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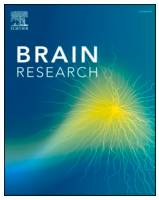

Immune cell imaging in the glioma microenvironment

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ABSTRACT

Glioblastoma remains a highly aggressive brain tumour with poor prognosis despite advances in standard therapies. The tumour microenvironment, comprising tumour cells, immune cells – predominantly tumour-associated microglia and macrophages (TAMMs) and extracellular matrix components, critically influences tumour progression and therapy resistance. TAMMs promote immunosuppression, tumour invasion, and angiogenesis, while T cells, although fewer, are suppressed by glioblastoma-mediated mechanisms, limiting anti-tumour immunity. Advances in non-invasive imaging technologies, including magnetic resonance imaging, positron emission transmission (PET), and optical methods, enable visualisation and characterisation of the immune microenvironment in vivo. Imaging agents targeting TAMM markers such as TSPO, CD163, CD68, CD206, and CX3CR1 have facilitated the mapping of immune cell distribution and functional states within gliomas. Additionally, emerging PET tracers allow monitoring of T-cell infiltration, activation, and exhaustion, providing insights into immunotherapy responses. Despite challenges such as blood brain barrier permeability, tracer specificity, and regulatory hurdles, multimodal imaging combined with radiomics and spatial transcriptomics offers promising avenues for personalised therapeutic strategies. Future directions focus on integrating immune cell imaging with theranostic approaches, nanoparticle delivery systems, and longitudinal monitoring to overcome tumour heterogeneity and improve treatment efficacy. This review highlights the evolving landscape of immune cell imaging in gliomas, emphasising its potential to enhance diagnosis, guide immunotherapy, and ultimately improve patient outcomes.

Abbreviations: TAMM, Tumour-associated Microglia and Macrophage; TME, Tumour Microenvironment; EGF, Epidermal Growth Factor; PDGF, Platelet-derived Growth Factor; IL, Interleukin; CXCL, Chemokine (C-X-C motif) Ligand; TNF, Tumour Necrosis Factor; FGF, Fibroblast Growth Factor; GM-CSF, Granulocyte-macrophage Colony-stimulating Factor; BBB, Blood Brain Barrier; SRCR, Scavenger Receptor Cysteine-Rich; MRI, Magnetic Resonance Imaging; USPIO, Ultra-small Iron Oxide Nanoparticles; ECM, Extracellular Matrix; PET, Positron Emission Tomography; MMP, Matrix Metalloproteinase; GAG, Glycosaminoglycans; TGF-β, Transforming Growth Factor Beta; TNF-α, Tumour Necrosis Factor***; PD-1, Programmed Death 1; SPECT, Single-photon Emission Computed Tomography; MIF, Migration Inhibitory Factor; Tregs, Regulatory T cells; CTLs, Cytotoxic T Lymphocytes; TIL, Tumour-Infiltrating Lymphocyte; TSPO, Translocator Protein; MIF, Macrophage Migration Inhibitory Factor; TSPO-PET, Translocator Protein-Positron Emission Tomography; CTL, Cytotoxic T Lymphocyte; PAI, Photoacoustic Imaging; CD, Cluster of Differentiation.

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1. Introduction

The prognosis of glioblastoma has not changed significantly over the past decades [Salvalaggio et al., 2022]. Increasing evidence suggests an important role for the tumour microenvironment (TME) in tumour progression [Karimian-Jazi et al., 2020]. The TME is a complex network including not only tumour cells but also immune cells (microglia, macrophages, T cells), reactive astrocytes, endothelial cells, pericytes and extracellular matrix (ECM) components, for example, fibrous proteins, glycoproteins, matrix metalloproteinases (MMPs), basement membrane proteins, and proteoglycans and glycosaminoglycans [Baghban et al., 2020]. In malignant gliomas, immune cells, primarily microglia and glioma-associated macrophages, with smaller contributions from monocytes, neutrophils, and tumour-infiltrating lymphocytes (TILs), can constitute up to 50% of the tumour cellularity. [Sharma P et al., 2023].

Tumour-associated microglia and macrophages (TAMMs) are the most prevalent immune cell population within glioblastoma TME. [Sharma P et al., 2023] TAMMs contribute to an immunosuppressive environment by secreting anti-inflammatory cytokines, such as IL-10 and transforming growth factor beta (TGF-β), which dampen T-cell activity and enhance tumour immune evasion [Allavena et al., 2008]. More specifically, glioblastoma-associated microglia and macrophages also help sustain local immune suppression. For example by recruiting CCR4 + regulatory T cells and CCR2 + monocytic myeloid-derived suppressor cells into the TME, thereby reinforcing both adaptive and innate immune suppression [Chang et al., 2016]. TAMMs also consist of brain-resident microglia, which plays a central role in glioblastoma progression [Quail et al., 2017]. Rather than attacking tumour cells, TAMMs are often co-opted to support the tumour by releasing pro-inflammatory cytokines, growth factors, and enzymes like MMPs that remodel the ECM, facilitating tumour invasion and spread [Quail et al., 2017; Allavena et al., 2008]. Given their dual roles in promoting tumour growth and suppressing immune responses, TAMMs are considered a promising target for therapeutic strategies aimed at reprogramming the TME to hinder glioblastoma progression.

T cells, though a smaller fraction of the immune cell population within the glioblastoma TME, play a vital role in immune surveillance and tumour suppression. These cells primarily include CD8 + cytotoxic T cells, which have the potential to directly target and kill tumour cells, and CD4 + helper T cells, which assist in orchestrating broader immune responses. [Noor et al., 2024] However, glioblastoma creates an immunosuppressive environment that severely limits T-cell efficacy. Mechanisms such as upregulation of immune checkpoint proteins (e.g., PD-L1) on tumour cells (and TAMMs), which engage PD-1 on T cells to inhibit their activity, and secretion of immunosuppressive cytokines like TGF-β and IL-10 create barriers to T cell function [Lou et al., 2017]. In glioblastoma specifically, tumour-derived TGF-β has been shown to impair effector T-cell infiltration, while regulatory T cells are enriched within the residual CD4 + compartment and contribute disproportionately to defective antitumour T-cell responsiveness [Lohr et al., 2011; Fecci et al., 2006]. In addition, PD-L1 expression has been documented in a subset of glioblastomas and is associated with worse clinical outcome, and glioblastoma-derived extracellular vesicles can also carry PD-L1 and directly suppress T-cell activation through PD-1 signalling [Nduom et al., 2016; Ricklefs et al., 2018]. Additionally, physical and molecular barriers within the TME restrict T-cell infiltration into tumour core regions, limiting their potential for tumour control. [Scott et al., 2021] This immune modulation has made T cells an important focus in glioblastoma research, with immunotherapies such as checkpoint inhibitors and CAR-T cell therapy aiming to enhance T-cell activation and persistence against tumour cells within the hostile TME [Scott et al., 2021].

As the understanding of the TME advances, more targeted therapies are being developed to exploit its components for therapeutic gain. These strategies aim to alter the TME to overcome immune suppression, limit tumour growth, or enhance treatment responses [Skytthe et al., 2020; Zhou et al., 2022]. However, significant challenges persist due to the inherent heterogeneity of the TME, both within individual tumours and across different glioblastoma patients, which complicates effective treatment [Akgül et al., 2019]. In addition, radiotherapy and chemotherapy can induce changes in the TME, promoting clonal evolution of tumour cells that are more resistant and harder to eradicate, while potentially fostering a more immunosuppressive environment. [Akgül et al., 2019]. Such variability necessitates personalised approaches and adaptive treatment strategies that consider these evolving changes, making the translation of TME insights into effective, standardised therapies particularly complex.

Since it is too invasive to frequently sample the TME in patients via tissue biopsy, imaging may offer a less invasive alternative for understanding the composition of the TME. This will allow for insight into the TME, thus contributing to (monitoring of) novel treatment options for glioblastoma patients. To date, the most commonly used modalities include magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT) [Puttick et al., 2015]. This review aims to comprehensively discuss the potential of imaging TAMMs and other immune cells within the glioblastoma TME. This review focuses primarily on glioblastoma while also incorporating selected evidence from other glioma subtypes where it helps clarify the biology, heterogeneity, and translational potential of immune cell imaging across the broader glioma landscape.

2. Methodology

To review the current literature on imaging techniques of glioblastoma immune cells, we searched the following databases: PubMed, EMBASE, the Cochrane Library, and Scopus. Only original, full-text articles written in English were included in our study. Key terms including "brain tumours," "brain cancer," "primary brain tumours," "intracranial tumours," "CNS tumours" "glioblastoma" "high-grade glioma," "glioma subtypes," "glioblastoma" "tumour stroma," "tumour microenvironment," and "immune cells," "immune infiltration," "lymphocytes," "myeloid cells," "NK cells," "immune cell phenotypes," "immune cells in brain tumours," "brain tumour immune profile" were used in combination with additional terms including "imaging techniques," "in vivo imaging," "neuroimaging," "microscopy in tumour imaging," "MRI immune cell tracking," "PET scans for immune cells," "functional imaging," "fluorescence imaging," "optical coherence tomography (OCT)," "confocal microscopy," "super-resolution imaging," "multimodal imaging" "tumour-associated macrophages," "macrophage polarisation," "M1 macrophages," "M2 macrophages," "microglia in tumours," "macrophage infiltration," "tumour-associated microglia and macrophages,", and "TAMMs".

In addition to the comprehensive database search, references cited in recent reviews focused on similar topics were manually examined to identify additional sources that could contribute to the search strategy. Standalone abstracts, case reports, posters, and unpublished or non-peer-reviewed studies were excluded. A summary of the methodology employed is presented in supplementary Table 1.

