Title:

Tumor infiltrating immune cells in gliomas and meningiomas

Authors:

Patrícia Domingues[1,2], María González-Tablas[2], Álvaro Otero[3],Daniel Pascual[3], David Miranda[3], Laura Ruiz[3], Pablo Sousa[3], Juana Ciudad[2], Jesús María Gonçalves[3], María Celeste Lopes[1], Alberto Orfao[2] , María Dolores Tabernero[2,3,4]

Institutions:

[1]Centre for Neurosciences and Cell Biology and Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

[2]Centre for Cancer Research (CIC-IBMCC; CSIC/USAL; IBSAL) and Department of Medicine, University of Salamanca, Salamanca, Spain

[3]Neurosurgery Service of the University Hospital of Salamanca, Salamanca, Spain

[4]Instituto de Estudios de Ciencias de la salud de Castilla y León (IECSCYL-IBSAL) and Research Unit of the University Hospital of Salamanca, Salamanca, Spain

Corresponding Author:

María Dolores Tabernero Redondo, M.D. Ph.D.

Research Unit of the University Hospital of Salamanca

Paseo San Vicente 58-182, Salamanca, Spain

Phone: +34923-29-12-30. Fax: +34923-29-46-24

E-mail: taberner@usal.es

Abstract

Tumor-infiltrating immune cells are part of a complex microenvironment that promotes and/or regulates tumor development and growth. Depending on the type of cells and their functional interactions, immune cells may play a key role in suppressing the tumor or in providing support for tumor growth, with relevant effects on patient behavior. In recent years, important advances have been achieved in the characterization of immune cell infiltrates in central nervous system (CNS) tumors, but their role in tumorigenesis and patient behavior still remain poorly understood. Overall, these studies have shown significant but variable levels of infiltration of CNS tumors by macrophage/microglial cells (TAM) and to a less extent also lymphocytes (particularly T-cells and NK cells, and less frequently also B-cells). Of note, TAM infiltrate gliomas at moderate numbers where they frequently show an immune suppressive phenotype and functional behavior; in contrast, infiltration by TAM may be very pronounced in meningiomas, particularly in cases that carry isolated monosomy 22, where the immune infiltrates also contain greater numbers of cytotoxic T and NK-cells associated with an enhanced anti-tumoral immune response. In line with this, the presence of regulatory T cells, is usually limited to a small fraction of all meningiomas, while frequently found in gliomas. Despite these differences between gliomas and meningiomas, both tumors show heterogeneous levels of infiltration by immune cells with variable functionality. In this review we summarize current knowledge about tumor-infiltrating immune cells in the two most common types of CNS tumors–gliomas and meningiomas–,as well as the role that such immune cells may play in the tumor

microenvironment in controlling and/or promoting tumor development, growth and control.

Keywords

Brain tumors, glioma, meningioma, microenvironment, immune cells, lymphoid cells, myeloid cells, immune infiltration, multiparameter flow cytometry.

Introduction

Tumor development and growth typically requires an appropriate microenvironment, in addition to genetic/molecular alteration of tumor cells. Such tumor microenvironment consists of a complex network of distinct cell types and extracellular matrix components, in which neoplastic cells interact with fibroblasts, vascular endothelial cells, a variety of infiltrating immune cells (including a network of cytokines and chemokines released by these cells) and extracellular matrix proteins,among other components. Although tumor development and growth largely depend on an adequate microenvironment, the tumor cells *per se* also induce significant changes in the tissue where they home and grow(1). Because of this, patients may show behavioral changes including neuropsychiatric symptoms and/or cognitive effects depending on the affected region of the brain and/or the local immune response(2).

Immune cells present in the tumor typically include T lymphocytes, natural killer (NK) cells, macrophages, dendritic cells (DC), polymorphonuclear leukocytes and occasional B cells(1, 3). Overall, infiltration by immune cells is a hallmark of virtually every tumor(4), and it is frequently associated with tumor behavior and patient outcome(3). In this regard, while multiple reports in the literature have linked the presence of inflammatory infiltrates in human tumors with an improved prognosis and a better patient outcome(3, 5, 6), many others have found no significant association,or they have even linked immune cell infiltration with a poorer prognosis(3). Such apparent discrepancy may be due to the type and functional state of immune cells

