironment. This review discusses multiplex protein imaging, array-based spatial transcriptomics, and in situ RNA imaging as complementary approaches for studying BrM and summarizes findings on regional differences in immune access and tumor cell states, CD8⁺ T cell localization and tumor contact, macrophage-associated and perivascular niches, tertiary lymphoid structure (TLS) signatures and organization, and potentially tumor-reactive or progenitor-like exhausted T cell populations. The clinical significance of these features varies. TLS signatures and several immune cell measures have been associated with survival, whereas differences in T cell infiltration and perivascular macrophage organization have been reported between ICB-treated and ICB-naïve BrM specimens. However, most studies are retrospective, rely on selected surgical specimens, and establish prognostic or treatment-associated relationships rather than prospectively validated predictors of anti-PD-1/PD-L1 benefit. Finally, the review considers how targeted, reproducible spatial measures could be benchmarked with conventional biomarkers and validated in prospective, tumor-type-specific intracranial cohorts before clinical use.

Keywords:

brain metastases; spatial omics; anti-PD-1 therapy; tumor microenvironment; predictive biomarkers; immune checkpoint; tertiary lymphoid structures

1. Introduction

Brain metastases (BrM) are a frequent and clinically consequential complication of advanced melanoma, non-small cell lung cancer (NSCLC), and breast cancer, and are associated with substantial neurological morbidity and poor survival. Immune checkpoint blockade (ICB) targeting programmed cell death protein 1 (PD-1) or programmed death-ligand 1 (PD-L1) has expanded treatment options for selected patients with BrM by restoring antitumor T cell activity [1,2]. The evidence for intracranial activity is particularly strong in melanoma. In the CheckMate 204 trial, nivolumab

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combined with ipilimumab achieved an intracranial benefit rate of 57% in patients with untreated melanoma BrM [3]. These findings demonstrated that durable immune-mediated control within the brain is attainable. Nevertheless, responses remain inconsistent in routine clinical practice. In a retrospective cohort of patients with NSCLC BrM treated with ICB, brain lesions responded differently from extracranial lesions in the same patient [4]. This discordance indicates that treatment response is shaped not only by tumor-intrinsic properties but also by the local brain microenvironment.

Conventional biomarkers only partially explain this variability. Although PD-L1 expression, tumor mutational burden (TMB), and microsatellite instability (MSI) are used to support treatment selection in extracranial disease [5–8], their capacity to predict intracranial response remains uncertain. In a retrospective cohort of patients with resected NSCLC BrM treated with ICB, higher intracranial PD-L1 expression was associated with longer intracranial progression-free survival, although the reported cutoff requires external validation and cannot be considered universally applicable [9]. In BrM, biomarker interpretation is further complicated by regional differences among the tumor core, invasive margin, perivascular compartment, and tumor-brain interface. Consequently, a specimen obtained from a single region may incompletely represent the lesion. The blood-tumor barrier (BTB) and the specialized cellular composition of brain tissue introduce additional variability by influencing drug penetration, antigen presentation, and immune cell trafficking [10–12].

The importance of spatial context in tumor immunology was recognized well before the advent of modern spatial omics. Immunoscore studies demonstrated that prognosis is related not only to the type and density of immune cells but also to their location within the tumor [13]. Spatially resolved technologies now extend this principle by enabling tumor, immune, vascular, stromal, and neural compartments to be examined within intact tissue architecture. These methods can reveal whether T cells are excluded from tumor nests, whether suppressive myeloid cells accumulate around blood vessels, and whether multicellular niches that support or restrain antitumor immunity coexist within the same lesion.

This review examines how spatial profiling has refined current understanding of BrM biology, with particular emphasis on intratumoral and intertumoral heterogeneity, immune-cell organization, vascular remodeling, glial responses, and multicellular interactions within the brain metastatic niche. It critically assesses the evidence supporting candidate spatial biomarkers, including tumor-immune proximity, T cell localization, macrophage-associated niches, tertiary lymphoid structures (TLS), and potentially tumor-reactive T cell states. It also considers the practical barriers to clinical implementation, the complementary role of liquid biopsy, and the potential of artificial intelligence and digital pathology to translate complex spatial measurements into clinically scalable tools.

The development of BrM proceeds through a series of spatially and temporally coordinated events. Circulating tumor cells (CTCs) released from primary tumors become arrested within brain capillaries, cross the blood-brain barrier (BBB), and initially proliferate along perivascular spaces before establishing independent metastatic colonies. Colonization subsequently reshapes the brain microenvironment. Resident microglia and astrocytes become reactive, whereas endothelial cells exposed to inflammatory factors (TNF- α , IL-1 β) upregulate the expression of adhesion molecules (VCAM-1, E-selectin). Tumor-derived factors (COX2, MMP9) further compromise barrier integrity. In turn, reactive glial cells provide signals that support tumor cell survival and outgrowth.

2. Spatial Omics Technologies: Overview and Applications

2.1. Evolution of Spatial Omics Technologies

Spatial omics has evolved in response to a central challenge in tissue biology: how to measure an increasing number of molecules while preserving their anatomical context. Early multiplex immunofluorescence (mIF) offered a practical means of visualizing several protein markers within intact tissue architecture, thereby linking immune phenotypes to their spatial locations. A major advance occurred in 2014, when imaging mass cytometry (IMC) and multiplexed ion beam imaging (MIBI) introduced metal-isotope-based detection. By avoiding much of the spectral overlap inherent in fluorescence-based methods, these platforms substantially expanded the number of proteins that could be measured simultaneously [14,15]. Spatial transcriptomics advanced in parallel. Multiplexed error-robust fluorescence in situ hybridization (MERFISH) enabled error-robust imaging of hundreds to thousands of RNA species at single-cell and even subcellular resolution [16]. The array-based spatial transcriptomics approach reported in 2016 subsequently made it possible to profile transcriptomes across tissue sections using positional barcodes, laying the groundwork for subsequent platforms such as Visium [17].

Subsequent platforms evolved along two complementary directions. Iterative antibody-based imaging methods, including co-detection by indexing (CODEX, now commercialized as PhenoCycler) and cyclic immunofluorescence (CycIF), enhanced protein multiplexing at single-cell resolution. Digital spatial profiling (DSP), Visium, and Slide-seqV2 expanded regional or near-cellular transcriptomic coverage [18]. More recent platforms have further narrowed the long-standing trade-off between molecular breadth and spatial resolution. CosMx Spatial Molecular Imager and Xenium In Situ can directly map targeted RNA molecules in formalin-fixed, paraffin-embedded (FFPE) tissue at single-cell or subcellular resolution, whereas Stereo-seq uses DNA nanoball arrays to support sequencing-based profiling across relatively large tissue areas [19–21]. Together, these methods have transformed the study of tumor tissues. Rather than merely counting individual markers, investigators can now examine how tumor, immune, stromal, and vascular cells are arranged into spatially defined multicellular niches. This shift has already yielded biologically meaningful insights in cancer research. For example, CODEX analysis identified a prognostically favorable MT2 immune niche in small-cell lung cancer, and studies integrating cytometry by time of flight (CyTOF), single-cell RNA sequencing (scRNA-seq), and spatial imaging have linked B cell-rich TLS with immunotherapy response in melanoma and renal cell carcinoma [22,23].

2.2. Relevance of Spatial Omics Technologies to Brain Metastases

In BrM research, these platforms are best regarded as complementary tools rather than competing alternatives (Table 1). Protein-based imaging methods are particularly useful when the primary aim is to define immune phenotypes or measure direct tumor-immune, immune-immune, and perivascular interactions. Whole-transcriptome spatial approaches are more suitable for discovery studies, particularly for comparing transcriptional programs across the tumor core, invasive margin, and adjacent brain tissue. High-plex in situ RNA platforms offer an additional advantage in retrospective clinical cohorts, because they can interrogate selected cell states, signaling programs, and ligand-receptor neighborhoods in archival FFPE samples. Therefore, in most BrM studies, the optimal platform is not necessarily the one with the highest plex or resolution, but the one that best matches the biological question, available tissue, field of view, turnaround time, and computational support. A practical workflow may begin with broad, sequencing-based spatial profiling for discovery, followed by a smaller, pathology-compatible imaging panel for validation and eventual clinical application [20].

Table 1. Comparison of spatial omics technology platforms for brain metastasis analysis.