3. Overview of immune cell imaging in gliomas; history, current and emerging technologies, and clinical applicability

Traditional imaging techniques like contrast-enhanced MRI are useful for identifying tumour mass and vascular permeability but lack the specificity to differentiate between neoplastic tissue and inflammatory changes, especially under immunotherapeutic interventions. Immune cell imaging thus represents a paradigm shift, enabling clinicians and researchers to visualise the immune milieu of gliomas in vivo, track immune responses over time, and potentially distinguish treatment-induced inflammation (e.g., pseudoprogression) from true tumour progression [Antonios et al., 2017].

3.1. Historical Context

Knowledge about immune cells in gliomas emerged from histopathological analyses, which showed high TAMM infiltration and minimal lymphocyte presence. This set the stage for imaging approaches that could noninvasively assess immune cell presence. One of the earliest tools was TSPO PET imaging, using ligands such as [^11C]-(R) PK11195 to detect activated microglia and macrophages in glioma patients [Su et al., 2013]. These studies confirmed that TSPO expression and tracer uptake correlate with glioma grade, validating immune cell activity as an imaging target.

Simultaneously, the use of ultrasmall superparamagnetic iron oxide nanoparticles (USPIOs), like ferumoxytol, provided an MRI-based method to visualise phagocytic cells in gliomas. Clinical studies demonstrated that ferumoxytol-enhanced MRI could highlight TAMM-rich regions that might not be apparent on standard gadolinium-enhanced scans [Stoller et al., 2023]. Two-photon intravital microscopy enabled real-time imaging of immune cells in live mouse gliomas. Using fluorescent lineage tracing, researchers distinguished microglia from infiltrating monocyte-derived macrophages, revealing distinct behaviors and demonstrating the utility of live-cell imaging in studying immune dynamics. [Chen et al. 2019].

3.2. Current and emerging technologies and their mechanisms

Modern immune imaging in gliomas now includes a suite of modalities such as MRI, PET, and optical imaging, each offering unique insights into immune cell function, location, and behavior. These technologies are complemented by emerging agents and tracers that allow better specificity and resolution.

Iron oxide nanoparticles such as ferumoxytol are phagocytosed by TAMMs and produce a T2 signal drop on MRI. This allows clinicians to map macrophage-rich areas in glioblastomas, sometimes revealing infiltrative tumour regions missed by gadolinium [Stoller et al., 2023]. Another innovative approach usesˆ19F MRI, in which perfluorocarbon nanoparticles are ingested by macrophages and visualised through a distinct fluorine signal; this technique successfully tracked TAMMs longitudinally in glioma models [Croci et al., 2022].

TSPO PET imaging targets the translocator protein expressed by activated microglia/macrophages and some glioma cells showed that TSPO PET could reveal spatial heterogeneity in immune cell distribution and monitor changes during therapy [Zinnhardt et al. 2021]. PET imaging of immune cell metabolism is another avenue. Researchers have used [^18F]-FAC PET to detect deoxycytidine kinase activity in activated T cells in mouse gliomas following immunotherapy, highlighting areas of immune activation [Antonios et al. 2017]. Immuno-PET offers a direct approach by targeting specific immune cell surface markers. Studies have developed a CD8-targeted PET tracer, allowing visualisation of cytotoxic T lymphocytes within gliomas [Nagle et al. 2021]. Finally, intravital two-photon microscopy offers high-resolution imaging of immune cell dynamics in live animals, enabling single-cell tracking of macrophages and T cells in real time [Chen et al., 2019].

In order to clarify the translational status of the modalities discussed, it is necessary to differentiate between those modalities that are currently established technologies and those that are still emerging. Ferumoxytol enhanced MRI and TSPO PET are clinically available technologies that are already used in human studies. Originally approved as an iron replacement therapy, ferumoxytol has been repurposed for macrophage imaging in gliomas because of its uptake by phagocytic cells in gliomas [Stoller et al., 2023]. TSPO PET imaging with [^{18}F]GE-180 has been shown to be useful for glioma grading and monitoring immune activity during treatment, but its specificity is limited by off target expression in astrocytes and tumour cells [Zinnhardt et al., 2021]. These well established tools have immediate clinical relevance, allowing whole brain, noninvasive assessment of immune cell dynamics.

In contrast, techniques such asˆ19F MRI, [^18F]-FAC PET, CD8 immuno-PET, and two-photon intravital microscopy are considered emerging.ˆ19F MRI, while promising in preclinical models for its high specificity and absence of background signal, remains constrained to animal studies due to the need for specialised fluorine imaging infrastructure [Croci et al., 2022]. Tracking T cell activation post immunotherapy can also be done with [^18F]-FAC PET, but its rapid metabolism in humans requires optimisation or replacement with more stable analogs such as [^18F]-CFA [Antonios et al., 2017]. While CD8 immuno-PET is effective at mapping cytotoxic lymphocytes in experimental gliomas, it is still in early stage development and safety evaluation [Nagle et al., 2021]. Finally, although it provides unmatched resolution for single cell tracking in live tissues, two photon intravital microscopy is invasive and animal based and therefore not translatable to clinical diagnostics [Chen et al., 2019].

3.3. Advantages

Each modality offers distinct benefits. MRI and PET allow noninvasive, whole-brain imaging, crucial for capturing the infiltrative nature of gliomas. Ferumoxytol MRI enables high-resolution detection of macrophage-rich regions, while TSPO PET delineates inflammation and immune activity with molecular specificity [Zinnhardt et al., 2021].ˆ19F MRI adds the ability to quantify immune cell burden longitudinally without background noise [Croci et al., 2022].

The specificity of certain tracers, such as [^18F]-FAC for T cells or CD8-directed PET tracers, allows precise mapping of immune subsets, enabling functional imaging of anti-tumour immunity [Antonios et al., 2017; Nagle et al., 2021]. These techniques also permit longitudinal monitoring of immune responses, crucial for assessing dynamic therapy effects. Importantly, many of these methods have translational potential—ferumoxytol and TSPO PET tracers are already in human use, making clinical implementation more feasible [Stoller et al., 2023].

3.4. Limitations

However, limitations remain, as tracer specificity is imperfect. For example, TSPO is not exclusive to macrophages and may also be expressed by reactive astrocytes and tumour cells, complicating interpretation [Zinnhardt et al., 2021]. Another issue is the need for exogenous contrast agents, which adds cost, regulatory complexity, and patient burden. PET tracers require access to cyclotron facilities, limiting widespread use. Furthermore, some preclinical tracers like [^18F]-FAC do not perform optimally in humans due to rapid metabolism, necessitating alternatives like [^18F]-CFA [Antonios et al., 2017]. Table 1 summarises the immune imaging technologies in gliomas, along with their clinical applicability, advantages, and limitations.

4. TAMMs and their role in glioma microenvironment

TAMMs are found to be crucial in tumour progression [Tong et al., 2021]. Conventionally, microglia and macrophages were classified as classically activated and alternatively activated, depending on the inflammatory markers they secrete [Tong et al., 2021]. However, it has become increasingly clear that this model is too simplistic with recent evidence suggesting that macrophages are of a spectrum with multiple subtypes [Wang et al., 2022]. Classically activated microglia and macrophages are primarily involved in the pro-inflammatory function, secreting chemokines such as several cytokines like tumour necrosis factor (TNF), interleukin (IL) −1, IL-6, IL-8, and IL-12 which aid in the activation, proliferation, and generation of effector cells in gliomas [Guadagno et al., 2018]. From an imaging perspective, this phenotypic diversity is important because TAMMs are not only biologically active but also potentially imageable through their abundance, spatial distribution, iron handling, and surface-marker expression, making them

Table 1
Immune Imaging Technologies in Gliomas: Clinical Applicability, Advantages, and Limitations.

Technology	Type	Clinical Applicability in Gliomas	Advantages	Limitations
Ferumoxytol MRI (Iron oxide nanoparticles) [Stoller et al., 2023]	Current	Used in glioblastoma patients to map TAMMs; highlights infiltrative, macrophage-rich tumour zones missed by gadolinium contrast.	Noninvasive; detects macrophage-rich infiltrative regions with high resolution	Requires exogenous contrast; iron handling varies between patients
ˆ19F MRI (Perfluorocarbon nanoparticles) [Croci et al., 2022]	Emerging	Demonstrated in glioma mouse models to track macrophage burden over time; not yet available for clinical glioma imaging.	Quantifies immune cell burden longitudinally; no background noise from endogenous fluorine	Limited to animal models; requires fluorine imaging infrastructure
TSPO PET [Zinnhardt et al., 2021]	Current	Used in glioma patients to noninvasively visualise activated microglia/macrophages; enables mapping of immune infiltration and monitoring of immunotherapy response.	Molecular specificity for inflammation; tracks immune distribution and therapy response	TSPO is expressed by astrocytes and tumour cells, reducing specificity
[ˆ18F]-FAC PET [Antonios et al., 2017]	Emerging	Preclinical imaging of T cell activation in glioma-bearing mice post-immunotherapy promising for evaluating immune response to T cell-based treatments.	Detects T cell activation; functional imaging of immune response post-immunotherapy	Metabolised too rapidly in humans; alternatives like [ˆ18F]-CFA are needed
CD8 Immuno-PET [Nagle et al., 2021]	Emerging	Enables preclinical visualisation of CD8 + cytotoxic T cell infiltration in glioma models; potential clinical tool for monitoring T cell–based immunotherapy.	Visualises cytotoxic T cells; enables precise mapping of immune subsets	Early-stage; potential off-target effects and immunogenicity not fully understood
Two-photon Intravital Microscopy [Chen et al., 2019]	Emerging	Used in murine glioma models to directly observe dynamic immune cell behaviors (e.g., microglia vs. monocyte-derived macrophages) in the live TME.	High-resolution, real-time imaging of individual immune cells in live animals	Invasive; limited to research use; not applicable for clinical diagnostics

Abbreviations
MRI; Magnetic Resonance Imaging, TAMMs; Tumour-associated Microglia and Macrophage, PET; Positron Emission Tomography, TSPO; Translocator Protein, TME; Tumour Microenvironment.

suitable targets for MRI, PET, and radiomic immune profiling strategies in glioma [Khalili et al., 2023; Zinnhardt et al., 2021].