infiltrating the tumor. In fact, the different types of infiltrating immune cell populations vary not only according to the type of cancer, but also from patient to patient within the same type of tumor or at different time points within a patient (e.g. at diagnosis vs recurrence); these observations suggest that different immune cell microenvironments may have distinct effects/roles in tumor control and progression(3). In addition, the same immune cells present in the tumor microenvironment may modulate their anti-or pro-tumoral functions, being able to play dual roles with potential to either suppress or promote malignancy(7);usually, the latter predominates as the tumor cells acquire mechanisms for 'immune evasion'. Thus, in such circumstances the tumor, not only manages to escape from the host immune system, but it also develops a phenotype capable of manipulating immune cells (e.g. via secretion of chemokines and cytokines), and modifying their function to create a microenvironment that would favor tumor progression(8). To date, many mechanisms of immune evasion by tumor cells have been identified (Table 1), including inhibition of immune cell functions or apoptosis of anti-tumor effector cells, together with production of both growth factors and angiogenic factors that stimulate tissue repair and vascularization, and consequently also, tumor growth(1). In case of CNS tumors, immune responses may also contribute to induce changes in patient symptoms and behavior, depending on tumor localization and the specific types of immune cells and mediators involved. In this regard, it is considered that younger patients presenting with acute signs and symptoms of neurologic disease are investigated earlier, and consequently, referred more promptly for treatment(9). Conversely, patients with organic brain lesions in neurologically silent brain areas might present with milder

symptoms and/or isolated psychiatric symptoms such as depression, anxiety disorders, schizophrenia, anorexia nervosa, or cognitive dysfunction(10, 11). In such later cases, differential diagnosis between a brain tumor vs. a psychiatric disorder is required, final diagnosis being frequently delayed for variable periods of time(2, 12).

Table 1. Mechanisms that have been frequently associated with immune escape by tumor cells.

Cell feature	Mechanism of immune escape	Tumor cell-associated	Immune cell-associated
↓Expression of TAA	Lack of susceptibility to effector immune cells	+	-
↓ HLA Expression on tumor cells	Immune selection of resistant variants	+	-
↓Co-stimulatory molecules	Activation of signaling pathways for tumor cell survival	+	+
↑Death-receptor/ligand signaling	↑Immune cell death/apoptosis	+	+
Defective antigen presentation by DC	Altered T cell function	-	+

Altered T-cell receptor (TCR) signaling	Suppression of immune cells (e.g. T cells) by Tregs or MDSC	-	+
Secretion of chemokines and cytokines	Suppression of immune response	+	+

TAA,tumor associated antigens; Treg, regulatory T-cell; MDSC, myeloid-derived suppressor cell.

Diagnostic subtypes of glioma and meningioma

CNS tumors are rather heterogeneous and they vary widely by site of origin, morphological and histophatological features, growth potential and extent of invasion. At present, classification of gliomas is mainly based on the existence morphological evidence of differentiation of tumor cells along the astrocytic (70% of the cases) and less frequently the oligodendroglial and mixed astrocytic-oligodendroglialcell lineages in addition to ependymal tumors(13). The specific cell(s) targeted during neoplastic/malignant transformation of gliomas currently remains unknown, although tumor cells from primary brain tumors mimic the morphologic and phenotypic profiles of glial cells, or their precursors, from which they potentially originate. Because of their distinct cell appearance, gliomas are therefore classified into four major groups: astrocytomas, oligodendrogliomas, oligoastrocytomas (tumors presenting morphological features of both astrocytes and oligodendrocytes) and ependymomas, depending on their differentiation-associated features and their morphological similarities with normal/reactive glial cells. These tumors are further subclassified

according to their histopathological gradeinto grade I to grade IV tumors,>80% of all diffuse gliomas being high-grade (grade III/IV) tumors, from whichglioblastoma (GBM) is the most common in adults. Thus, according to the WHO criteria, patients are distributed into: i) astrocytomas (grade I pilocytic astrocytomas, grade II diffuse astrocytomas, grade III anaplastic astrocytomas, grade IV glioblastomas, and grade IV gliosarcomas; ii) oligodendrogliomas (grade II oligodendroglioma, and grade III anaplastic oligodendrogliomas); iii) mixed oligoastrocytomas (grade II and grade III anaplastic oligoastrocytomas) and; ependymal tumors (subependymoma and myxopapillary ependymoma grade I; ependymoma grade II and anaplastic ependymoma grade III) (Table 2).