Technology Platform	Spatial Resolution	Detection Throughput	Tissue Compatibility	Key Advantages	Limitations	Typical Applications in BrM Studies
Multiplex IF (mIF)	Single-cell	4–8 markers	FFPE and fresh frozen	Preserved cell morphology, quantitative accuracy	Limited throughput	Quantification of T-cell infiltration and checkpoint-receptor expression [33]
Imaging Mass Cytometry (IMC)	Subcellular (1 μm)	40+ markers	FFPE and fresh frozen	High-plex protein imaging with minimal spectral overlap	High cost, slow acquisition, and limited field-of-view throughput	Single-cell mapping of immune populations, cellular interactions, and regional neighborhoods [32]
MIBI-TOF	Subcellular	40+ protein markers	FFPE and fresh frozen	High-dimensional protein maps with low channel crosstalk	Ablative acquisition, high cost, limited availability, and restricted field-of-view throughput	NA
MERFISH	Single-cell/Subcellular	Thousands of targeted RNAs	Fresh frozen; FFPE compatibility	Error-robust barcoding and high plex	Complex imaging/decoding, optical crowding, and	NA

			depends on implementation		lengthy acquisition	
Visium	55 μ m spots	Whole transcriptome	Fresh frozen; FFPE-compatible workflows available	Unbiased transcriptomic analysis	Limited resolution	ICB-associated T-cell infiltration and perivascular CD206+ macrophage remodeling [41]
PhenoCycler-Fusion (formerly CODEX)	Single-cell	40–60 markers	FFPE and fresh frozen	High-throughput, preserved tissue architecture	Specialized equipment required	Mapping tumor-state-specific immune neighborhoods [26]
DSP	Regional (10–600 μ m)	Whole transcriptome /96 proteins	FFPE and fresh frozen	Flexible ROI selection	Non-single-cell resolution	Identification of immunosuppressive and fibrotic transcriptional programs [29]
SlideSeq V2	10 μ m beads	Whole transcriptome	Primarily fresh frozen	Near single-cell resolution	Complex data analysis	Metabolic heterogeneity mapping [28]
CosMx SMI	Single-cell/Subcellular	Hundreds to thousands of targeted RNAs	FFPE and fresh frozen	Direct transcript localization with morphology and cell interactions	Targeted panels, long imaging runs, and segmentation and transcript-assignment errors	NA
Xenium In Situ	Single-cell/Subcellular	Hundreds to thousands of targeted RNAs	FFPE and fresh frozen	High transcript sensitivity and scalable in situ workflow	Targeted design, segmentation/spillover artifacts, and substantial data processing	Single-cell resolution of survival-associated macrophage-tumor interactions, integrated with snRNA-seq and Visium data [27]
Stereo-seq	Nanoscale	Whole transcriptome	Primarily fresh frozen	Centimeter-scale field of view with broad transcriptomic coverage	High sequencing/computational burden; binning and cell-boundary uncertainty	NA

3. Spatial Architecture of Brain Metastases

3.1. Tumor Heterogeneity and Evolution

Spatial profiling has revealed marked intratumoral heterogeneity in BrM. In lung adenocarcinoma BrM, Xu et al. combined scRNA-seq and spatial transcriptomics and found that tumor cell populations are organized into distinct transcriptional programs [24]. The authors proposed a trajectory involving mesenchymal-epithelial transition (MET), with the transcription factor ATF3 acting as a potential regulator. They also implicated macrophage-derived LGALS3-ANXA2 signaling in promoting the MET program. These findings suggest that metastatic outgrowth is influenced not only by tumor-intrinsic changes but also by local interactions within the metastatic niche.

A complementary perspective was reported by Gonzalez et al., who analyzed 15 pan-cancer BrM samples and identified multiple macrophage states and eight functional tumor cell programs. These programs reflected distinct biological functions, including proliferation and inflammatory stress responses, suggesting that metastatic cells adapt to local microenvironmental pressures [25]. Yang et al. used PhenoCycler-Fusion 2.0 (formerly CODEX) with a 60-marker immune-oncology panel to profile 23 pan-cancer BrM specimens. The analysis distinguished major immune, stromal, vascular, and malignant cell populations and further classified malignant cells into epithelial, intermediate, proliferating epithelial, mesenchymal, and CD57+ neural-like states. Spatial neighborhood analysis demonstrated that

mesenchymal and CD57⁺ neural-like tumor cells were preferentially represented within 15 μ m of CD8⁺ and CD4⁺ T cells, whereas Ki-67⁺ epithelial tumor cells exhibited strong self-clustering [26]. These findings revealed distinct spatial patterns of tumor-immune organization in BrM, linking tumor cell states to local T-cell distribution. More recently, Khatri et al. integrated single-nucleus RNA sequencing (snRNA-seq) data from 23 BrM specimens with Visium profiling of 12 specimens and Xenium profiling of 4 specimens. This study identified spatially distinct macrophage-tumor interactions associated with clinical outcome. HLA-DR⁺ inflammatory macrophages in direct contact with tumor cells at the tumor boundary were associated with favorable treatment response and longer survival, whereas macrophages expressing extracellular matrix programs and TGF- β 1 were associated with poorer survival [27]. Pronounced spatial metabolic heterogeneity has also been documented. In melanoma BrM, Slide-seqV2 profiling revealed adjacent regions with distinct metabolic states, along with region-specific differences in antigen-presentation and interferon-related programs [28]. Together, these studies demonstrate that BrM heterogeneity extends beyond differences in cellular composition to the spatial organization of tumor states, immune-cell neighborhoods, and region-specific transcriptional and metabolic programs. Such features may be obscured by bulk profiling approaches.

3.2. Unique Features of the Brain Metastatic Niche

The brain metastatic niche is actively remodeled as tumor cells colonize and expand within the brain (Figure 1). Spatial transcriptomic analysis of NSCLC BrM from 44 patients demonstrated that BrM develop an immunosuppressive and fibrotic-like microenvironment, with reduced antigen-presentation activity and increased infiltration of neutrophils, macrophages, reactive astrocytes, and altered microglial states [29]. Importantly, these cell populations are unevenly distributed across the lesion; hence, their functional roles cannot be inferred from overall abundance alone.

Vascular remodeling is another defining feature of the BrM niche. Replacement of the BBB by a variably permeable BTB generates regional differences in immune-cell and drug access [30]. This altered vasculature influences where immune cells and therapeutic agents can enter the tumor. It may also induce hypoxia and metabolic stress within poorly perfused regions.

Reactive astrocytes further contribute to the spatial complexity of the brain metastatic niche. Priego et al. identified a pSTAT3⁺ astrocyte population surrounding BrM that promoted tumor progression [31]. These cells suppressed CD8⁺ T cell activation and promoted the expansion of CD74⁺ microglia/macrophages, thereby linking a glial state to local immune inhibition. In the same study, Legasil, a silibinin-based nutraceutical shown to impair STAT3 activation, was evaluated in a small, non-randomized cohort of patients with lung cancer BrM and demonstrated encouraging intracranial activity. However, these findings warrant cautious interpretation, as most patients received concomitant chemotherapy.

Together, these findings underscore the necessity to consider the spatial organization of the brain metastatic niche. Tumor-immune interactions, vascular access, and glial-derived immunosuppression vary across regions of the lesion, which may partly explain why immune infiltration does not consistently translate into effective antitumor activity.

3.3. Comparison with Primary Brain Tumors

Direct comparison with primary brain tumors helps to distinguish site-specific from lineage-specific features. In an IMC study comprising 389 high-dimensional histopathology images from 139 high-grade glioma and 46 BrM patient tumors, Karimi et al. found that BrM contained higher frequencies of natural killer cells (NK cells), neutrophils, T cells, regulatory T cells, classical monocytes, and macrophages than glioblastomas [32]. BrM tumor cells formed more compact territories and exhibited stronger spatial avoidance of most nonmalignant lineages. Nine cellular neighborhoods were identified across the cohort: tumor-compartment neighborhoods predominated in BrM cores, whereas margins shared features with glioblastoma, indicating that the local anatomical region can be as influential as tumor origin. The degree of inflammation also differed according to primary cancer type. Melanoma BrM exhibited the most prominent CD8⁺ T cell accumulation at tumor margins, whereas breast cancer BrM harbored the least inflammatory infiltrate. These differences argue against treating BrM as a single immunological entity.