Alternatively activated microglia and macrophages are believed to play an anti-inflammatory role by secreting and expressing markers, such as IL-4, IL-10, and TNF-β. Unlike classically activated macrophages, these alternatively activated microglia and macrophages can foster anti-inflammatory T-cell responses, particularly those involving Th2-type cytokines, which contribute to the inflammatory response in gliomas [Franco et al., 2015]. These immunosuppressive and pro-tumoural properties are directly relevant to biomarker design because they justify imaging approaches that quantify myeloid-cell burden, immune-suppressive niches, or regional inflammatory activity rather than relying only on gross tumour morphology; in practice, this has motivated the development of TAMM-relevant methods such as ferumoxytol-enhanced MRI, CD11b-directed immunoPET, and TSPO PET in glioma [Iv et al., 2019; Nigam et al., 2020; Zinnhardt et al., 2021].

Classically activated TAMMs, which exhibit anti-tumour and pro-inflammatory characteristics, predominate in the early stages of glioma [Tong N et al., 2021]. In contrast, the group also demonstrated the presence of alternatively activated TAMMs, which display immuno-suppressive properties in high-grade murine glioma, characterised by restricted T cell trafficking and activation. Furthermore, they identified a high expression of CD74 and its binding partner, macrophage migration inhibitory factor (MIF), in specific TAMM populations. This finding was subsequently validated in human samples, suggesting the CD74-MIF axis as a potential therapeutic target for TAMMs [Zeiner et al., 2015]. Additionally, studies have also reported the roles of TAMMs in the glioma TME in promoting tumour growth, tumour invasion, and angiogenesis [Zhu et al., 2017].

Beyond glioblastoma, TAMM heterogeneity is also evident in other gliomas. In IDH mutant astrocytomas and oligodendrogliomas, single cell studies show that microglial and macrophage states vary by subtype, grade, and recurrence, with higher grade lesions showing greater macrophage associated programmes and recurrent disease showing further myeloid remodelling [Venteicher et al., 2017; Blanco-Carmona et al., 2023]. Mechanistically, IDH mutant gliomas can shape infiltrating myeloid cells through altered tryptophan metabolism and cholesterol handling, supporting distinct immune phenotypes within the TME [Friedrich et al., 2021; Wang et al., 2023]. Similar patterns are seen in paediatric low grade glioma, where tumour associated microglia support tumour maintenance in NF1 associated optic glioma and pilocytic astrocytoma shows progressive microglial enrichment and increasingly anti inflammatory features over time [Solga et al., 2015; AlShakweer et al., 2011; Stone et al., 2025].

For the purposes of immune-cell imaging, these observations should be translated into a clearer biomarker framework. High TAMM abundance supports bulk-cell imaging approaches, regional macrophage accumulation and iron uptake support contrast-enhanced MRI methods, and surface-marker or activation-state expression support targeted PET tracers. In glioma, this has already been demonstrated in several forms: ferumoxytol-enhanced MRI has been used as a noninvasive biomarker of iron-containing macrophages in high-grade gliomas, with imaging measurements correlating with macrophage-rich tissue sampling; CD11b immunoPET has enabled noninvasive quantification of glioblastoma-infiltrating myeloid cells in preclinical models; and TSPO PET has been applied to assess multiple cellular components of the glioma TME in vivo [Iv et al., 2019; Nigam et al., 2020; Zinnhardt et al., 2021].

TAMMs in the glioma TME are shown to secrete factors such as epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) that aid in the promotion of tumour growth [Kennedy et al., 2013; Liu et al., 2018; Wang et al., 2018]. Next, TAMMs can promote the invasion and progression of glioma by secreting proteases and MMPs that aid in the destruction of the ECM, which is key in cell-to-cell communication [Das et al., 2011; Yang et al., 2017; Yu et al., 2017]. Furthermore, the secretion of growth factors by TAMMs aids in transforming the cells to a more mesenchymal phenotype [Das et al., 2011; Yang et al., 2017; Yu et al., 2017].

Clinically, the value of imaging these TAMM-associated processes lies in improving noninvasive immune profiling, monitoring microenvironmental response to therapy, and potentially distinguishing biologically active inflammatory niches from changes seen on conventional structural imaging alone [Khalili et al., 2023; Zinnhardt et al., 2021].

Finally, TAMMs can promote angiogenesis with the expression of angiogenic factors such as VEGF, PDGF, FGF1, FGF2, and placental growth factors in gliomas [Lorger et al., 2012; Sharma et al., 2023; Parmigiani et al., 2021]. Fig. 1 shows the role of TAMMs in the brain TME, where it has multiple surface cell receptors to interact with surrounding stromal cells, producing cytokines and chemokines such as interleukin-10 (IL-10) that promote an immunosuppressive environment.

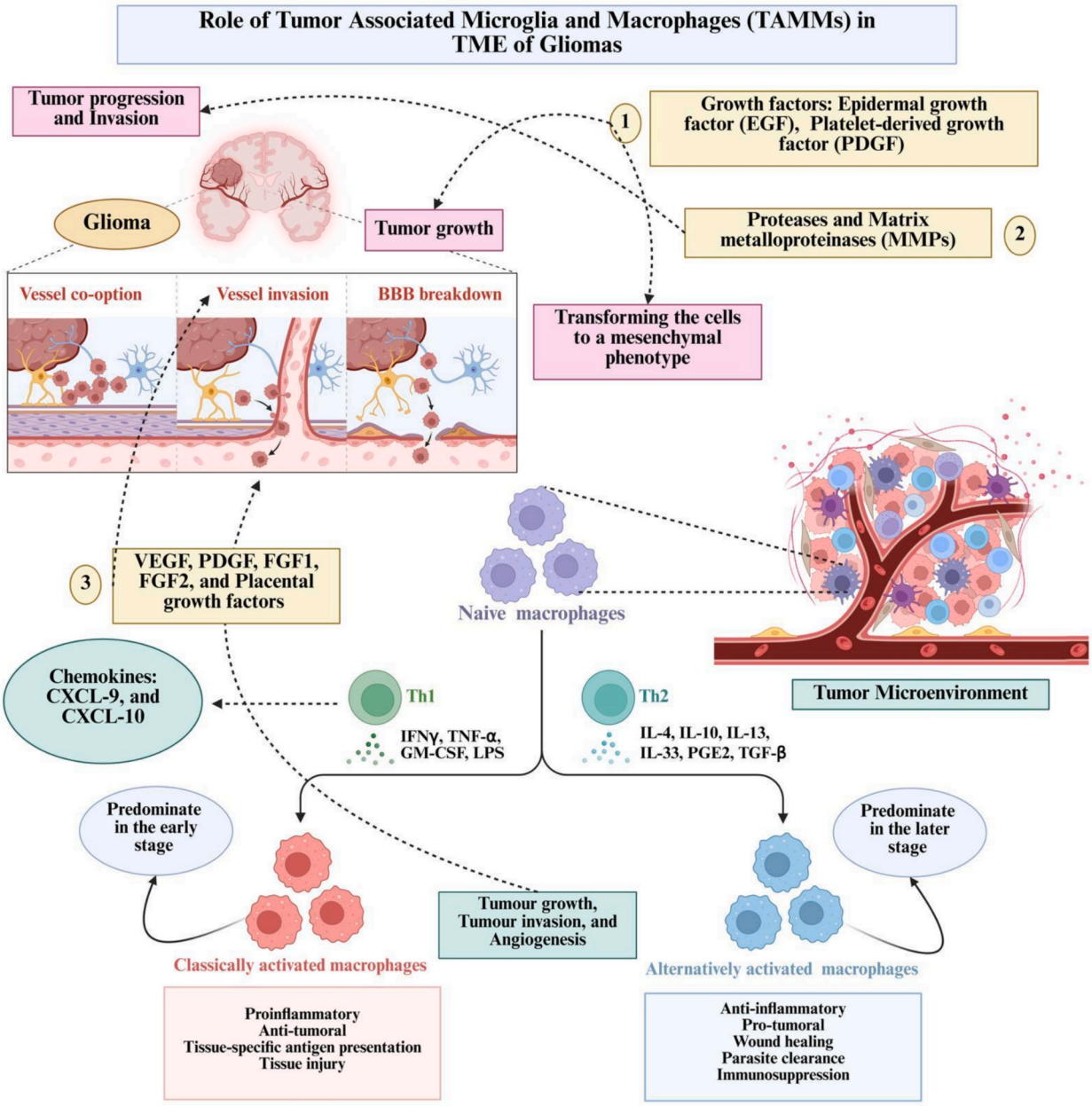

Fig. 1. The Role of Tumour-Associated Microglia and Macrophages (TAMM) in the Tumour Microenvironment of Gliomas. TAMMs have numerous cell surface receptors that enable it to interact with surrounding cells, secreting various chemokines and cytokines after changes at the transcription level. **Abbreviations:** TAMM; Tumour Associated Microglia and Macrophage, TME; Tumour Microenvironment, EGF; Epidermal Growth Factor, VEGF; Vascular Endothelial Growth Factor, FGF; Fibroblast Growth Factor, IFN-γ; Interferon Gamma, LPS; Lipopolysaccharide, Epidermal Growth Factor; PDGF; Platelet-derived Growth Factor, IL; Interleukin, CXCL; Chemokine (C-X-C motif) Ligand, TNF-α; Tumour Necrosis Factor, TNF-β; Tumour Necrosis Factor Beta, FGF; Fibroblast Growth Factor, GM-CSF; Granulocyte-macrophage Colony-stimulating Factor, BBB; Blood Brain Barrier, PGE2; Prostaglandin E2, MMP; Matrix Metalloproteinase, Th1: T Helper cell type 1, Th2: T Helper Cell Type 2.