Conversely, all meningiomas originate from the meningeal coverings of the brain and the spinal cord. The vast majority of meningiomas are considered to be benign and slow-growing neoplastic lesions. However, these tumors present with a great clinical heterogeneity as regards the symptoms of the disease, histopathology, recurrence rates, clinical aggressiveness, and outcome. Overall, the majority of meningiomas are intracranial tumors, with up to 60% being located in the convexity, parasagittal, tuberculum sellae, and sphenoid wing regions, the clinical signs and symptoms associated with an underlying meningioma being directly related to the size and localization of the tumor. Despite this, general CNS-associated symptoms such as personality changes, neuropsychological deficits, headache, aphasia, sensory-motor or visual symptoms, as well as seizures, also occur rather frequently(14). From the histopathological point of view, meningiomas are currently classified according to the WHO grading system into three major (prognostic) categories which include: benign

(WHO grade I), atypical (WHO grade II), and anaplastic (WHO grade III) meningiomas, with several histopathological variants. Such variants include: i) grade I meningothelial, fibroblastic, transitional, psammomatous, angiomatous, microcystic, secretory, lymphoplasmacyte-rich and metaplastic meningiomas; ii) grade II atypical, clear-cell and chordoid tumors and; iii) grade III anaplastic, rhabdoid and papillary meningiomas(13). WHO grade I/benign meningiomas represent around 90% of all meningiomas, the meningothelial, fibroblastic, and transitional variants being the most common ones(15). By definition, these meningiomas do not invade the brain and they display a benign clinical behavior(Table 2); despite this, a significant proportion of cases show recurrence of the disease after complete tumor resection, with different recurrence rates (range: 7% to 20% of cases) for distinct histopathological subtypes.

Table 2. Histopathologic WHO classification of glioma and meningiomas and distribution of the histopathologic subtypes according to tumor grade.

Tumor type	WHO GRADE				
	Grade I	Grade II	Grade III	Grade IV	
Glioma	Pilocytic astrocytoma	Diffuse astrocytoma Pylomyxoid Pleomorphic Xanthoastrocytoma	Anaplastic Astrocytoma	GBM	Gliosarcoma
	-	Oligodendroglioma	Anaplastic Oligodendroglioma		-
	-	Oligoastrocytoma	Anaplastic oligoastrocytoma		-
	Subependymoma Myxopapillary	Ependymoma grade II	Anaplastic ependymoma		
Meningioma	Meningothelial Fibroblastic Transitional Psammomatous Angiomatous Microcystic Secretory Lymphoplasmacyte-rich Metaplastic	Atypical Clear-cell Chordoid	Anaplastic Rhabdoid Papillary		

GBM, glioblastoma multiforme.

The CNS microenvironment in brain tumors

The CNS has unique microenvironmental conditions which as a whole, differ significantly from most other organs and tissues. To a certain extent, this relates to an active blood brain barrier (BBB), that confers a selective permeability around most CNS blood vessels (16, 17); such selective permeability, limits diffusion of molecules from the blood to the tissue, limiting the exposure of the brain parenchyma to circulating

antigens and metabolites. The BBB comprises tight junctions between endothelial cells surrounding the vessel and astrocyte foot processes(18). The pericytes, a population of cells resident in the perivascular space, share a common basement membrane with the capillaries and provide mechanical stability to the endothelial-based capillaries(19).

Other unique features of the CNS are related to its cellular composition, which includes several cells with potent immunoregulatory properties, in the absence of a (standard) lymphatic drain system(20, 21). Taken together, these factors contribute to explain the "immune privilege" of the brain, which is often described as a tissue with diminished or absent immune responses(22). However, this concept is more complex because this status is not uniform throughout the brain. Some brain regions are not protected from the (systemic) immune system in the same way as the brain parenchyma(18). In addition, the resident myeloid cell populations are distinct in different regions of the brain(e.g. the ventricles containing the choroid plexus and the cerebrospinal fluid-CSF-, the meninges and the perivascular space have distinct immunological properties)(22).

Despite all the above, at present it is known that circulating (systemic) immune cells are capable of migrating from cerebral vessels into both the perivascular space and the brain parenchyma, in response to various stimuli and signals(23). Migration of leukocytes is believed to occur in post-capillary venules, where the BBB is less strictly selective, with lower density of tight junctions and a perivascular space surrounding the vessels that does not exist in the brain capillaries(23). The mechanisms by which circulating leukocytes can cross the endothelial cell layer remain largely unknown; however, lymphocytes and leukemic cells, appear to migrate (transcellularly) across

the endothelial cell layer e.g. using the VLA-4/VCAM-1 ligand-receptor complex to adhere to endothelial cells in the brain vasculature(24). Following attachment of circulating immune system cells onto the vessel wall, these cells are often subsequently activated locally by multiple factors such as chemokines(24) with potential effects also on CNS cells including glial cells and neurons.