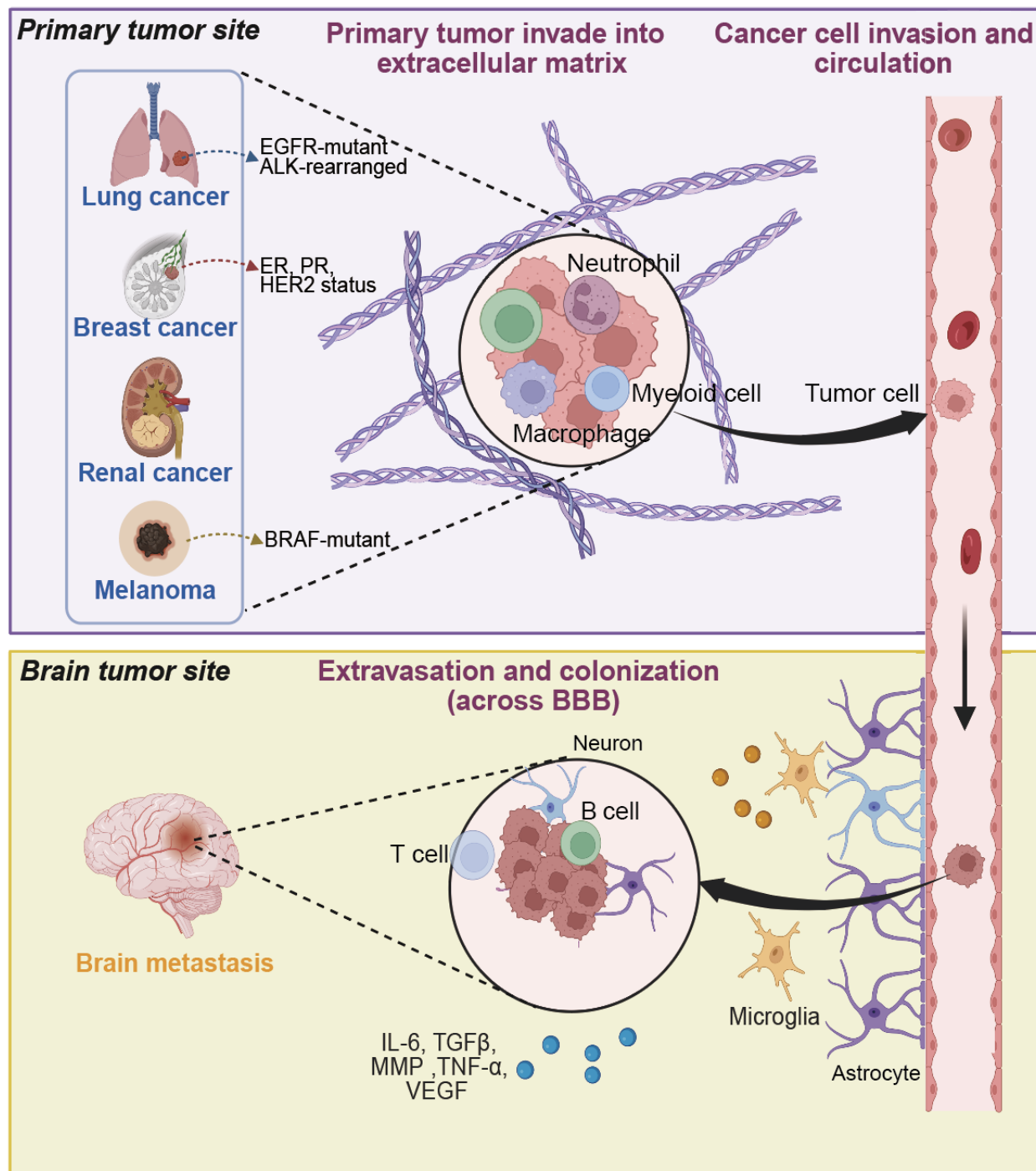


Figure 1. Brain metastasis formation.

4. Immune Landscape of Brain Metastases

4.1. Immune Cell Composition and Spatial Distribution

Antitumor immunity in BrM is shaped not only by immune cell abundance but also by their spatial distribution and

interactions within the tumor microenvironment (Figure 2a). Spatial analyses have demonstrated that tumor-associated macrophages (TAMs) and T cells frequently occupy adjacent regions in BrM. However, such proximity does not necessarily reflect productive antitumor immunity. CD4⁺ T cells within these niches may exhibit anergic states, CD8⁺ T cells may be exhausted, and neighboring TAMs may express immunoregulatory molecules, including PD-L1/PD-L2, IDO-1/IDO-2, and CD80. Thus, spatial contact may create opportunities for immune recognition while simultaneously sustaining local immunosuppression [11]. The distribution of immune cells also varies across anatomical regions within individual lesions. Karimi et al. observed a pronounced accumulation of CD8⁺ T cells at the tumor margins of melanoma BrM [32]. This regional pattern illustrates how aggregate CD8⁺ T cell counts may obscure differences in immune localization.

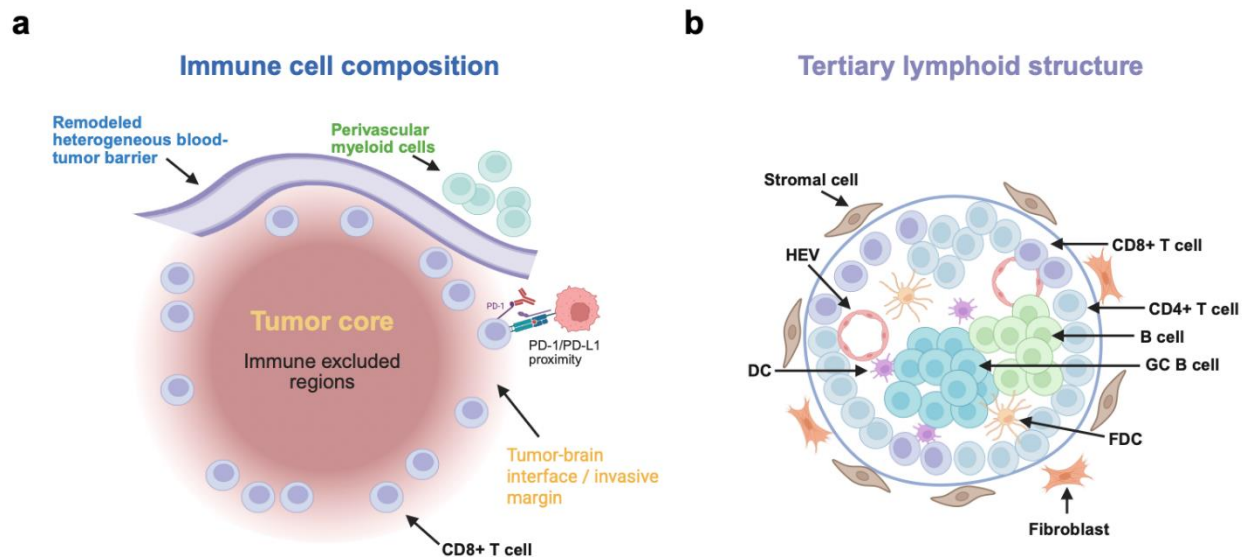


Figure 2. Spatial immune architecture of the brain metastatic microenvironment. (a) Schematic representation of the immune cell composition in BrM, highlighting immune-cell accumulation at the tumor-brain interface and perivascular spaces, heterogeneous blood-tumor barrier remodeling, immune-excluded tumor regions, and CD8⁺ T cell penetration into the tumor core. (b) Schematic representation of tertiary lymphoid structures composed of organized B cell follicles, T cell zones, DCs, and HEVs that support local antigen presentation, lymphocyte activation, and immune priming.

4.2. T Cell States and Exhaustion Patterns

Checkpoint-receptor expression in BrM may not carry the same significance as in extracranial tumors. Using mIF, Lu et al. compared 65 lung cancer BrM with 41 primary lung tumors [33]. BrM contained fewer CD3⁺, CD4⁺, CD8⁺, and FOXP3⁺ cells, lower granzyme B expression, and reduced PD-1, TIM-3, and LAG-3 expression on CD3⁺ T cells, despite comparable PD-L1 levels at both sites. Unexpectedly, higher expression of TIM-3 and LAG-3 on CD3⁺ T cells was associated with longer overall survival (OS) in BrM but not in primary lung tumors. These findings challenge the simplistic view that receptors such as PD-1 or TIM-3 always mark irreversibly exhausted T cells [34]. The results emphasize that immune receptor expression should be interpreted not in isolation but in conjunction with the functional state and location of the cells.

The origin of tumor-reactive T cells may also influence treatment response. In melanoma BrM treated with ICB, scRNA-seq revealed that patients with partial responses had stronger clonal expansion of T cells in the peripheral blood, rather than only within the brain lesion [35]. T cell clones shared between blood and brain also appeared less exhausted than those confined to the brain. These findings suggest that circulating T cells may constitute a more functional immune source for BrM. Therefore, vascular access and immune cell trafficking may be important determinants of immunotherapy response rather than merely background anatomical features.

4.3. Tertiary Lymphoid Structures

TLS are organized, non-encapsulated lymphoid aggregates that develop in chronically inflamed tissues. Mature TLS typically comprise spatially segregated B cell follicles and T cell zones, together with dendritic cells (DCs), follicular dendritic cell (FDC) networks, and high endothelial venules (HEVs) (Figure 2b). This organization distinguishes TLS from unstructured lymphocyte accumulation and may facilitate local antigen presentation, B cell maturation, antibody production, and the maintenance of antitumor T cell responses.

Direct evidence in BrM remains limited. Mughal et al. performed RNA sequencing and spatial immune-cell analysis in BrM samples from 95 patients. They developed a 32-gene TLS signature and corroborated the transcriptomic classification using mIF, identifying organized aggregates containing CD20+ B cells, CD3+ T cells, and LAMP3+ dendritic cells [36]. TLS signatures were detected more frequently in BrM derived from lung cancer and melanoma than in breast cancer BrM and were more prevalent in treatment-naïve lesions. Among patients with lung cancer BrM, higher TLS signature scores were associated with longer OS, and this association was reproduced in an independent transcriptomic cohort. This observation is consistent with previous findings in non-small cell lung cancer, breast cancer, and colorectal cancer [37–39]. Collectively, these findings suggest that the organization of immune cells into TLS may be biologically and clinically relevant in BrM.

4.4. Tumor-Reactive and Progenitor-like T Cell States

BrM can also be stratified on the basis of the abundance of potentially tumor-reactive T cells (pTRT cells). Approximately half of the analyzed BrM samples displayed a pTRT cell-high microenvironment characterized by abundant CXCL13-expressing CD39+CD8+ T cells within tumor nests. These cells were clonally expanded and exhibited minimal overlap with circulating T cell clones, supporting their putative tumor reactivity. In pTRT cell-high BrM, microglia and monocyte-derived macrophages showed enhanced interferon response, antigen presentation, and T cell recruitment programs, including increased expression of CXCL9, CXCL10, and CXCL11. However, their high IDO1 expression also indicated immunosuppressive potential, suggesting that distinct macrophage populations may exert opposing functions within these tumors [40].