5. Imaging targets and tracers used to identify TAMMs in gliomas

A range of biomarkers and imaging targets have been identified for TAMMs within the glioblastoma TME. These biomarkers, including CD163, CD68, Translocator Protein (TSPO), CD206, and CX3CR1, exhibit specific expression patterns and cellular interactions that distinguish TAMMs, enabling both enhanced diagnostic imaging and targeted therapeutic strategies [Matias et al., 2018; Ammer et al., 2020]. The diversity of these markers potentially allows for nuanced imaging of the TME, providing insights into tumour progression and immune dynamics. Importantly, beyond their role in improving diagnostic accuracy, these biomarkers also facilitate therapeutic interventions aimed at reprogramming TAMMS to promote antitumour responses [Matias et al., 2018; Ammer et al., 2020].

5.1. CD163

CD163, a member of the scavenger receptor cysteine-rich (SRCR) family, plays a pivotal role in glioma imaging due to its selective expression on specific macrophage populations and its dual function as both a diagnostic and therapeutic target [Matias et al., 2018].

This 130 kDa membrane protein, expressed on macrophages, binds haemoglobin-haptoglobin complexes and facilitates their clearance via

endocytosis, thereby protecting tissues from oxidative damage caused by these complexes [Liu et al., 2019]. In the context of brain tumour imaging and monitoring, CD163 is particularly significant for its specificity to alternatively activated macrophages, including perivascular and meningeal macrophages. These macrophages exhibit a distinct flattened, elongated morphology along blood vessels, a feature that typically excludes microglia from this category [Liu et al., 2019]. This specificity enhances the ability to differentiate these macrophages from other phagocytic cells in the CNS, such as microglia, thereby improving the accuracy of imaging in human studies.

The integration of CD163-targeted imaging with radiomics-based analysis has demonstrated enhanced tumour segmentation and phenotyping. Radiomics features, such as texture and shape metrics from MRI, when combined with PET-CD163 uptake, can improve differentiation between pro-tumoural and anti-tumoural immune profiles [Zhou et al., 2024]. Fig. 2 summarises these imaging modalities in identifying TAMMs through CD163 expression, and the pathologies that this is particularly relevant for.

Beyond glioblastoma, CD163 also shows important relevance across diffuse glioma subtypes. Large scale transcriptomic analyses demonstrated that CD163 expression increases with glioma grade, is significantly enriched in IDH wild type tumours, and is particularly elevated in the mesenchymal molecular subtype, suggesting that its value extends beyond GBM alone and may help stratify biologically aggressive non GBM gliomas [Liu et al., 2019]. In addition, more recent single cell and machine learning based work identified a CD163 + FPR3 + macrophage subset across glioma cohorts, with this signature showing strong prognostic value across six independent datasets and outperforming many conventional clinical variables and previously published signatures [Zhou et al., 2024]. Together, these findings suggest that CD163 related macrophage profiling may have utility not only for glioblastoma imaging, but also for characterising lower grade and molecularly distinct

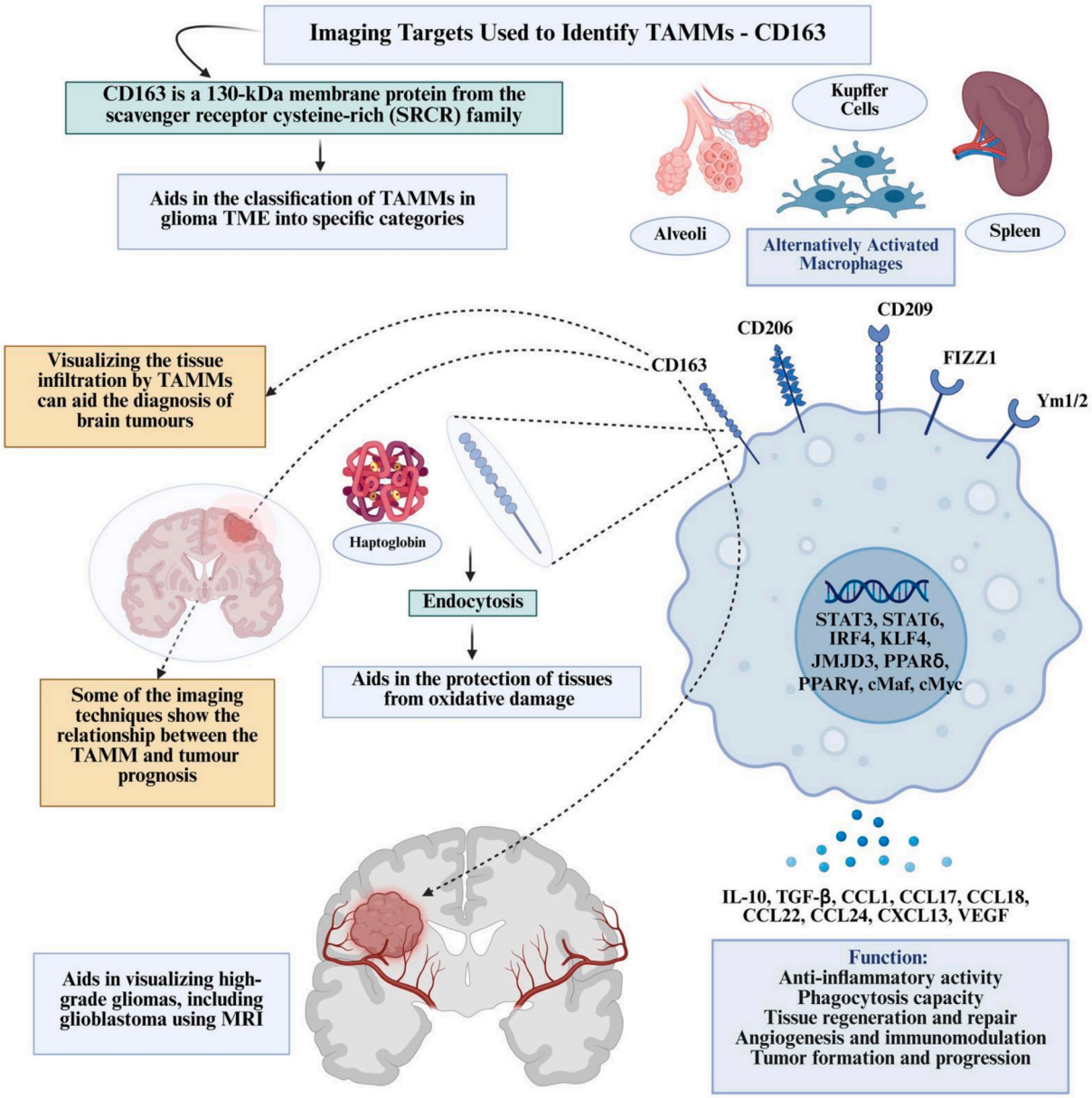

Fig. 2. The Imaging Targets Used to Identify Brain Tumour TAMMs-CDI63. TAMMs-CD163 can be used across different pathologies due to the role of TAMMs across these conditions. **Abbreviations:** TAMM; Tumour Associated Microglia and Macrophage, TME; Tumour Microenvironment, SRCR; Scavenger Receptor Cysteine-Rich, EGF; Epidermal Growth Factor; IL; Interleukin, CXCL; Chemokine (C-X-C motif) Ligand, TNF-β; Tumour Necrosis Factor Beta, FGF; Fibroblast Growth Factor; MRI; Magnetic Resonance Imaging, VEGF; Vascular Endothelial Growth Factor.

gliomas with immunosuppressive microenvironments [Liu et al., 2019; Zhou et al., 2024].

Despite strong preclinical evidence, CD163-targeted imaging has not yet entered routine clinical use. However, its unique expression profile and functional implications in glioma support its inclusion in future clinical trials. One limitation to consider is the potential loss of CD163 expression under certain inflammatory or activation states, which could reduce specificity in longitudinal monitoring [Chen et al., 2019].

In summary, CD163 represents a clinically actionable imaging biomarker for TAMM detection in glioblastoma. Its value lies not just in improved tumour visualisation but also in patient stratification and therapeutic monitoring, highlighting the need for translational studies to bridge preclinical insights into clinical practice.