For decades, it is well-established that the limited delivery of systemic drugs across the BBB is one of the major obstacles for the treatment of CNS disease/tumors. Only when specific drugs (e.g. vaccine peptides, some humanized monoclonal antibodies) and chemotherapeutic agents are administrated locally or at relatively high doses, they may cross the BBB. Under physiological conditions, direct contact between immune cells and CNS cells is hindered by the BBB, although the precise mechanisms controlling the effect of the BBB on CNS tumor cells are poorly understood; at the same time, the relationship between the growth of tumor vasculature and the entrance of immune cells, and their impact on the tumor as well as the surrounding brain tissue have not been fully established. Despite this, it is accepted that immune escape of CNS tumors relates at least in part to the modulation of immune cell entry into the brain by the BBB and the expression of specific profiles of chemokines and chemokine receptors. Thus, changes on the permeability of the BBB have been demonstrated in response to inflammatory mediators which involve endothelial cells, astrocytes, microglial cells, pericytes and extracellular components involved in the brain response to immune stimuli (25). Of note, such inflammatory responses may contribute to specific neuropsychiatric symptoms and changes in patient behavior,

such as depression, anxiety, irritability, apathy and hallucinations together with other neurological and cognitive symptoms depending on the specific cells involved(26, 27).

CNS resident immune cells

In the healthy CNS, there are several different subsets of myeloid cells which reside in the brain and other CNS tissues. Thus, parenchymal microglial cells are considered to be CNS resident macrophages(28, 29). Myeloid cells which populate other brain compartments are generally referred to as macrophages, prefixed with their localization, e.g. choroid plexus, meningeal or perivascular macrophages(22). Phenotypically, the distinction between the parenchymal microglia and other brain macrophages has been based on the levels of expression of the CD45 common leukocyte antigen: microglial cells are characterized by low CD45 expression whereas, other macrophages are $CD45^{high}$ (21, 22); similarly to other monocytic/macrophage cells, human parenchymal microglial cells have further been reported to express CD11b(30). However, in many studies the term microglia, macrophages or microglia/macrophages is used to describe potentially mixed cell populations. In turn, perivascular macrophages participate in antigen-presentation at the BBB, they have a high turnover rate and they are constitutively replenished by circulating monocytes(31, 32). In contrast, parenchymal microglia are differentiated tissue macrophages which are supposed to take up residency in the brain during embryonic development(28, 33).

The spectrum of functional properties and activities of these cells in the brain is as wide as for conventional macrophages, including scavenger functions, phagocytosis,

antigen presentation and migration(29). Some of the chemokines that have been related to migration of microglia/macrophages into the brain include: CCL21 via interaction with its CXCR3 receptor, CX3CL1 and CX3CR1, SDF-1α (CXCL12) via CXCR4, and the monocyte chemotactic protein 1 (MCP-1, CCL2) via CCR2(22, 23). Among other phagocytic and signaling cell receptors, these cells might express the Toll-like receptors (TLRs) 1-9, immunoglobulin Fc receptors, scavenger receptors and complement receptors, phagocytosis being a major function of activated microglia/macrophages(22, 33). In contrast, the ability of these cells for antigen presentation to T cells is more controversial.

A prerequisite of antigen presenting cells (APCs) is related to the expression of major histocompatibility complex(HLA) class II (for CD4 T cells) and HLA class I (for CD8 T cells) molecules together with costimulatory molecules (e.g. CD86). Microglial cells are usually considered the primary immune effector cells in the CNS, which are capable of generating significant immune responses(29). It has been suggested that upon stimulation, resident microglial cells can be rapidly activated via at least two functionally distinct morphological states, leading to activated microglia (which only express HLA-I) and reactive/amoeboid microglia (which express both HLA-I and HLA-II in association with an increased antigen presenting capacity)(33, 34). However, several reports have identified those cells residing in the perivascular space or the meninges as those displaying the greatest ability to present antigens to infiltrating T cells, for their subsequent stimulation and activation(22, 35). As macrophages infiltrate the perivascular space, infiltrated T lymphocytes recognize the antigens presented by these APCs and they will subsequently act as effector adaptative immune cells(35).