By integrating scRNA-seq with spatial transcriptomics, Sun et al. identified T cell factor 1 (TCF-1)-expressing progenitor-like exhausted CD8+ T cells (CD8-Tprog.ex) in BrM [41]. These cells can self-renew and give rise to terminally exhausted descendants, properties considered essential for sustained responses to ICB. A greater abundance of these cells was associated with longer OS. This result suggests that therapeutic potential may depend less on the total number of CD8+ T cells than on whether a lesion contains a spatially accessible reservoir of progenitor-like cells capable of replenishing the effector pool.

4.5. Vascular and Stromal Remodeling

The distribution of immune cells in BrM is closely linked to the local vasculature. Remodeling of the BBB into the BTB alters both barrier permeability and the routes through which circulating immune cells enter metastatic lesions. Monocytes recruited to the tumor margins and perivascular regions can differentiate into macrophages with immunosuppressive phenotypes. These cells may restrict T-cell activity even in the presence of nearby lymphocytes [11]. Myeloid cells may also contribute at an earlier stage of metastasis. Cathepsin S released by macrophages and microglia can disrupt endothelial junctions and facilitate tumor-cell passage across the BBB [42].

The vascular niche may also be altered by treatment. Sun et al. identified cuffs of CD206+ macrophages around blood vessels in untreated BrM [41]. These structures became less prominent following immune checkpoint blockade, although the study did not establish whether their loss directly enhanced T-cell entry into the tumor. Bejarano et al. further demonstrated that vascular structure influences immune cell access. In preclinical models, reduced pericyte coverage was associated with increased vascular permeability and greater immune cell infiltration [43]. These observations suggest that vascular features may help to identify which tumor regions are accessible to immune cells. Any attempt to modify this compartment therapeutically must nevertheless account for the protective function of the BBB in normal brain tissue.

4.6. Critical Appraisal of Current Spatial Evidence

The available evidence is biologically coherent but remains insufficient for clinical qualification. Several patterns

recur across studies, including myeloid cell enrichment, immune accumulation at tumor margins or perivascular sites, heterogeneous vascular permeability, and limited penetration of cytotoxic lymphocytes into tumor nests. Nevertheless, important uncertainties remain. The relative abundance and functional roles of microglia, infiltrating macrophages, and exhausted T cell states differ across cohorts, probably because studies vary in primary tumor type, corticosteroid exposure, prior radiotherapy, systemic treatment, sampled region, and analytical platform. Most datasets are retrospective, single-center, and cross-sectional, and many include only a small number of surgically resected lesions. Such samples may not be representative of small, deep, or non-surgical metastases. Therefore, candidate spatial features, including CD8⁺ T cell proximity, TLS organization, macrophage-enriched neighborhoods, and progenitor-like exhausted T cells, should currently be regarded as discovery-stage or early translational biomarkers. None of these features has yet been prospectively validated in a multicenter trial using a prespecified assay, cutoff, and intracranial endpoint.

5. Therapeutic Implications and Biomarker Discovery

The translational promise of spatial omics lies in its capacity to identify targets and biomarkers that are not apparent from bulk measurements. For BrM, the most informative candidates are likely to integrate tumor-intrinsic expression with the vascular, stromal, and immune compartments that determine whether a therapy can reach and engage its target. Candidate spatial features of potential clinical relevance are summarized in Table 2.

Table 2. Candidate spatial features with potential clinical relevance in brain metastases.

Candidate Spatial Feature	Definition	Reported Association	Evidence Setting	Reference	Clinical Validation
TLS signature and organization	A 32-gene RNA-based TLS signature, supported by mIF detection of organized CD20 ⁺ B cell, CD3 ⁺ T cell, and LAMP3 ⁺ dendritic cell aggregates	Higher TLS signature scores were associated with longer OS after BrM diagnosis in lung cancer BrM	Retrospective human BrM cohorts; prognostic analysis	[36]	Early translational: prognostic association reproduced in an independent transcriptomic cohort; no prospective validation of ICI predictive value
Intratumoral CD3⁺ T cell infiltration	CD3 ⁺ T cell infiltration quantified using multiplex quantitative immunofluorescence	Higher CD3 ⁺ infiltration was associated with longer OS in lung cancer BrM	Retrospective human BrM cohort; prognostic analysis	[33]	Exploratory prognostic evidence; no validated cutoff or prospective assessment
TIM-3 and LAG-3 expression on CD3⁺ T cells	Quantitative expression on CD3 ⁺ T cells, dichotomized at the median	Higher TIM-3 and LAG-3 expression was associated with longer OS in lung cancer BrM	Retrospective human BrM cohort; prognostic analysis	[33]	Exploratory prognostic evidence; no validated cutoff or prospective assessment
Progenitor-exhausted CD8⁺ T cell abundance	Relative abundance of the scRNA-seq-defined CD8 ⁺ Tprog.ex population, with spatial distribution assessed by spatial transcriptomics	Greater abundance was associated with longer OS, particularly in ICB-treated BrM patients	Retrospective, heterogeneous human BrM cohort	[41]	Discovery stage: no standardized tissue assay, clinical cutoff, or prospective validation
Perivascular CD14⁺CD206⁺ macrophage organization	Localization of CD14 ⁺ CD206 ⁺ macrophages around α -SMA ⁺ vessels,	Perivascular macrophage accumulation was reduced after ICB and	Comparison of ICB-naïve and ICB-treated human BrM specimens	[41]	Mechanistic and treatment-associated feature; causality and

assessed using spatial transcriptomics and mIF	was accompanied by increased T-cell infiltration	predictive value remain unvalidated
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B7-H3 (CD276) represents one such candidate. It is expressed by tumor cells in breast cancer BrM [44] and is also upregulated in the vasculature of BrM arising from several primary cancers [43,45]. Its expression across malignant and stromal compartments could broaden the therapeutic reach of B7-H3-directed approaches. At the same time, spatial mapping will be required to establish which cellular source predicts response and whether expression in normal or reactive brain tissue causes clinically relevant toxicity.

Spatial studies also indicate opportunities for intervention before macroscopic metastases are established. In lung adenocarcinoma BrM, Xu et al. implicated tumor cell ATF3 and macrophage-mediated LGALS3/ANXA2 signaling in the MET program associated with metastatic outgrowth [24]. If experimentally validated, these pathways could be targeted during seeding or early colonization. The clinical challenge is temporal: biomarkers would need to identify high-risk patients while the disease remains at a stage at which disrupting colonization is still feasible.

TIM-3 exemplifies why therapeutic targets cannot be interpreted independently of disease stage and anatomical site. In models of lung adenocarcinoma precursors, Zhu et al. found that TIM-3-associated features were enriched early in tumor development and that TIM-3 blockade reduced tumor burden when administered at the precancerous stage, but not during advanced disease [46]. Early intervention enhanced antigen presentation and T cell activation and shifted macrophage polarization toward a higher M1/M2 ratio. These findings support TIM-3 as a potential target for cancer interception.

However, the BrM data point in a more complex direction. Lu et al. reported that lung cancer BrM contained fewer T cells and lower expression of granzyme B, PD-1, TIM-3, and LAG-3 than primary lung tumors, yet higher TIM-3 and LAG-3 expression on CD3⁺ T cells was associated with longer survival specifically in BrM [33]. Taken together, these two studies suggest that TIM-3 marks different biological states across stages and tissues. Clinical development will therefore require spatially and phenotypically defined patient selection rather than reliance on aggregate TIM-3 expression alone.

TLS organization may also serve as a candidate spatial biomarker in BrM because it captures coordinated local immune activity rather than the abundance of a single immune cell population. However, the current evidence is prognostic rather than predictive. It remains unclear whether TLS-positive BrM derive greater benefit specifically from PD-1/PD-L1 blockade or whether TLS merely identify tumors with a generally more favorable immune microenvironment.

Spatial metrics may provide information beyond aggregate marker levels by revealing whether immune cells are positioned to interact with tumor cells. Karimi et al. observed a prominent accumulation of CD8⁺ T cells at the tumor margins of melanoma BrM [32]. This regional pattern demonstrates how aggregate CD8⁺ T cell counts may obscure differences in immune localization. Whether margin-associated CD8⁺ T cell accumulation predicts intracranial treatment response remains to be determined and should be evaluated in prospective ICB-treated BrM cohorts.

6. Clinical Translation and Current Immunotherapy Trials

Prospective intracranial evidence is most mature in melanoma. Phase II studies demonstrated intracranial activity with pembrolizumab monotherapy in selected patients with active melanoma or NSCLC BrM [47,48]. The randomized Anti-PD-1 Brain Collaboration (ABC) study and CheckMate 204 support the use of nivolumab plus ipilimumab as a highly active regimen for asymptomatic melanoma BrM [1,3,49,50]. In the CheckMate 204 trial, the intracranial clinical benefit rate was 57.4% among asymptomatic patients but only 16.7% in the small symptomatic cohort, in which low-dose corticosteroids were permitted [50].