5.2. CD68

CD68 is expressed in cells of the monocyte-macrophage lineage, including macrophages, but is also present in microglia, which are resident immune cells of the central nervous system. A study reports the overexpression of CD68 in TAMMs, where CD68 plays a role in both physiological and pathological mechanisms, including atherosclerosis, inflammation, autoimmunity, and tumourigenesis [Chistiakov et al., 2017]. Though CD68 is a useful marker of TAMMs, it is not specific to TAMMs as it is also overexpressed on tumour cells. With an overexpression of CD68 in TAMMs and tumour cells, it is useful in highlighting the TME. The expression of CD68 is also reported to distinguish between the classically activated/alternatively activated subtypes of

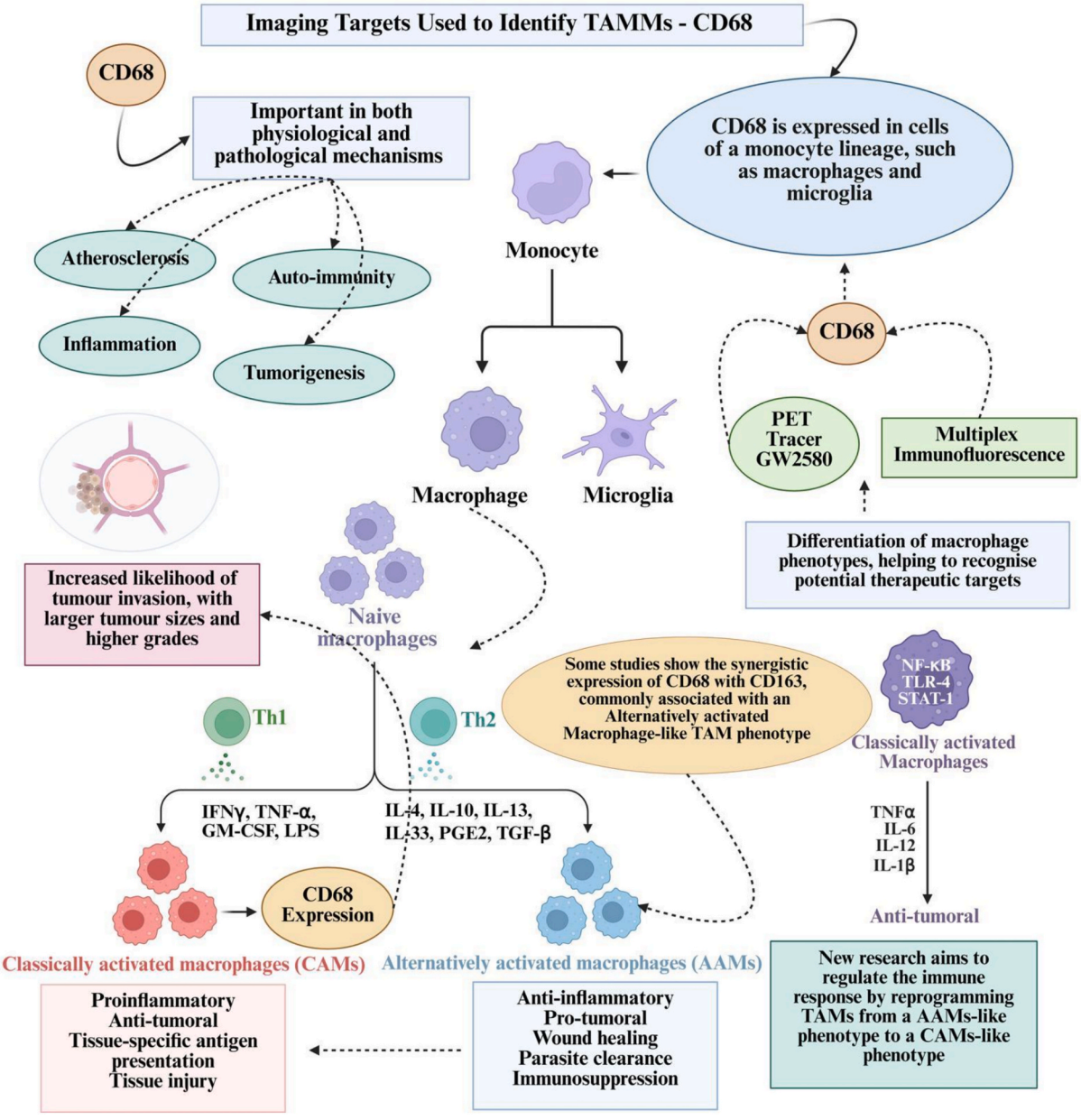

Fig. 3. CD68 as a Marker of Tumor-Associated Microglia and Macrophages in Glioma: Roles in Monocyte-Lineage Differentiation, Immune Polarization, Inflammation, and Tumor Progression Within the Tumor Microenvironment **Abbreviations:** AAM: Alternatively Activated Macrophages, CAM: Classically Activated Macrophages, PET: Positron Emission Tomography, Th1: T Helper cell type 1, Th2: T Helper Cell Type 2, GM-CSF: Granulocyte-Macrophage- Colony-Stimulating Factor, LPS: Lipopolysaccharide, TNF-β; Tumour Necrosis Factor Beta, TNF-α; Tumour Necrosis Factor, IFN: Interferon, IL: Interleukin, TGF: Transformation growth factor, PG: Prostaglandin, STAT4: Signal Transducer and Activator of Transcription 4, TLR-4: Toll-like receptor 4, NF-kB; Nuclear Factor-kappa B, TAMM; Tumour Associated Microglia and Macrophage, IFN-γ; Interferon Gamma.

macrophages. Specifically, the classically activated TAMMs express CD68 and support the anti-tumour pro-inflammatory response [Wang L et al., 2018].

Although CD68-targeted radiotracers are still under development, multiplex immunohistochemistry (mIHC) and immuno-PET approaches are increasingly being used in translational research settings. mIHC allows for co-detection of CD68 with other markers (e.g., CD163), providing spatial resolution and insight into macrophage phenotypes within patient tumour biopsies. [Hu et al., 2024] This is possible due to the morphological heterogeneity (ameboid, round, and oval shapes) of CD68-positive cells. Consequently, this may guide immunotherapy stratification or post-surgical evaluation of immune infiltration. [Hu et al., 2024].

CD68 expression has been associated with glioma grade and invasiveness, with higher CD68 + macrophage densities correlating with larger tumour volumes and poorer prognosis [Melcher et al., 2021]. In glioblastoma patients, immunohistochemical CD68 + scores have also been proposed as predictive biomarkers for treatment response, particularly in response to anti-angiogenic or immune-modulating therapies.

Emerging therapeutic strategies aim to reprogram CD68 + TAMMs from a pro-tumourigenic (alternatively activated) to an anti-tumourigenic (classically activated) phenotype. [Tanaka et al., 2021] Thus, targeting CD68 is not only useful for diagnosis but is also effective for monitoring glioma progression. Fig. 3 depicts the role of CD68 as an imaging target used to identify TAMMs in brain tumours.

5.3. TSPO

TSPO is becoming an increasingly valuable biomarker for identifying TAMMs in brain tumours, especially gliomas. Overexpression of TSPO in glioma cells and TAMMs reveals that TSPO is an indicator of TME inflammatory status and malignancy. TSPO expression has been shown to correlate with tumour grade and aggressiveness and is a reliable marker for differentiating high-grade gliomas from lower-grade types and normal brain tissue [Ammer et al., 2020]. In a larger histologically verified cohort including WHO grade II to IV gliomas, 18F-GE-180 uptake showed a strong grade-dependent pattern beyond glioblastoma, with median TBRmax values rising from 1.63 in WHO grade II gliomas to 3.63 in WHO grade III gliomas and 5.15 in WHO grade IV gliomas; importantly, all TSPO-negative cases were WHO grade II diffuse astrocytomas or oligodendrogliomas, whereas high-grade gliomas were almost uniformly PET-positive [Unterrainer et al., 2019].

Clinically, TSPO is most commonly imaged using PET with radioligands such as [^{18}F]GE-180, [^{18}F]DPA-714, and [^{11}C]PK11195. These tracers allow non-invasive, in vivo visualisation of neuroinflammation, particularly the distribution and density of TSPO-expressing TAMMs, which can reflect tumour progression or immune infiltration. [Buck et al., 2015]. For example, in an imaging study of glioma patients, Unterrainer et al. (2019) demonstrated that [^{18}F]GE-180 PET imaging enabled clear demarcation of active tumour regions, supporting its role in surgical planning, treatment monitoring, and recurrence detection. [Unterrainer et al., 2019]. Non-glioblastoma data from the same tracer also suggest utility in lower-grade and intermediate-grade gliomas. In WHO grade III tumours, TSPO uptake remained clearly detectable, and in recurrent lower-grade astrocytomas, emergence of high focal 18F-GE-180 uptake was associated with histologically confirmed malignant transformation to anaplastic astrocytoma or glioblastoma, whereas recurrent diffuse astrocytomas without malignant transformation showed no visually detectable uptake [Unterrainer et al., 2019]. Moreover, TSPO-PET imaging has shown utility in differentiating between tumour progression, which are frequent challenges in glioma management. This capability improves clinical decision-making, particularly in selecting patients for further surgery, chemotherapy, or immunotherapy [Albert et al., 2017].

TSPO imaging also offers prognostic value. Elevated TSPO signal intensity has been associated with high tumour grade and poor overall survival, especially in IDH-wildtype glioblastomas. [Zinnhardt et al., 2021; Filippi et al., 2023] This suggests TSPO could be incorporated into imaging-based risk stratification models. Therapeutically, while TSPO ligands were initially used only for imaging, recent research suggests that certain ligands may modulate immune cell activity or induce apoptosis in tumour cells. This points to a potential theranostic role, where the same molecular target is used for both imaging and therapy, offering a new paradigm in personalised glioma treatment [Rechichi et al., 2008; Janczar et al., 2015].

In summary, TSPO is more than a molecular marker. It is a clinically actionable target with growing relevance in glioma imaging, patient stratification, and potentially treatment monitoring. Integration of TSPO-PET into routine clinical practice may enhance diagnostic accuracy, guide immunotherapy selection, and provide earlier, more reliable indicators of therapeutic efficacy.

5.4. CD206

CD206, a marker for alternatively activated macrophages, is emerging as a clinically relevant biomarker in gliomas due to its central role in shaping the tumour's immunosuppressive microenvironment. TAMMs that express CD206 support glioma progression by facilitating immune evasion and tumour growth through immunosuppressive mechanisms [Tanaka et al., 2021]. Importantly, the significance of CD206 in glioma is not uniform across all tumours, because glioma immune composition varies substantially between molecular subtypes and even between distinct tumour regions within the same lesion. Accordingly, CD206 should be interpreted as a context dependent immune biomarker, whose biological and potential imaging relevance is shaped by subtype specific microenvironmental features rather than assumed to have a single meaning across all diffuse gliomas [Zeiner et al., 2019; Khalili et al., 2023].