Indirect evidences also indicate that DC constitute another subgroup of candidate APC to migrate from the brain to lymph nodes(35, 36); CCR7-mediated chemotaxis of DC facilitates lymph node entry through the high endothelial venules, promoted by a CCL21 chemokine gradient(22).

The cellular composition of the cerebrospinal fluid

In recent years, several reports have provided detailed information about the composition of human cerebrospinal fluid (CSF) as regards its immune cell components(37-39). Overall, CSF is a paucicellular sample which mainly contains leukocytes typically at counts below 5 cells/µL. Around two-thirds of the whole CSF white blood cell populations correspond to T cells (mainly $CD4^+$ and to a less extent also $CD8^+$ T-lymphocytes) and around 25% are monocytes. In contrast, B-lymphocytes, NK-cells, DC, as well as neutrophils, are only detected in a small fraction ofall CSF samples from normal individuals, typically at lower numbers(40).

Immune cell infiltrates in brain tumors

Several distinct subtypes of immune cells have been reported to infiltrate brain tumors, where they have been associated with a wide spectrum of functions(19). From the different subtypes of brain tumors, GBM is among the most investigated ones, due to its relatively high incidence and aggressive clinical behavior. Overall, these studies have shown that despite the presence of immune cells in GBM, the overall tumor

environment is highly immunosuppressive. In this section we will briefly review the methods that have been used to characterize the cellular composition of brain tumor tissues and the main populations of immune cells that have been reported to infiltrate brain tumor tissues (e.g. myeloid cells and lymphoid cells) and their contribution to the behavior of the tumor, with special emphasis on gliomas and meningiomas; in turn, we will not discuss the role of the immune infiltrates in the patient symptoms and behavior which have been poorly investigated so far (41).

Evaluation of the cellular composition of tumor tissues. Brain tumors typically display a heterogeneous and variable cellular composition. Until now, several different techniques have been used for the identification and characterization of the different cell populations coexisting in tumor tissues. Among such techniques, immunohistochemistry (IHC) is the most widely used method in diagnostic surgical pathology of solid tumors. This technique combines staining with antibodies for localization and identification of specific antibody-targeted antigens in a cell or tissue specimen by light microscopy(42). Therefore, it allows the observer to distinguish between cancer cells and other different types of non-neoplastic cells, through combined assessment of cell morphology and detection/recognition of specific molecules in one or more subsets of cells. This method permits semi-quantitative evaluation of the cellular components of a tumor sample, and determination of the specific localization of a cell population in the tumor tissue(43). However, it also has some limitations, which are related to: i) the subjective nature of data interpretation with a relatively high degree of inter-observer variability; ii) usually it does not allow

simultaneous immunophenotypic identification of all different cell populations in the sample, and; iii) the identified cells cannot be isolated in sufficient numbers for their further complete (e.g. molecular) characterization, even when laser-microdissection techniques are used(44) (Table 3).

More recently, flow cytometry (FCM) has also been used for the identification and characterization of heterogeneous cell populations coexisting in tumor samples. However, whereas FCM is currently applied in routine clinical diagnosis and classification of hematological malignancies (e.g. leukemia and lymphoma)(39), its application to the study of solid tumor tissue samples remains rather limited(45). This is mainly due to the fact that FCM cannot be directly applied to the study of solid tumors tissues, since it requires prior preparation of single cell suspensions from the tumor tissue specimen(46). The key advantage of flow cytometry is that a very large number of cells can be evaluated in a very short time, information being generated for multiple parameters in a single cell basis; this confers FCM unique analytical capabilities *vs.* other technologies. In contrast, the major disadvantage of FCM for the study of solid tissues relies on the loss of all information about the architecture of the tissue and the spatial relationship between the different cells coexisting in a tumor sample(47). Such type of analysis is also associated with the presence of increased amounts of dying cells and both cellular and tissue debris, due to the need to apply mechanical and/or enzymatically tissue disaggregation procedures for the preparation of single cell suspensions, even when DNA and apoptotic cell dyes (e.g. DAPI or DRAQ5) are used to positively select for viable cells showing a high DNA content (46). Despite all the above, FCM immunophenotyping has been used for the analysis of

tumor infiltrating macrophages/microglia (30, 48-54), myeloid-derived suppressor cells (MDSC) (55) and lymphocyte subsets such as T-cells (56-58), both in experimental models and primary human glioma samples (49). In contrast with gliomas, few studies have been reported in which FCM immunophenotyping has been used to characterize neoplastic and/or infiltrating immune cell populations in meningiomas (59, 60).