For NSCLC BrM, pembrolizumab has shown intracranial activity in a phase II cohort of patients with PD-L1-positive tumors and limited, asymptomatic brain lesions [48,51].

The optimal integration of stereotactic radiosurgery (SRS) with checkpoint blockade remains unresolved, because treatment timing, lesion selection, and radio necrosis can confound intracranial response assessment [52]. Evidence beyond PD-1 blockade remains preliminary. Dedicated BrM trials of LAG-3 blockade are emerging, including a trial evaluating nivolumab plus relatlimab (NCT05704647) in active melanoma BrM [53]. Anti-TIGIT and anti-TIM-3 strategies have not yet been prospectively validated in active BrM.

7. Limitations and Requirements for Clinical Implementation

7.1. Technical, Analytical, and Operational Constraints

Routine use of spatial omics in BrM is limited primarily by tissue availability and quality. Most specimens are obtained during surgery and therefore derive mainly from large, symptomatic, or surgically accessible lesions. Small or deeply located metastases are underrepresented in existing cohorts. The available material may also vary in ischemic time, fixation, storage duration, and prior treatment. Although FFPE-compatible platforms permit the study of archival samples, RNA degradation and altered antigen detection can compromise assay performance. Fresh-frozen tissue generally supports broader transcriptomic analysis, but its collection requires coordination at the time of surgery and is not feasible at all centers.

Practical limitations persist beyond tissue collection. Spatial assays require specialized equipment, substantial data storage, and expertise in pathology and bioinformatics. Their turnaround time may also be incompatible with urgent treatment decisions. Data analysis is particularly challenging in small BrM cohorts, where technical batch effects may be confounded with differences in primary tumor type, sampled region, or previous therapy. Batch correction can mitigate technical variation, but excessive correction may also eliminate genuine regional differences. For clinical use, the most promising findings will therefore need to be translated into smaller, more reproducible assays that are compatible with limited tissue.

7.2. Artificial Intelligence (AI) and Digital Pathology

Digital pathology may offer a more accessible means of evaluating some spatial features. Models applied to whole-slide hematoxylin and eosin (H&E) images can examine larger tissue areas than manually selected fields of view and can identify tumor, necrosis, blood vessels, immune-rich regions, and adjacent brain tissue [54,55]. Graph-based methods can also represent cells and their spatial relationships, making them well suited to the analysis of tumor-immune contact and cellular neighborhoods [56]. Histological information could also be integrated with spatial omics, scRNA-seq, magnetic resonance imaging (MRI), and clinical data [57].

One potential clinical application would be to screen routine H&E slides before ordering a more expensive spatial assay. Cases exhibiting a recognizable spatial pattern might be assessed directly, whereas equivocal cases could undergo additional molecular profiling. Such an approach could extend spatial assessment to hospitals lacking dedicated spatial-omics facilities and to retrospective cohorts containing only conventional slides.

Several challenges remain unresolved. A model may learn to recognize differences in staining, scanners, tissue processing, or institutional annotation rather than biologically relevant features. This concern is particularly pertinent in BrM, where morphology also varies according to the primary cancer, prior radiotherapy, hemorrhage, necrosis, and corticosteroid exposure. Performance within a single institutional dataset is therefore insufficient. Before clinical use, models should be validated across centers and technical settings, calibrated for the intended population, and compared with expert pathological assessment [58].

7.3. Liquid Biopsy as a Longitudinal Complement

A spatial assay characterizes the tissue collected at a particular time and location. It cannot capture how an intracranial lesion evolves during treatment unless another tissue sample is obtained. Liquid biopsy could provide complementary longitudinal information without repeated neurosurgical sampling.

However, its performance in BrM depends on the source of the sample and the distribution of disease. Plasma-derived circulating tumor DNA (ctDNA) may be difficult to detect when extracranial tumor burden is low, partly because the BBB and BTB limit the release of tumor-derived DNA into the peripheral circulation. Cerebrospinal fluid (CSF) ctDNA may more accurately reflect intracranial genomic alterations in selected patients; however, lumbar puncture is invasive, and diagnostic yield varies with lesion location and proximity to CSF spaces [59,60]. Spatial profiling and liquid biopsy are therefore more likely to be complementary than interchangeable. Tissue collected at baseline could characterize the cellular organization of the lesion, while plasma or CSF ctDNA and MRI could be employed for monitoring during follow-up. Discordant findings would require interpretation in relation to extracranial disease, treatment history, imaging changes, and the anatomical site sampled. Repeat tissue assessment should be reserved for cases in which it is clinically justified and can be performed safely.

8. Conclusions and Future Directions

PD-L1 expression, TMB, and MSI can inform ICB treatment selection in specific cancer settings, but their ability to predict intracranial response in patients with BrM remains uncertain. For an effective response to occur, T cells must infiltrate the tumor, migrate beyond the perivascular or peripheral regions, recognize malignant cells, and remain functional despite local suppressive signals [61]. Spatial profiling may provide complementary information on immune cell location and cell-cell contact. CD8+ T cell infiltration into tumor regions, tumor-immune proximity, TLS organization, and macrophage-associated niches represent candidate features [32,36,41].

Within BrM, blood-tumor barrier permeability and perivascular myeloid organization vary regionally [30,41,43]. These observations may help explain why some patients exhibit discordant intracranial and extracranial responses, although direct longitudinal evidence remains limited. Paired, longitudinal sampling of brain and extracranial disease during treatment would help to determine which differences are specific to the brain microenvironment.

Several spatial features warrant further clinical validation. These include the balance between microglia and infiltrating macrophages, the integrity of the BTB at the tumor core and margin, the presence of macrophage-rich perivascular regions, and the extent to which CD8+ T cells penetrate tumor nests. These features should be studied alongside established biomarkers rather than treated as substitutes. Sampling strategy will also be critical. Spatial maps generated from surgical specimens may guide biopsy strategies by identifying regions that better capture immune access, exclusion, or perivascular niche biology, but these strategies must balance safety, tissue availability, and treatment-related changes caused by corticosteroids, radiotherapy, or systemic therapy.

Future studies should evaluate whether spatial measurements improve prediction of intracranial ICB benefit. Prospective multicenter cohorts should prespecify tissue regions, assays, quality-control thresholds, intracranial endpoints, and analysis plans before enrollment. Validation should be stratified by primary tumor type and include symptomatic or corticosteroid-treated patients. Candidate measurements could include CD8+ T cell-tumor contact, TLS organization, macrophage-vascular neighborhoods, and progenitor T cell niches. AI-assisted multimodal models could integrate these spatial features with genomic profiles, digital pathology, MRI, and longitudinal liquid biopsy data to predict intracranial ICB benefit.

9. List of Abbreviations

ABC	Anti-PD-1 Brain Collaboration
AI	Artificial Intelligence
BBB	Blood-Brain Barrier
BrM	Brain Metastases
BTB	Blood-Tumor Barrier
CODEX	Co-Detection by Indexing
CSF	Cerebrospinal Fluid
CTCs	Circulating Tumor Cells
ctDNA	Circulating Tumor DNA
CycIF	Cyclic Immunofluorescence
CyTOF	Cytometry by Time of Flight
DC/DCs	Dendritic Cell/Dendritic Cells
DSP	Digital Spatial Profiling
FDC	Follicular Dendritic Cell
FFPE	Formalin-Fixed, Paraffin-Embedded
GC B cell	Germinal Center B Cell
H&E	Hematoxylin and Eosin
HEVs	High Endothelial Venules
ICB	Immune Checkpoint Blockade
IMC	Imaging Mass Cytometry
LAG-3	Lymphocyte Activation Gene-3
MERFISH	Multiplexed Error-Robust Fluorescence in Situ Hybridization
MET	Mesenchymal-Epithelial Transition
MIBI	Multiplexed Ion Beam Imaging

mIF	Multiplex Immunofluorescence
MRI	Magnetic Resonance Imaging
MSI	Microsatellite Instability
NK cells	Natural Killer Cells
NSCLC	Non-Small Cell Lung Cancer
OS	Overall Survival
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Death-Ligand 1
PD-L2	Programmed Death-Ligand 2
pTRT cells	Potentially Tumor-Reactive T Cells
scRNA-seq	Single-Cell RNA Sequencing
snRNA-seq	Single-Nucleus RNA Sequencing
SRS	Stereotactic Radiosurgery
TAMs	Tumor-Associated Macrophages
TCF-1	T Cell Factor 1
TIGIT	T-Cell Immunoreceptor with Ig and ITIM Domains
TIM-3	T-Cell Immunoglobulin and Mucin-Domain Containing-3
TLS	Tertiary Lymphoid Structures
TMB	Tumor Mutational Burden

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Consent for Publication

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Conflicts of Interest

The authors declare no conflicts of interest related to this manuscript. The first author is affiliated with the same institution as the Guest Editor. To ensure editorial impartiality, the Guest Editor was not involved in the handling of, or decision-making related to, this manuscript.