Clinically, CD206 has gained attention for its potential in non-invasive immune profiling. Studies have shown elevated CD206 expression in induced microglia-like cells (iMGs) derived from peripheral blood mononuclear cells of glioma patients. These iMGs, generated via density gradient centrifugation, monocyte enrichment, and a 14-day induction with GM-CSF and IL-34, mirror the immune characteristics of gliomas [Tanaka et al., 2021]. The upregulation of CD206 in these circulating iMGs suggests a minimally invasive approach to monitor the glioma immune environment and potentially track disease progression or response to treatment [Hata et al., 2020].

To address molecular heterogeneity more systematically, CD206 should be discussed in relation to IDH defined glioma biology, rather than only as a general macrophage marker. In a large histopathologic analysis across astrocytic glioma subgroups, CD206-positive glioma-associated microglia and macrophages showed a heterogeneous distribution across tumour compartments and molecular classes, with marked variability even within IDH1R132H wt glioblastoma, indicating that biomarker interpretation depends on both molecular subtype and sampling location within the tumour [Zeiner et al., 2019]. More broadly, immune cell imaging in glioma is increasingly understood as a personalised, subtype-aware strategy, because molecular background influences the abundance, spatial distribution, and interpretability of immune biomarkers detected by tissue, blood-based surrogate assays, and imaging methods [Khalili et al., 2023].

In glioblastoma, CD206-positive glioma-associated microglia and macrophages show substantial spatial and inter-patient heterogeneity, with preferential localisation in perivascular and perinecrotic regions; this supports the view that CD206 is best interpreted as a subtype- and region-sensitive biomarker of the glioma immune microenvironment, rather than a uniform readout across all cases [Zeiner et al., 2019]. This has practical translational importance, because any future CD206-directed immune imaging or macrophage-targeted stratification strategy will need to account for molecular subclass, local tumour architecture, and compartment specific immune cell distribution if it is to be

clinically meaningful [Khalili et al., 2023]. These findings support the use of CD206 not only as a prognostic marker but also as a tool for stratifying patients based on the immune profile of their tumour. Furthermore, CD206′s association with the immunosuppressive phenotype of TAMMs highlights its promise as a therapeutic target. Modulating macrophage polarisation through CD206 could potentially enhance the effectiveness of immune-based therapies [Liu et al., 2024].

In summary, CD206 is best viewed as a heterogeneity-sensitive biomarker of the glioma myeloid microenvironment rather than a universally interpretable macrophage marker. Its detection in blood-derived iMGs supports the development of minimally invasive immune monitoring, while tissue-based studies show that its significance varies according to molecular subtype, intratumoural compartment, and local immune architecture [Tanaka et al., 2021; Zeiner et al., 2019]. Importantly, the Tanaka cohort suggests that this variability extends to IDHmut diffuse gliomas, with high CD206-positive cell numbers in grade II gliomas but weaker expression in grade III gliomas, while paired peripheral iMG profiles mirrored these tissue-level patterns in several non-GBM cases [Tanaka et al., 2021]. For that reason, the translational value of CD206 lies not only in prognosis or macrophage targeting, but also in its potential contribution to personalised immune profiling frameworks for glioma, in which molecular subtype and microenvironmental context guide biomarker interpretation and future imaging applications [Khalili et al., 2023].

5.5. CX3CR1

CX3CR1, a chemokine receptor involved in inflammatory signalling, has emerged as a marker for identifying TAMMs within the brain TME. In particular, CX3CR1 is upregulated in monocyte-derived TAMMs (TAMM-MDMs), suggesting its role in shaping the immune landscape of brain tumours [Schulz et al., 2021]. This upregulation is linked to crosstalk between glial cells and neurons, contributing to tumour formation and progression. Importantly, CX3CR1 relevance is not confined to glioblastoma. In human tissue analyses, CX3CR1 was overexpressed at both mRNA and protein level in gliomas across different malignancy grades, including WHO grade I to III tumours as well as glioblastoma, and was localised predominantly to Iba1-positive and CD11b/c-positive glioma-infiltrating microglia/macrophages. This indicates that CX3CR1 marks glioma-associated myeloid cells across diffuse glioma biology more broadly, rather than only in glioblastoma [Held-Feindt et al., 2010].

From a translational perspective, CX3CR1 expression has been associated with angiogenesis and TAMM accumulation, making it a potential biomarker for tumour progression and prognosis in brain tumours [Lee et al., 2020]. Its involvement in the CX3CL1–CX3CR1 signalling axis also highlights its potential utility in therapeutic targeting and immune modulation. In human glioma-infiltrating microglia/macrophage enriched fractions, CX3CL1-triggered CX3CR1 activation promoted adhesion and migration and increased expression of matrix metalloproteases MMP2, MMP9, and MMP14, supporting a tumour-promoting role of this axis at the level of stromal myeloid cell behaviour. Because this was demonstrated in human astrocytoma and glioblastoma material rather than in GBM alone, it further supports CX3CR1 as a biologically relevant marker across glioma grades [Held-Feindt et al., 2010]. In vivo work in a PDGFB-driven proneural glioma model showed that loss of CX3CR1 increased tumour incidence and shortened survival by promoting infiltration of inflammatory monocytes into perivascular regions and increasing IL1β-associated tumour-supportive signalling, indicating that intact CX3CR1 signalling may restrain certain protumour myeloid effects in at least some glioma contexts [Feng et al., 2015]. These findings suggest that CX3CR1 could serve as a dynamic indicator of tumour–immune interactions, particularly in glioblastomas. However, conflicting data indicate that complete loss of CX3CR1 may also facilitate micrometastatic progression [Erreni et al., 2010], underlining the complexity of its role in tumour evolution.

Importantly, CX3CR1 has therapeutic relevance, particularly in the context of immunotherapy. Blocking CX3CL1–CX3CR1 signalling has been shown to enhance the efficacy of anti-PD-1 immune checkpoint inhibitors by reshaping the myeloid cell compartment, resulting in improved survival in preclinical glioma models [Chaudhri et al., 2023]. These findings support the potential for CX3CR1 as a therapeutic target, especially in combination treatment strategies.

In summary, CX3CR1 plays a multifaceted role in brain tumours, with implications for prognosis, immunotherapy, and tumour imaging. Its expression in TAMMs highlights its value as a biomarker for immune profiling and a potential target in combinatorial therapeutic approaches, as illustrated in Fig. 4.

6. Glioma imaging modalities and T-cells

Characterisation of immune infiltration and prediction of response to immunotherapy in gliomas (astrocytomas and oligodendrogliomas) requires medical imaging of the immune microenvironment. In vivo tracking of T cells and immune activity in gliomas is now possible with advanced PET and MRI techniques. To provide a more structured framework, T-cell imaging in glioma can be organised into four complementary categories: (1) cytotoxic and infiltrating CD8 + T cells, (2) activated T cells, (3) suppressive or checkpoint-associated T-cell states including Tregs and exhausted T cells, and (4) supportive myeloid imaging that contextualises T-cell function within the glioma immune microenvironment [Khalili et al., 2023; Vincze et al., 2024]. Mechanistically, this imaging framework is supported by evidence that brain tumour antigens drain to the deep cervical lymph nodes, where antigen-presenting cells prime T cells and imprint CNS-homing programs before these cells encounter profound local immunosuppressive signalling within the glioma microenvironment [Broekman et al., 2018] (see Fig. 5).

CXCR4-targeted and CD8-specific radiotracers have been used to noninvasively image T cell distribution and activity in gliomas using PET imaging. More specifically, CD8 immunoPET has been used in orthotopic glioblastoma models to quantify early intratumoural CD8 + T-cell infiltration after combination immunotherapy, showing that direct imaging of CD8 + cells may help identify treatment response earlier than conventional imaging alone [Gallegos et al., 2024]. Lymphocyte trafficking is dependent on CXCR4 and radiolabeled CXCR4 antagonists can reveal T cell accumulation in glioblastomas and associated lymphoid structures. T-cell activation has been shown in tumour-draining lymph nodes and treated glioma sites in studies using GL261 and NSCL61 glioma models [Nobashi et al., 2021]. In addition to trafficking-based approaches, activation-sensitive imaging has become feasible. CD69 immunoPET has been shown to visualise T-cell activation in murine glioblastoma and to predict response to immunotherapy, while TIGIT immunoPET has enabled imaging of an immune checkpoint associated with suppressive or dysfunctional lymphocyte states in the glioma microenvironment [Nisnboym et al., 2023; Vincze et al., 2024]. In addition to this, ferumoxytol-enhanced MRI visualises iron laden macrophages that interact with infiltrating T cells which indirectly assesses immune engagement within the glioma microenvironment [Iv et al., 2019]. It complements direct T cell imaging and reflects local immune cell density and activity.