Myeloid cells. Myeloid cell populations that have been shown to infiltrate brain tumors include microglial cells and tumor-associated macrophages (TAM); in addition, MDSC and DC have also been identified among immune cell infiltrates in CNS tumors. Of note, all such myeloid cell populations partially overlap with native CNS tissue myeloid cells, which may make it difficult to determine in many tumor samples, whether these cells correspond to tissue resident or newly-recruited cells.

*Microglial cells & tumor-associated macrophages (TAM).*Several studies have recurrently reported infiltration by microglial cells and TAM in both primary –e.g. gliomas (30, 49, 61) and meningiomas (59-62)– and metastatic brain tumors (18, 61). Although a clear discrimination between both subtypes of myeloid cells may still require full definition, several studies have proposed the existence of highly discriminating phenotypes based on marker combinations such as those provided by the CD45/CD11b expression profile (30).

In this regard, Parney et al. (30) reported a mean percent of 1.65% $CD45^{dim}CD11b^{+}$ (microglial) cells, and 6.25% $CD45^{bright}CD11b^{+}$ (macrophage) cells in 9 newly-diagnosed malignant gliomas by FCM immunophenotyping. In addition, several

studies which focused on the understanding of the mechanisms of tumor evasion from immune surveillance, and the immunosuppressive environment of gliomas, reported glioma-associated microglial cells/macrophages to lack on the expression of costimulatory molecules critical for T-cell activation (e.g. CD86, CD80, and CD40)(49), as well as on the ability to secrete cytokines (interleukin1β (IL-1β), interleukin6 (IL-6), TNFα) that are critical for developing effective innate immune responses (49), to have an impaired capacity to upregulate the expression of HLA class II molecules (52), and to display increased expression of immunosuppressive surface ligands such as B7-H1 and the Fas ligand (49, 53, 54).

Table 3. Overall composition of tumor immune cell infiltrates in gliomas and meningiomas as assessed by flow cytometry (FCM) and immunohistochemistry (IHC).

Tumor infiltrating immune cells	Gliomas				Meningiomas			
	FCM		IHC		FCM		IHC	
	% of cells	N.of cases	% of cells	N.of cases	% of cells	N.of cases	% of cells	N.of cases
Myeloid cells								
TAM/microglia	4%	8 (62)	6.8%	18 (61)	24%	27 (62)	1.7%	18 (61)
(CD68+)	0.8%	6 (50)	2%-11%[a]	36 (63)	22%[b]	51 (59)		
			34%	91 (64)				
Macrophages	6%[c]	9 (30)						
Microglia	2%[d]	9 (30)						
M1			130-472/mm^2[e]					
M2			23-287/mm^2[f]					
			49-368/mm^2[g]	79 (65)				
Dendritic cells								
Myeloid	0.13%[h]	6 (49)						
Plasmacytoid	0.02%[i]	6 (49)						
Lymphoid cells								
Total T cells (CD3$^+$)	2%	30 (57)	50%[j]	14 (56)	5%; 0.5%[k]	11 (57)	9.5%-1.8%[l]	62 (68)
	25%	10 (66)			1.7	9 (56)		
	1%	14 (56)					25%	9 (56)
	2%	9 (30)			1%	51(59)		
	2%	4 (67)						
CD8$^+$ T cells	0.04		9%-18%[m]	130(70)	1%	51(59)	80%	28 (72)
	26%	8 (69)	62%	91(64)				
			8/mm^2	93(67)				
			78%	67(71)				
CD4$^+$ T cells	6%	10 (66)	0.10%				20%	28 (72)
			0.005%-0.2%	60(49)				
	0.1%	6 (50)	1%-12%[n]	130 (70)				
	29%	8 (69)	12/mm^2	93 (67)				
			22%67 (71)					
Tregs	6%	10 (66)	3%	10 (66)	0.6%	14(56)	10%	28 (72)
			34%	67 (71)	5%	10 (73)		
	11%- 25%[o]	19 (58)						
	3%-12%[p]	19 (58)						
	4%[q]	14 (56)						
	15%[r]	29 (73)						
	4.4%[s]	4 (67)						
B cells	0.03[t]	5 (50)			0.03%	51(59)	60%	28 (72)
	0.7%	8 (69)						
NK cells	2.1[u]	8 (69)	49%		0.2%	51(59)	Small	91 (64)
			58%	91 (64)			populations	