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References

- [1] Long, G.V.; Atkinson, V.; Lo, S.; Guminski, A.D.; Sandhu, S.K.; Brown, M.P.; Gonzalez, M.; Scolyer, R.A.; Emmett, L.; McArthur, G.A.; et al. Five-Year Overall Survival from the Anti-PD1 Brain Collaboration (ABC

- Study): Randomized Phase 2 Study of Nivolumab (Nivo) or Nivo+Ipilimumab (ipi) in Patients (pts) with Melanoma Brain Metastases (mets). *J. Clin. Oncol.* **2021**, *39*, 9508. https://doi.org/10.1200/jco.2021.39.15_suppl.9508.
- [2] Chen, D.S.; Mellman, I. Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* **2013**, *39*, 1–10. <https://doi.org/10.1016/j.immuni.2013.07.012>.
 - [3] Tawbi, H.A.; Forsyth, P.A.; Algazi, A.; Hamid, O.; Hodi, F.S.; Moschos, S.J.; Khushalani, N.I.; Lewis, K.; Lao, C.D.; Postow, M.A.; et al. Combined Nivolumab and Ipilimumab in Melanoma Metastatic to the Brain. *N. Engl. J. Med.* **2018**, *379*, 722–730. <https://doi.org/10.1056/nejmoa1805453>.
 - [4] Kang, S.; Jeong, H.; Park, J.E.; Kim, H.S.; Kim, Y.H.; Lee, D.H.; Kim, S.W.; Lee, J.C.; Choi, C.M.; Yoon, S. Central Nervous Systemic Efficacy of Immune Checkpoint Inhibitors and Concordance Between Intra/Extracranial Response in Non-Small Cell Lung Cancer Patients with Brain Metastasis. *J. Cancer Res. Clin. Oncol.* **2023**, *149*, 4523–4532. <https://doi.org/10.1007/s00432-022-04251-3>.
 - [5] Reck, M.; Rodríguez-Abreu, D.; Robinson, A.G.; Hui, R.; Csőszi, T.; Fülöp, A.; Gottfried, M.; Peled, N.; Tafreshi, A.; Cuffe, S.; et al. Pembrolizumab Versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2016**, *375*, 1823–1833. <https://doi.org/10.1056/nejmoa1606774>.
 - [6] Herbst, R.S.; Baas, P.; Kim, D.-W.; Felip, E.; Pérez-Gracia, J.L.; Han, J.-Y.; Molina, J.; Kim, J.-H.; Arvis, C.D.; Ahn, M.-J.; et al. Pembrolizumab Versus Docetaxel for Previously Treated, PD-L1-Positive, Advanced Non-Small-Cell Lung Cancer (KEYNOTE-010): A Randomised Controlled Trial. *Lancet* **2016**, *387*, 1540–1550. [https://doi.org/10.1016/s0140-6736\(15\)01281-7](https://doi.org/10.1016/s0140-6736(15)01281-7).
 - [7] Cao, D.; Xu, H.; Xu, X.; Guo, T.; Ge, W. High Tumor Mutation Burden Predicts Better Efficacy of Immunotherapy: A Pooled Analysis of 103078 Cancer Patients. *Oncoimmunology* **2019**, *8*, e1629258. <https://doi.org/10.1080/2162402x.2019.1629258>.
 - [8] Le, D.T.; Uram, J.N.; Wang, H.; Bartlett, B.R.; Kemberling, H.; Eyring, A.D.; Skora, A.D.; Luber, B.S.; Azad, N.S.; Laheru, D.; et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N. Engl. J. Med.* **2015**, *372*, 2509–2520. <https://doi.org/10.1056/nejmoa1500596>.
 - [9] Wasilewski, D.; Onken, J.; Horicke, P.; Bukatz, J.; Murad, S.; Fruh, A.; Shaked, Z.; Misch, M.; Kuhl, A.; Klein, O.; et al. Predictive Role of Intracranial PD-L1 Expression in a Real-World Cohort of NSCLC Patients Treated with Immune Checkpoint Inhibition Following Brain Metastasis Resection. *J. Neurooncol.* **2024**, *167*, 155–167. <https://doi.org/10.1007/s11060-024-04590-w>.
 - [10] Achrol, A.S.; Rennert, R.C.; Anders, C.; Soffiatti, R.; Ahluwalia, M.S.; Nayak, L.; Peters, S.; Arvold, N.D.; Harsh, G.R.; Steeg, P.S.; et al. Brain metastases. *Nat. Rev. Dis. Primers* **2019**, *5*, 5. <https://doi.org/10.1038/s41572-018-0055-y>.
 - [11] Klemm, F.; Maas, R.R.; Bowman, R.L.; Kornete, M.; Soukup, K.; Nassiri, S.; Brouland, J.P.; Iacobuzio-Donahue, C.A.; Brennan, C.; Tabar, V.; et al. Interrogation of the Microenvironmental Landscape in Brain Tumors Reveals Disease-Specific Alterations of Immune Cells. *Cell* **2020**, *181*, 1643–1660.e1617. <https://doi.org/10.1016/j.cell.2020.05.007>.
 - [12] Harter, P.N.; Bernatz, S.; Scholz, A.; Zeiner, P.S.; Zinke, J.; Kiyose, M.; Blasel, S.; Beschorner, R.; Senft, C.; Bender, B.; et al. Distribution and Prognostic Relevance of Tumor-Infiltrating Lymphocytes (TILs) and PD-1/PD-L1 Immune Checkpoints in Human Brain Metastases. *Oncotarget* **2015**, *6*, 40836–40849. <https://doi.org/10.18632/oncotarget.5696>.
 - [13] Galon, J.; Costes, A.; Sanchez-Cabo, F.; Kirilovsky, A.; Mlecnik, B.; Lagorce-Pagès, C.; Tosolini, M.; Camus, M.; Berger, A.; Wind, P.; et al. Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. *Science* **2006**, *313*, 1960–1964. <https://doi.org/10.1126/science.1129139>.
 - [14] Giesen, C.; Wang, H.A.; Schapiro, D.; Zivanovic, N.; Jacobs, A.; Hattendorf, B.; Schöffler, P.J.; Grolimund, D.; Buhmann, J.M.; Brandt, S.; et al. Highly Multiplexed Imaging of Tumor Tissues with Subcellular Resolution by Mass Cytometry. *Nat. Methods* **2014**, *11*, 417–422. <https://doi.org/10.1038/nmeth.2869>.
 - [15] Angelo, M.; Bendall, S.C.; Finck, R.; Hale, M.B.; Hitzman, C.; Borowsky, A.D.; Levenson, R.M.; Lowe, J.B.; Liu, S.D.; Zhao, S.; et al. Multiplexed Ion Beam Imaging of Human Breast Tumors. *Nat. Med.* **2014**, *20*, 436–442. <https://doi.org/10.1038/nm.3488>.
 - [16] Chen, K.H.; Boettiger, A.N.; Moffitt, J.R.; Wang, S.; Zhuang, X. Spatially Resolved, Highly Multiplexed RNA Profiling in Single Cells. *Science* **2015**, *348*, aaa6090. <https://doi.org/10.1126/science.aaa6090>.
 - [17] Ståhl, P.L.; Salmén, F.; Vickovic, S.; Lundmark, A.; Navarro, J.F.; Magnusson, J.; Giacomello, S.; Asp, M.; Westholm, J.O.; Huss, M.; et al. Visualization and Analysis of Gene Expression in Tissue Sections by Spatial Transcriptomics. *Science* **2016**, *353*, 78–82. <https://doi.org/10.1126/science.aaf2403>.