From an imaging perspective, suppressive T-cell states in glioma are currently assessed more convincingly through checkpoint-associated imaging than through established glioma-specific Treg-only tracers; for example, PD-L1 immunoPET and TIGIT immunoPET provide in vivo access to immunosuppressive niches that are highly relevant to Treg-rich and exhausted T-cell states [Sharma et al., 2023; Vincze et al., 2024]. Regulatory T cells (Tregs) play a central role in the suppression of anti-tumour immunity in glioblastoma, contributing significantly to immune evasion and poor responses to immunotherapy. These cells are consistently enriched in the glioblastoma TME, where they inhibit cytotoxic $CD8^{+}$ T cell function, suppress dendritic cell activity, and secrete

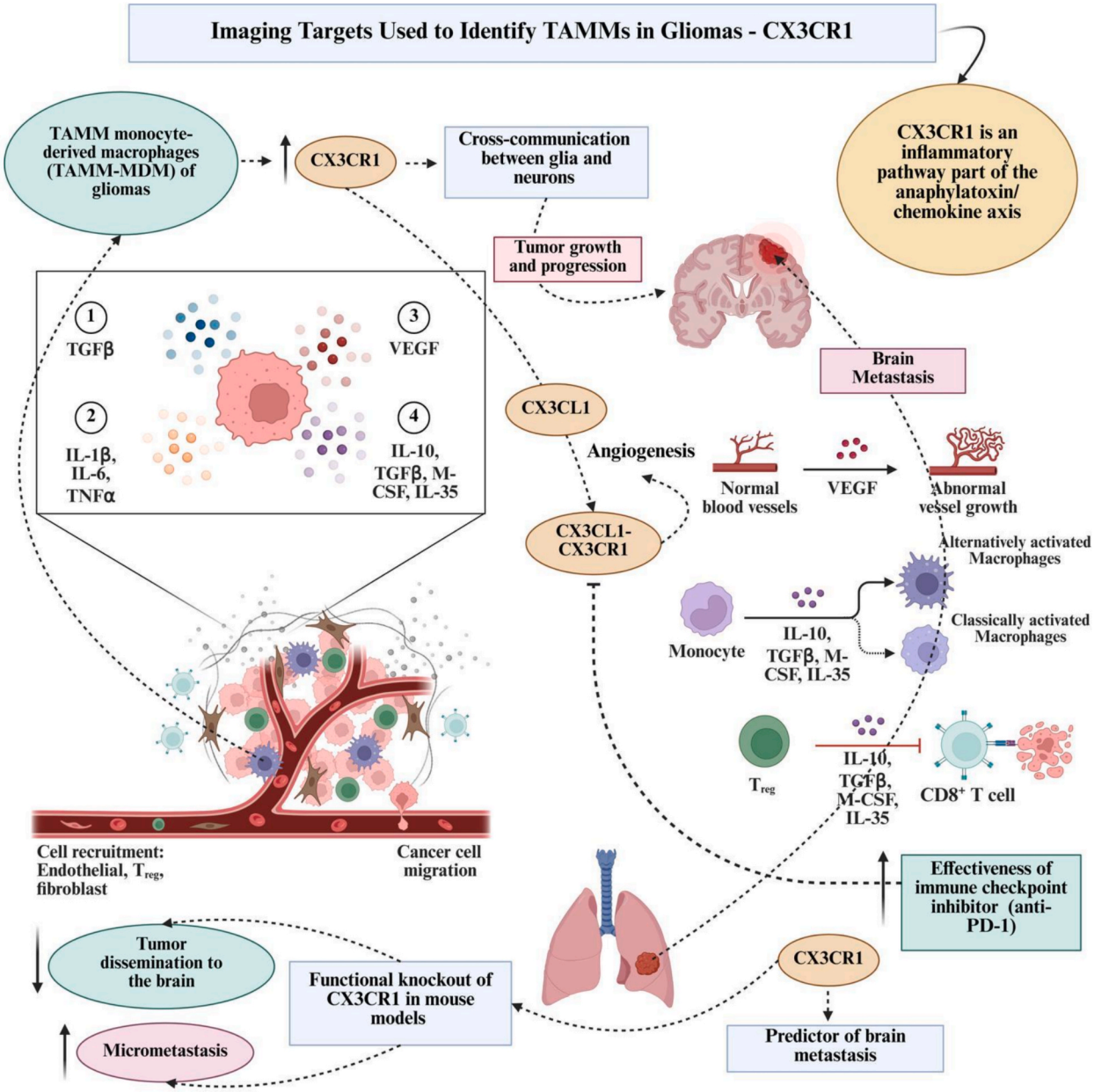

Fig. 4. The role of CX3XR1 as an imaging target used to identify TAMMs in brain tumours. CX3CR1 is involved in the lineage differentiation of TAMMs that have different roles in brain tumour progression, and therefore monitoring. **Abbreviations:** VEGF: Vascular Endothelial Growth Factor, TGF: Transformation Growth Factor, TNF: Tumour Necrosis Factor, IL: Interleukin, GM-CSF: Granulocyte-Macrophage Colony-stimulating Factor, CXCL; Chemokine (C-X-C motif) Ligand, TAMM; Tumour Associated Microglia and Macrophage, TAM-MDM; Tumour-associated Macrophage-Monocyte-Derived Macrophage, PD; Programmed Death, IL-10; Interleukin-10, M−CSF; Macrophage Colony-Stimulating Factor, TNF-β; Tumour Necrosis Factor Beta, TNF-α; Tumour Necrosis Factor.

immunosuppressive cytokines such as TGF-β and IL-10, thereby promoting tumour progression [Han et al., 2014; See et al., 2015; Lu et al., 2021].

Tregs in glioblastoma also include highly suppressive subsets such as T follicular regulatory (Tfr) cells, which particularly target $CXCR5^{+}$ $CD8^{+}$ T cells, markers of potent anti-tumour activity [Lu et al., 2021]. Imaging and histological studies show that TILs, including Tregs, often express immune checkpoint molecules like PD-1, TIM-3, and CTLA-4, indicating states of functional exhaustion [Amy, Heimberger et al., 2008; Liu et al., 2016; Lucca et al., 2015]. Notably, glioma-intrinsic expression of ICOSLG fosters expansion of IL-10–producing Tregs, further enhancing the immunosuppressive milieu [Iwata et al., 2019]. The dominance and functional diversity of Tregs within the glioma-infiltrating $CD4^{+}$ T cell compartment underscore their contribution to the failure of immune-based therapies. Targeted depletion or modulation of Tregs has shown promise in preclinical glioblastoma models and remains a key strategy for enhancing antitumour immunity.

Building upon this, recent studies have further detailed the complex landscape of T cell exhaustion in glioblastoma, particularly among both effector and regulatory subsets. T cell exhaustion is a well-characterised and severe dysfunction in glioblastoma, marked by the upregulation of inhibitory receptors such as PD-1, TIM-3, and LAG-3, and leading to poor effector function and cytokine production among tumour-infiltrating lymphocytes [Woroniecka et al., 2018; Woroniecka & Fecci, 2018]. Subsets of T cells, especially CD8 + T cells, demonstrate distinct exhaustion profiles upon tumour entry, with triple-positive PD-1 + TIM-3 + LAG-3 + populations being particularly dysfunctional. In addition, tumour-infiltrating regulatory T cells (Tregs) also express

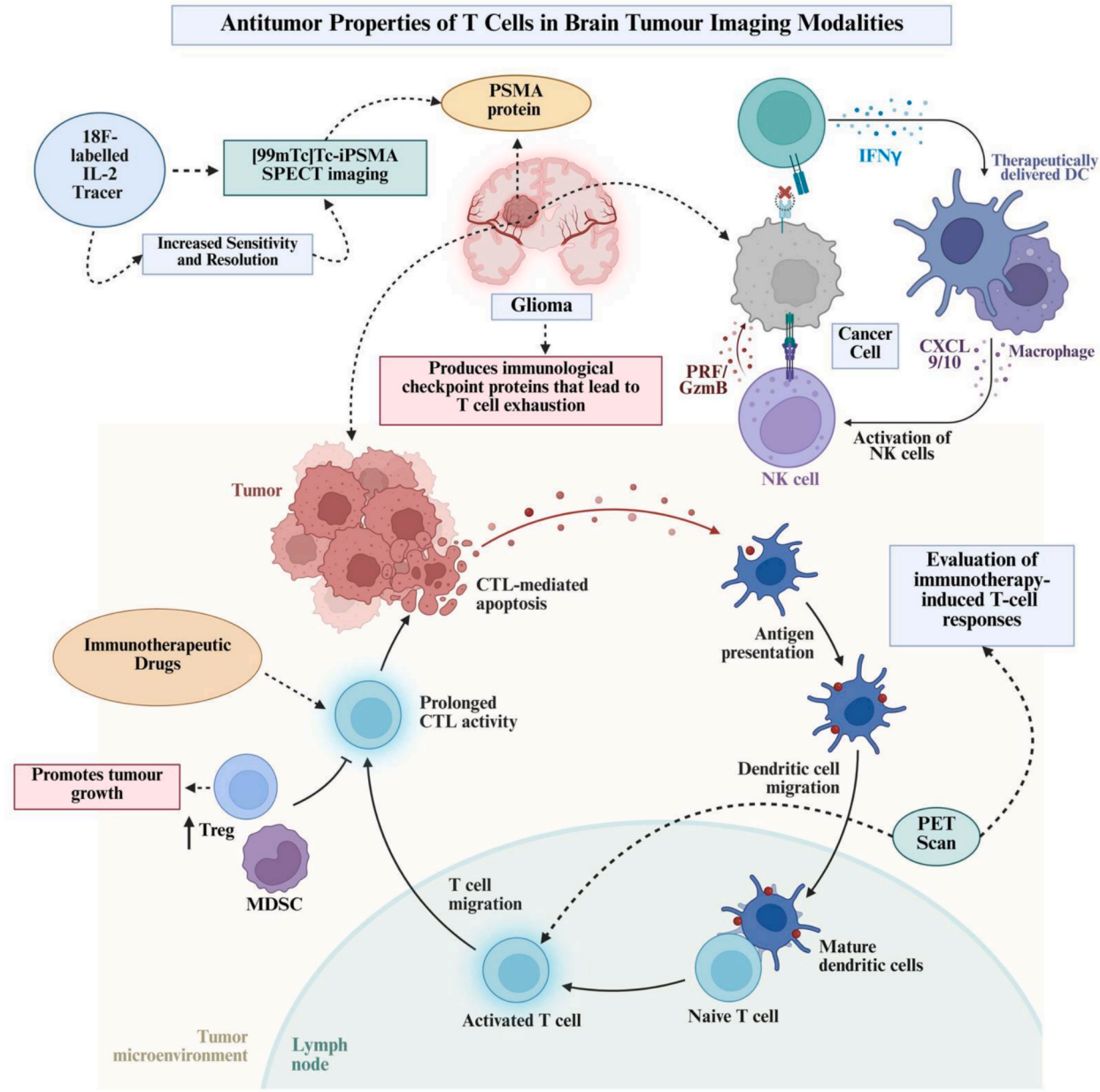

Fig. 5. Antitumor Functions of T Cells in Glioma: Mechanisms of Cytotoxicity, Immune Activation, Checkpoint Regulation, and Their Integration into Molecular Imaging Modalities **Abbreviations:** CTL: Cytotoxic T-Lymphocyte, NK cells: Natural Killer Cells, PET: Positron Emission Tomography, PRF: Perforin, GzmB: Granzyme B, SPECT: Single-photon Emission Computed Tomography, CXCL; Chemokine (C-X-C motif) Ligand, IFN-γ; Interferon Gamma, DC; Dendritic Cell, IL; Interleukin, MDSC; Myeloid-Derived Suppressor Cell.