Results expressed as median or mean ± one SP (range) percentage of cells from the whole tumor cellular or as number of cells/mm^2. [a]Percentage for grade II (2%), grade III (4%) and grade IV gliomas (11%), respectively. [b]CD68$^+$CD14$^+$HLADR$^+$ cells. [c]CD45^{bright}and plus CD11b$^+$. [d]CD45^{dim}plus CD11b$^+$. [e] Number of

cells/mm^2 for grade II (130/mm), grade III (221/mm) and grade IV gliomas (472/mm), respectively. [f]M2 marker was CD163 for grade II (23/mm), grade III (69/mm) and grade IV gliomas (287/mm), respectively. [g]M2 marker was CD204 for grade II (49/mm), grade III (138/mm) and grade IV gliomas (368/mm), respectively. [h]CD1c$^+$CD11c$^+$ BDCA-2$^-$ cells. [i]CD1c$^-$CD11C$^-$ BCDA-2$^+$ cells. [j] T cells determined by using CD3+CD56+ T/NK phenotype. [k] Mean of 5% in grade I meningiomas and 0.5% in grade II/III meningiomas.[l]Percentage for grade I (9.5%), grade II (2.4%) and grade III meningiomas (1.8%). [m]Percentage for grade II (9%), grade III (10%) and grade IV gliomas (18%), respectively. [n]Percentage for grade II (1%), grade III (3%) and grade IV gliomas (12%), respectively. [o] mean ±SD for grade II (11%±2%), grade III (16%±2%) and grade IV tumors (25%±3%), respectively. [p]mean ±SD for grade II (3%±0.1%), grade III (7%±0.2%) and grade IV tumors (12%±2%), respectively. [q]Tregs frequency from CD3$^+$ T cells using a CD4$^+$CD25$^+$ phenotype.[r] CD4$^+$CD25$^+$FoxP3$^+$ phenotype used to identify Tregs. [s]FoxP3 used to identify Tregs. [t]MHCII+ CD19+ markers to identify B cells. [u]CD56^{dim}CD16$^-$ markers used to identify NK cells.

In turn, Asai et al.(62) identified 24%±3.7% of CD68-positive macrophages and/or microglial cells in meningiomas, which appeared to be heterogeneous, potentially reflecting various functional states with a different role on the regulation of tumor growth. Similarly, our group applied FCM immunophenotyping to evaluate the cellular composition of meningiomas (Figure 1), our results showing systematic coexistence of CD45$^-$ neoplastic cells and CD45$^+$ immune infiltrating cells; the later included a major population of macrophages with a HLA-DR$^+$CD14$^+$CD45$^+$CD68$^+$CD16$^{-/+}$CD33$^{-/+}$ phenotype and high phagocytic/endocytic activity, together with lymphocytes (mostly T CD8$^+$- and NK-cells) present at lower levels(59).