- [18] Dakshinamoorthy, G.; Singh, J.; Kim, J.; Nikulina, N.; Bashier, R.; Mistry, S.; Gallina, M.E.; Choksi, A.; Perera, M.; Wilson, A.; et al. Abstract 490: Highly Multiplexed Single-Cell Spatial Analysis of Tissue Specimens Using CODEX. *Cancer Res.* **2019**, *79*, 490. <https://doi.org/10.1158/1538-7445.am2019-490>.
- [19] He, S.; Bhatt, R.; Brown, C.; Brown, E.A.; Buhr, D.L.; Chantranuvatana, K.; Danaher, P.; Dunaway, D.; Garrison, R.G.; Geiss, G.; et al. High-Plex Imaging of RNA and Proteins at Subcellular Resolution in Fixed Tissue by Spatial Molecular Imaging. *Nat. Biotechnol.* **2022**, *40*, 1794–1806. <https://doi.org/10.1038/s41587-022-01483-z>.
- [20] Ozirmak Lermi, N.; Molina Ayala, M.; Hernandez, S.; Lu, W.; Khan, K.; Serrano, A.; Lubo, I.; Hamana, L.; Tomczak, K.; Barnes, S.; et al. Comparison of Imaging Based Single-Cell Resolution Spatial Transcriptomics Profiling Platforms Using Formalin-Fixed Paraffin-Embedded Tumor Samples. *Nat. Commun.* **2025**, *16*, 8499. <https://doi.org/10.1038/s41467-025-63414-1>.
- [21] Chen, A.; Liao, S.; Cheng, M.; Ma, K.; Wu, L.; Lai, Y.; Qiu, X.; Yang, J.; Xu, J.; Hao, S.; et al. Spatiotemporal Transcriptomic Atlas of Mouse Organogenesis Using DNA Nanoball-Patterned Arrays. *Cell* **2022**, *185*, 1777–1792.e1721. <https://doi.org/10.1016/j.cell.2022.04.003>.
- [22] Chen, H.; Deng, C.; Gao, J.; Wang, J.; Fu, F.; Wang, Y.; Wang, Q.; Zhang, M.; Zhang, S.; Fan, F.; et al. Integrative Spatial Analysis Reveals Tumor Heterogeneity and Immune Colony Niche Related to Clinical Outcomes in Small Cell Lung Cancer. *Cancer Cell* **2025**, *43*, 519–536 e515. <https://doi.org/10.1016/j.ccell.2025.01.012>.
- [23] Helmink, B.A.; Reddy, S.M.; Gao, J.; Zhang, S.; Basar, R.; Thakur, R.; Yizhak, K.; Sade-Feldman, M.; Blando, J.; Han, G.; et al. B cells and Tertiary Lymphoid Structures Promote Immunotherapy Response. *Nature* **2020**, *577*, 549–555. <https://doi.org/10.1038/s41586-019-1922-8>.
- [24] Xu, C.; Bao, J.; Pan, D.; Wei, K.; Gao, Q.; Lin, W.; Ma, Y.; Lou, M.; Chang, C.; Jia, D. Single-Cell and Spatial Transcriptomics Reveal a Potential Role of ATF3 in Brain Metastasis of Lung Adenocarcinoma. *Transl. Lung Cancer Res.* **2025**, *14*, 209–223. <https://doi.org/10.21037/tlcr-24-784>.
- [25] Gonzalez, H.; Mei, W.; Robles, I.; Hagerling, C.; Allen, B.M.; Hauge Okholm, T.L.; Nanjaraj, A.; Verbeek, T.; Kalavacherla, S.; van Gogh, M.; et al. Cellular Architecture of Human Brain Metastases. *Cell* **2022**, *185*, 729–745.e720. <https://doi.org/10.1016/j.cell.2021.12.043>.
- [26] Yang, Z.; Wei, S.; Duan, H.; Wang, X.; Zhang, D.; Kiang, K.M.-Y.; Deng, Y.; Yang, Y.; Ku, Y.; Hu, W.; et al. A Proteogenomic Atlas of 1032 Brain Metastases Identifies Molecular Subtypes, Immune Landscapes, and Therapeutic Vulnerabilities. *Nat. Commun.* **2026**, *17*, 2038. <https://doi.org/10.1038/s41467-026-68748-y>.
- [27] Khatri, A.; McKernan, C.M.; Van Swearingen, A.E.D.; Jain, V.; Thrash, H.L.; Towne, A.; Gu, J.J.; Gregory, S.G.; Anders, C.K.; Pendergast, A.M. Spatially Resolved Transcriptomics in Human Brain Metastases Identifies Macrophage-Tumor Interactions Associated with Survival. *iScience* **2026**, *29*, 116517. <https://doi.org/10.1016/j.isci.2026.116517>.
- [28] Biermann, J.; Melms, J.C.; Amin, A.D.; Wang, Y.; Caprio, L.A.; Karz, A.; Tagore, S.; Barrera, I.; Ibarra-Arellano, M.A.; Andreatta, M.; et al. Dissecting the Treatment-Naïve Ecosystem of Human Melanoma Brain Metastasis. *Cell* **2022**, *185*, 2591–2608.e2530. <https://doi.org/10.1016/j.cell.2022.06.007>.
- [29] Zhang, Q.; Abdo, R.; Iosef, C.; Kaneko, T.; Cecchini, M.; Han, V.K.; Li, S.S.-C. The Spatial Transcriptomic Landscape of Non-Small Cell Lung Cancer Brain Metastasis. *Nat. Commun.* **2022**, *13*, 5983. <https://doi.org/10.1038/s41467-022-33365-y>.
- [30] Arvanitis, C.D.; Ferraro, G.B.; Jain, R.K. The Blood–Brain Barrier and Blood–Tumour Barrier in Brain Tumours and Metastases. *Nat. Rev. Cancer* **2020**, *20*, 26–41. <https://doi.org/10.1038/s41568-019-0205-x>.
- [31] Priego, N.; Zhu, L.; Monteiro, C.; Mulders, M.; Wasilewski, D.; Bindeman, W.; Doglio, L.; Martínez, L.; Martínez-Saez, E.; Ramón y Cajal, S.; et al. STAT3 Labels a Subpopulation of Reactive Astrocytes Required for Brain Metastasis. *Nat. Med.* **2018**, *24*, 1024–1035. <https://doi.org/10.1038/s41591-018-0044-4>.
- [32] Karimi, E.; Yu, M.W.; Maritan, S.M.; Perus, L.J.M.; Rezanejad, M.; Sorin, M.; Dankner, M.; Fallah, P.; Doré, S.; Zuo, D.; et al. Single-Cell Spatial Immune Landscapes of Primary and Metastatic Brain Tumours. *Nature* **2023**, *614*, 555–563. <https://doi.org/10.1038/s41586-022-05680-3>.
- [33] Lu, B.Y.; Gupta, R.; Aguirre-Ducler, A.; Gianino, N.; Wyatt, H.; Ribeiro, M.; Chiang, V.L.; Contessa, J.N.; Adeniran, A.J.; Jilaveanu, L.B.; et al. Spatially Resolved Analysis of the T Cell Immune Contexture in Lung Cancer-Associated Brain Metastases. *J. Immunother. Cancer* **2021**, *9*, e002684. <https://doi.org/10.1136/jitc-2021-002684>.
- [34] Wherry, E.J.; Kurachi, M. Molecular and Cellular Insights into T Cell Exhaustion. *Nat. Rev. Immunol.* **2015**, *15*, 486–499. <https://doi.org/10.1038/nri3862>.

- [35] Alvarez-Breckenridge, C.; Markson, S.C.; Stocking, J.H.; Nayyar, N.; Lastrapes, M.; Strickland, M.R.; Kim, A.E.; de Sauvage, M.; Dahal, A.; Larson, J.M.; et al. Microenvironmental Landscape of Human Melanoma Brain Metastases in Response to Immune Checkpoint Inhibition. *Cancer Immunol. Res.* **2022**, *10*, 996–1012. <https://doi.org/10.1158/2326-6066.cir-21-0870>.
- [36] Mughal, S.S.; Reiss, Y.; Felsberg, J.; Meyer, L.; Macas, J.; Schlue, S.; Starzetz, T.; Köhrer, K.; Fehm, T.; Müller, V.; et al. Identification and Characterization of Tertiary Lymphoid Structures in Brain Metastases. *Acta Neuropathol. Commun.* **2025**, *13*, 91. <https://doi.org/10.1186/s40478-025-02007-x>.
- [37] Germain, C.; Gnjjatic, S.; Tamzalit, F.; Knockaert, S.; Remark, R.; Goc, J.; Lepelley, A.; Becht, E.; Katsahian, S.; Bizouard, G.; et al. Presence of B Cells in Tertiary Lymphoid Structures Is Associated with a Protective Immunity in Patients with Lung Cancer. *Am. J. Respir. Crit. Care Med.* **2014**, *189*, 832–844. <https://doi.org/10.1164/rccm.201309-1611oc>.
- [38] Martinet, L.; Garrido, I.; Filleron, T.; Le Guellec, S.; Bellard, E.; Fournie, J.J.; Rochaix, P.; Girard, J.P. Human Solid Tumors Contain High Endothelial Venules: Association with T- and B-Lymphocyte Infiltration and Favorable Prognosis in Breast Cancer. *Cancer Res.* **2011**, *71*, 5678–5687. <https://doi.org/10.1158/0008-5472.can-11-0431>.
- [39] Di Caro, G.; Bergomas, F.; Grizzi, F.; Doni, A.; Bianchi, P.; Malesci, A.; Laghi, L.; Allavena, P.; Mantovani, A.; Marchesi, F. Occurrence of Tertiary Lymphoid Tissue Is Associated with T-Cell Infiltration and Predicts Better Prognosis in Early-Stage Colorectal Cancers. *Clin. Cancer Res.* **2014**, *20*, 2147–2158. <https://doi.org/10.1158/1078-0432.ccr-13-2590>.
- [40] Wischniewski, V.; Maas, R.R.; Aruffo, P.G.; Soukup, K.; Galletti, G.; Kornete, M.; Galland, S.; Fournier, N.; Lilja, J.; Wirapati, P.; et al. Phenotypic Diversity of T Cells in Human Primary and Metastatic Brain Tumors Revealed by Multiomic Interrogation. *Nat. Cancer* **2023**, *4*, 908–924. <https://doi.org/10.1038/s43018-023-00566-3>.
- [41] Sun, L.; Kienzler, J.C.; Reynoso, J.G.; Lee, A.; Shiuan, E.; Li, S.; Kim, J.; Ding, L.; Monteleone, A.J.; Owens, G.C.; et al. Immune Checkpoint Blockade Induces Distinct Alterations in the Microenvironments of Primary and Metastatic Brain Tumors. *J. Clin. Investig.* **2023**, *133*, e169314. <https://doi.org/10.1172/jci169314>.
- [42] Sevenich, L.; Bowman, R.L.; Mason, S.D.; Quail, D.F.; Rapaport, F.; Elie, B.T.; Brogi, E.; Brastianos, P.K.; Hahn, W.C.; Holsinger, L.J.; et al. Analysis of Tumour- and Stroma-Supplied Proteolytic Networks Reveals a Brain-Metastasis-Promoting Role For Cathepsin S. *Nat. Cell Biol.* **2014**, *16*, 876–888. <https://doi.org/10.1038/ncb3011>.
- [43] Bejarano, L.; Kauzlaric, A.; Lamprou, E.; Lourenco, J.; Fournier, N.; Ballabio, M.; Colotti, R.; Maas, R.; Galland, S.; Massara, M.; et al. Interrogation of Endothelial and Mural Cells in Brain Metastasis Reveals Key Immune-Regulatory Mechanisms. *Cancer Cell* **2024**, *42*, 378–395 e310. <https://doi.org/10.1016/j.ccell.2023.12.018>.
- [44] Joshi, V.; Beecher, K.; Lim, M.; Stacey, A.; Feng, Y.; Jat, P.S.; Duijff, P.H.G.; Simpson, P.T.; Lakhani, S.R.; McCart Reed, A.E. B7-H3 Expression in Breast Cancer and Brain Metastasis. *Int. J. Mol. Sci.* **2024**, *25*, 3976. <https://doi.org/10.3390/ijms25073976>.
- [45] Miller, C.D.; Lozada, J.R.; Zorko, N.A.; Elliott, A.; Makovec, A.; Radovich, M.; Heath, E.I.; Agarwal, N.; McKay, R.R.; Garje, R.; et al. Pan-Cancer Interrogation of B7-H3 (CD276) as an Actionable Therapeutic Target Across Human Malignancies. *Cancer Res. Commun.* **2024**, *4*, 1369–1379. <https://doi.org/10.1158/2767-9764.crc-23-0546>.
- [46] Zhu, B.; Chen, P.; Aminu, M.; Li, J.R.; Fujimoto, J.; Tian, Y.; Hong, L.; Chen, H.; Hu, X.; Li, C.; et al. Spatial and Multiomics Analysis of Human and Mouse Lung Adenocarcinoma Precursors Reveals TIM-3 as a Putative Target for Precancer Interception. *Cancer Cell* **2025**, *43*, 1125–1140 e1110. <https://doi.org/10.1016/j.ccell.2025.04.003>.
- [47] Kluger, H.M.; Chiang, V.; Mahajan, A.; Zito, C.R.; Sznol, M.; Tran, T.; Weiss, S.A.; Cohen, J.V.; Yu, J.; Hegde, U.; et al. Long-Term Survival of Patients with Melanoma with Active Brain Metastases Treated with Pembrolizumab on a Phase II Trial. *J. Clin. Oncol.* **2019**, *37*, 52–60. <https://doi.org/10.1200/jco.18.00204>.
- [48] Goldberg, S.B.; Schalper, K.A.; Gettinger, S.N.; Mahajan, A.; Herbst, R.S.; Chiang, A.C.; Lilenbaum, R.; Wilson, F.H.; Omay, S.B.; Yu, J.B.; et al. Pembrolizumab for Management of Patients with NSCLC and Brain Metastases: Long-Term Results and Biomarker Analysis from a Non-Randomised, Open-Label, Phase 2 Trial. *Lancet Oncol.* **2020**, *21*, 655–663. [https://doi.org/10.1016/s1470-2045\(20\)30111-x](https://doi.org/10.1016/s1470-2045(20)30111-x).
- [49] Long, G.V.; Atkinson, V.; Lo, S.N.; Guminski, A.D.; Sandhu, S.K.; Brown, M.P.; Gonzalez, M.; McArthur, G.A.; Menzies, A.M. Ipilimumab Plus Nivolumab Versus Nivolumab Alone in Patients with Melanoma Brain