exhaustion markers such as PD-1 and exhibit impaired suppressive capacity, suggesting a broader spectrum of T cell dysfunction beyond effector cells [Lucca et al., 2015; Lowther et al., 2016]. The glioblastoma microenvironment further propagates T cell energy and suppressive Treg expansion, which correlates with T cell clonal constriction and terminal dysfunction [Woroniecka et al., 2018; Sambruni et al., 2023]. Including a detailed discussion of these dysfunctional T cell states and their subtypes would significantly enhance the review's immunological depth and highlight critical barriers to current immunotherapeutic strategies.

PET tracers have recently advanced to the point where it is possible to image immune checkpoint proteins and T cell activity in vivo. Functional insights into T cell engagement with glioma cells are provided by tracers targeting immune checkpoints (TIGIT) and costimulatory markers (OX40). Imaging of humanised OX40-agonist antibodies which are under clinical investigation for glioblastoma, can track therapeutic responses [Shibahara et al., 2015; Kasten et al., 2021]. Further, MRI derived radiomic signatures can stratify gliomas into immune subtypes, inflamed, intermediate or cold, based on T cell infiltration and immune gene expression patterns. IDH-wildtype astrocytomas tend to be T cell infiltrated and associated with imaging signal, while oligodendrogliomas are relatively immune excluded ("cold") [Duan et al., 2023; Su et al., 2015].

Syngeneic glioma models further demonstrate the utility of imaging in tracking CD8 + T cell retention, proliferation and cytotoxic function in vivo in adoptive T-cell therapy studies. Tools for imaging have confirmed that glioma-infiltrating T cells can mediate antitumour effects, especially in combination with Treg suppression or Fc-OX40L costimulatory agonists [Quail et al., 2017; Murphy et al., 2012].

A major translational advantage of this approach is that immune-

sensitive multimodal imaging may improve post-treatment response assessment, particularly in helping distinguish immunotherapy-related inflammatory change or pseudoprogression from true tumour progression, a problem that conventional MRI alone often handles poorly in glioma [Khalili et al., 2023]. By combining multiple imaging platforms (PET, MRI and radiomics), we achieve a detailed, noninvasive characterisation of T cell behavior in gliomas. Refining immunotherapeutic strategies and improving patient specific treatment planning requires this integration.

7. Imaging immune cells in brain tumours: challenges and future directions

The unique anatomical and physiological characteristics of the brain pose significant challenges to imaging immune cells within brain tumours. The BBB restricts the penetration of imaging agents and tracers, thereby limiting their utility for visualising immune cells in situ [Obermeier et al., 2013; Aum et al., 2014]. In response to these limitations, advanced imaging modalities such as optical imaging, fluorescence microscopy, photoacoustic imaging (PAI), and PET have been developed. These techniques offer non-invasive methods for monitoring molecular targets in vivo, enabling real-time assessment of immune cell dynamics and tumour progression [Wolf et al., 2020]. When integrated with radiogenomics and quantitative radiomics, these modalities facilitate the characterisation of tumour-infiltrating lymphocytes (TILs), a crucial component of cancer immunotherapy [Klemm et al., 2020].

Traditional imaging methods, such as MRI and CT, provide valuable macroscopic insights but lack the spatial resolution to distinguish immune cell types or their interactions within the TME. High-resolution techniques like multiphoton microscopy overcome this limitation but are hindered by shallow tissue penetration depths and reliance on invasive procedures, such as cranial windows, which are unsuitable for human studies [van der Heide et al., 2022]. Innovative approaches, such as ultra-small superparamagnetic iron oxide (USPIO) nanoparticle MRI, show promise by detecting monocyte infiltration in brain tumours as an early indicator of immune activation and therapeutic response [Helfen et al., 2018]. Similarly, PAI addresses several limitations by concurrently evaluating blood vessel architecture, oxygenation, and immune cell infiltration, offering a multi-dimensional view of the TME [Zhang et al., 2023].

A comprehensive understanding of immune cell dynamics in the brain TME necessitates real-time, high-resolution imaging with minimal invasiveness. However, technical challenges such as rapid acquisition speed, high sensitivity, and deep tissue penetration complicate this goal. While intravital microscopy enables dynamic imaging, its applicability is constrained by invasive surgical requirements and limited imaging depth [Engelhardt et al., 2017]. Recent advancements in PET imaging, such as [18F]FDB tracers, mitigate these challenges by minimising interference from TAMMs, which often cause false-positive signals in traditional PET tracers like [18F]FDG. Notably, the minimal uptake of [18F]FDB in glioblastoma demonstrates its potential as a glioblastoma-specific imaging modality free from confounding immune activity. Additionally, PET tracers such as 18F-AraG, which target activated T cells, allow precise evaluation of immune responses in the TME, particularly in the context of immunotherapy [Ellingson et al., 2021].

Advanced molecular imaging strategies, including PET imaging with 18F-labeled IL-2 tracers, show promise. These tracers, in combination with radiation or immunotherapy, exhibit enhanced tumour uptake, suggesting their utility in improving therapeutic outcomes [Hartimath et al., 2016, Ellingson et al., 2021]. Non-invasive imaging technologies are also being developed to visualise immune cell dynamics within the TME, potentially optimising cancer immunotherapy management [Zomer et al., 2022].

While invasive imaging modalities yield valuable insights in preclinical models, their translational potential is limited by differences in size, anatomy, and immune system function between humans and animals. For instance, genetically encoded fluorescent reporters used in animal models cannot be directly applied to human studies. Consequently, findings from animal models may not accurately reflect human tumour biology and immune interactions [Zamler et al., 2022]. Emerging spatial profiling technologies, such as spatial transcriptomics, offer a complementary approach by enabling in-depth, *in vitro* analysis of the cellular and spatial landscapes of the brain TME. These insights are instrumental in understanding therapy resistance and developing effective therapeutic strategies [Kalita-de Croft et al., 2021]. Translational innovations, including video-rate resonant scanning multiphoton microscopy (VR-MPLSM), hold the potential for real-time visualisation of cellular events in mouse models, providing a foundation for future clinical applications [Kirkpatrick et al., 2012].

The integration of diagnostic and therapeutic functionalities into single agents represents a significant advancement in overcoming the BBB and the complexity of the TME. Dual stimuli-responsive theranostic tracers, sensitive to the acidic and reductive conditions of the TME, enhance imaging sensitivity while simultaneously improving therapeutic efficacy [Wang et al., 2020]. Notable examples include targeted radiopharmaceutical therapy (TRT) directed at CD11b + tumour-associated myeloid cells, which has demonstrated the potential to augment immunotherapy efficacy in gliomas [Foster et al., 2021]. Similarly, Angiopep-2 conjugated hyaluronic acid nanoparticles (Thera-ANG cHANPs) provide a nano platform for localised, non-invasive drug delivery, enhancing both imaging and treatment outcomes [Wu et al., 2018; Costagliola et al., 2021]. Further innovations, such as polydopamine-based systems that degrade within the acidic TME to release reducing agents and generate toxic hydroxyl radicals, offer a synergistic approach to imaging-guided tumour treatment [Chen et al., 2020]. However, these technologies require rigorous validation and standardisation before clinical adoption.

8. Conclusion

In conclusion, imaging techniques that visualise immune cells, particularly TAMMs, within the glioblastoma TME are pivotal for advancing both our understanding of glioblastoma progression and the development of personalised therapeutic strategies. Non-invasive imaging methods, such as MRI, PET, and SPECT, combined with specific biomarkers, allow for precise monitoring of immune cell dynamics, tumour aggression, and treatment responses. As emerging imaging technologies and theranostics continue to evolve, they hold great promise in enhancing the targeting of immune cells and molecular features specific to glioblastoma, paving the way for more effective, individualised treatments and improved patient outcomes.

10. Consent to Participate

No original data from new patients were collected, consent to participate is not applicable.

11. Consent for Publication

Consent for publication is not applicable.

CRediT authorship contribution statement

Andrew Awuah Wireko: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Adam Ben-Jaafar:** Writing – review & editing, Writing – original draft, Methodology. **Joecelyn Kirani Tan:** Writing – review & editing, Writing – original draft, Methodology. **Sruthi Ranganathan:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Vivek Sanker:** Writing – review & editing, Writing – original draft, Visualization. **Princess Afia Nkrumah-Boateng:** Writing – review & editing, Writing – original draft, Methodology. **Krishitha Meenu Mannan:**

Writing – review & editing, Writing – original draft, Methodology. **Mubarak Jolayemi Mustapha:** Writing – review & editing, Writing – original draft, Methodology. **Aditya Gaur:** Writing – review & editing, Writing – original draft, Methodology. **Marike Broekman:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

9. Ethics approval

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Declaration of competing interest

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Availability of Data and Material

Not Applicable.

Appendix A. Supplementary data

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