- Metastases (ABC): 7-Year Follow-Up of a Multicentre, Open-Label, Randomised, Phase 2 Study. *Lancet Oncol.* **2025**, *26*, 320–330. [https://doi.org/10.1016/s1470-2045\(24\)00735-6](https://doi.org/10.1016/s1470-2045(24)00735-6).
- [50] Tawbi, H.A.; Forsyth, P.A.; Hodi, F.S.; Algazi, A.P.; Hamid, O.; Lao, C.D.; Moschos, S.J.; Atkins, M.B.; Lewis, K.; Postow, M.A.; et al. Long-Term Outcomes of Patients with Active Melanoma Brain Metastases Treated with Combination Nivolumab Plus Ipilimumab (CheckMate 204): Final Results of an Open-Label, Multicentre, Phase 2 Study. *Lancet Oncol.* **2021**, *22*, 1692–1704. [https://doi.org/10.1016/s1470-2045\(21\)00545-3](https://doi.org/10.1016/s1470-2045(21)00545-3).
- [51] Goldberg, S.B.; Gettinger, S.N.; Mahajan, A.; Chiang, A.C.; Herbst, R.S.; Sznol, M.; Tsiouris, A.J.; Cohen, J.; Vortmeyer, A.; Jilaveanu, L.; et al. Pembrolizumab for Patients with Melanoma or Non-Small-Cell Lung Cancer and Untreated Brain Metastases: Early Analysis of a Non-Randomised, Open-Label, Phase 2 Trial. *Lancet Oncol.* **2016**, *17*, 976–983. [https://doi.org/10.1016/s1470-2045\(16\)30053-5](https://doi.org/10.1016/s1470-2045(16)30053-5).
- [52] Qian, J.M.; Yu, J.B.; Kluger, H.M.; Chiang, V.L. Timing and Type of Immune Checkpoint Therapy Affect the Early Radiographic Response of Melanoma Brain Metastases to Stereotactic Radiosurgery. *Cancer* **2016**, *122*, 3051–3058. <https://doi.org/10.1002/cncr.30138>.
- [53] ClinicalTrials.gov. Phase II Study of Nivolumab in Combination with Relatlimab in Patients with Active Melanoma Brain Metastases 2023. Available online: <https://clinicaltrials.gov/study/NCT05704647> (accessed on).
- [54] Campanella, G.; Hanna, M.G.; Geneslaw, L.; Miralflor, A.; Werneck Krauss Silva, V.; Busam, K.J.; Brogi, E.; Reuter, V.E.; Klimstra, D.S.; Fuchs, T.J. Clinical-Grade Computational Pathology Using Weakly Supervised Deep Learning on Whole Slide Images. *Nat. Med.* **2019**, *25*, 1301–1309. <https://doi.org/10.1038/s41591-019-0508-1>.
- [55] Vorontsov, E.; Bozkurt, A.; Casson, A.; Shaikovski, G.; Zelechowski, M.; Severson, K.; Zimmermann, E.; Hall, J.; Tenenholtz, N.; Fusi, N.; et al. A Foundation Model for Clinical-Grade Computational Pathology and Rare Cancers Detection. *Nat. Med.* **2024**, *30*, 2924–2935. <https://doi.org/10.1038/s41591-024-03141-0>.
- [56] Ahmedt-Aristizabal, D.; Armin, M.A.; Denman, S.; Fookes, C.; Petersson, L. A Survey on Graph-Based Deep Learning for Computational Histopathology. *Comput. Med. Imaging Graph.* **2022**, *95*, 102027. <https://doi.org/10.1016/j.compmedimag.2021.102027>.
- [57] Waqas, A.; Naveed, J.; Shahnawaz, W.; Asghar, S.; Bui, M.M.; Rasool, G. Digital Pathology and Multimodal Learning on Oncology Data. *BJR Artif. Intell.* **2024**, *1*, ubae014. <https://doi.org/10.1093/bjrai/ubae014>.
- [58] Silberman, N.; Parwani, A.; McClintock, D.S.; Lujan, G.; Gondim, D.D.; Garcia, C.; Hanna, M.G.; Pantanowitz, L.; Lennerz, J.K.; Showalter, T.; et al. Recommendation Statement for the Validation, Implementation, and Clinical Application of Artificial Intelligence within a Clinical Laboratory from the Digital Pathology Association. *AI Precis. Oncol.* **2026**, *3*, 71–97. <https://doi.org/10.1177/2993091x261455975>.
- [59] De Mattos-Arruda, L.; Mayor, R.; Ng, C.K.Y.; Weigelt, B.; Martínez-Ricarte, F.; Torrejon, D.; Oliveira, M.; Arias, A.; Raventos, C.; Tang, J.; et al. Cerebrospinal Fluid-Derived Circulating Tumour DNA Better Represents the Genomic Alterations of Brain Tumours than Plasma. *Nat. Commun.* **2015**, *6*, 8839. <https://doi.org/10.1038/ncomms9839>.
- [60] Wu, J.; Liu, Z.; Huang, T.; Wang, Y.; Song, M.M.; Song, T.; Long, G.; Zhang, X.; Li, X.; Zhang, L. Cerebrospinal Fluid Circulating Tumor DNA Depicts Profiling of Brain Metastasis in NSCLC. *Mol. Oncol.* **2023**, *17*, 810–824. <https://doi.org/10.1002/1878-0261.13357>.
- [61] Pathak, R.; Amini, A.; Hill, A.; Massarelli, E.; Salgia, R. Immunotherapy in Non-Small Cell Lung Cancer Patients with Brain Metastases: Clinical Challenges and Future Directions. *Cancers* **2021**, *13*, 3407. <https://doi.org/10.3390/cancers13143407>.

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