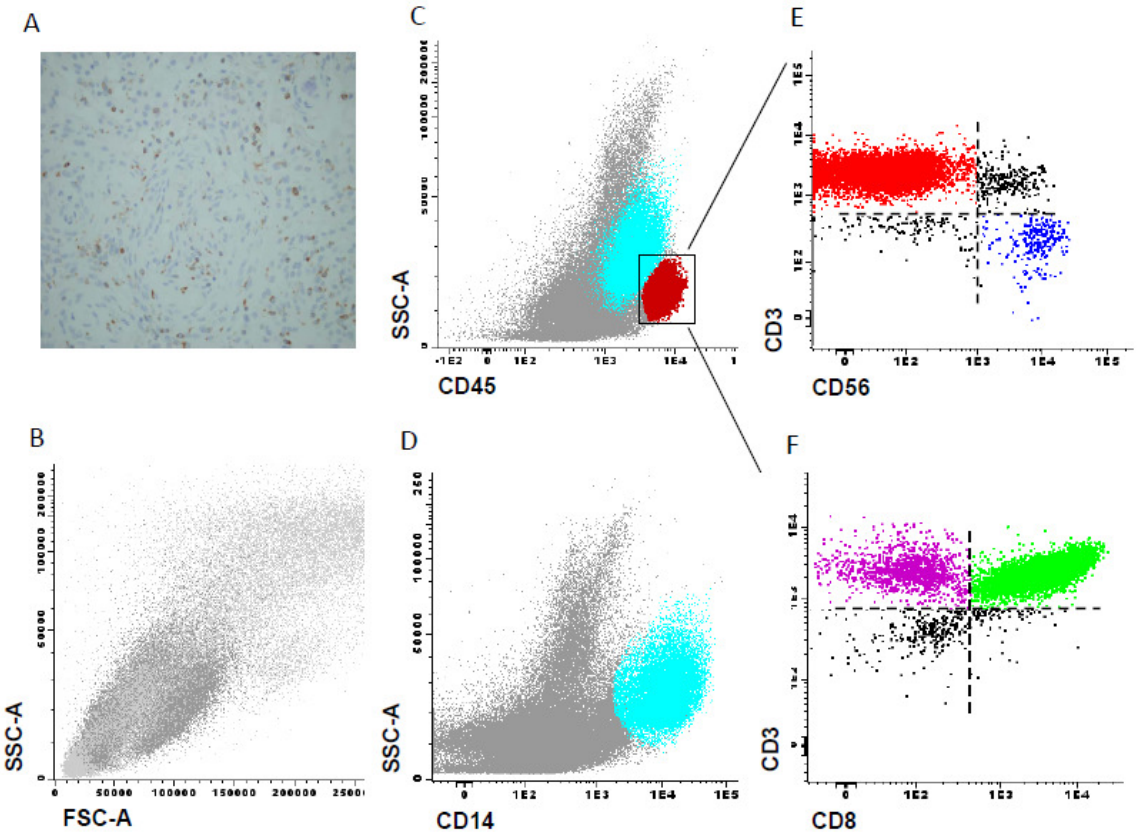

Figure 1. Illustrating example of the different subpopulations of tumor-infiltrating macrophages and lymphocytes ($CD45^{hi}$) in a meningioma carrying monosomy 22. In the upper panels, the whole tumor cellularity as identified by both immunohistochemistry (panel A, staining for CD68 positive macrophages depicted in brown color within the tumor parenchyma) and flow cytometry (panel B) is shown. In panel C, flow cytometry staining for CD45 is illustrated. In panels D to F, the represented bivariate dot plots show the presence of a high percentage of tumor-associated $CD14^{+}$macrophages (blue dots in panel D) and different subsets of $CD3^{+}CD56^{-}$(depicted as red dots in panel E) and $CD3^{+}CD56^{+}$ (identified as black dots in panel E) T-cells. $CD56^{+}$ $CD3^{-}$ NK-cells (depicted as blue dots in panel E) and both the major $CD3^{+}CD8^{+}$ T cytotoxic (green dots) and the $CD3^{+}CD8^{-}CD4^{+}$ T helper (violet dots) subsets are shown in panel F.

At present, it is well known that tumor cells can secrete several factors that might be responsible for the recruitment of microglial cells/macrophages; among

others, these include MCP-1 (CCL2) and MCP-3 (CCL7), the monocytic and/or granulo-monocytic colony stimulating factors (M-CSF and/or GM-CSF), the stromal cell-derived factor (SDF-1α) and the hepatocyte growth factor (HGF)(63, 74-76). Whether myeloid cells infiltrating brain tumor tissues are a cause or a consequence of tumor progression, still remains controversial. Nevertheless, it is tempting to model brain myeloid cells on current concepts about of macrophage plasticity, in which classically activated pro-inflammatory macrophages (M1) may promote anti-tumoral responses, whereas alternatively-activated immunoregulatory (M2) macrophages are predicted to be pro-tumoral. Polarization of TAM towards an M1 or M2 phenotype depends on the cytokine milieu and the local microenvironment (Figure 2).

Classically activated M1 macrophages are induced by IFNγ and/or Toll-like receptor (TLR) ligation through e.g. microbial stimuli/lipopolysaccharide (LPS), as well as by cytokines –TNF-α and GM-CSF– (77). Polarization towards M1 cells is typically observed in the presence of high interleukin 12 (IL-12) and interleukin 23 (IL-23) production, but low IL-10 levels (78); M1 macrophages participate as inducer and effector cells in polarized Th1 responses, through production of pro-inflammatory cytokines, including interleukin 1 (IL-1), TNFα and interleukin 6 (IL-6) (78, 79). In turn, these cells up-regulate nitric oxide synthase 2 (NOS2) expression and thereby, they also produce reactive oxygen and nitrogen species (78). M1 macrophages have antigen presentation capacity, and they mediate innate immune responses against intracellular parasites and tumor cells (7, 8, 80).

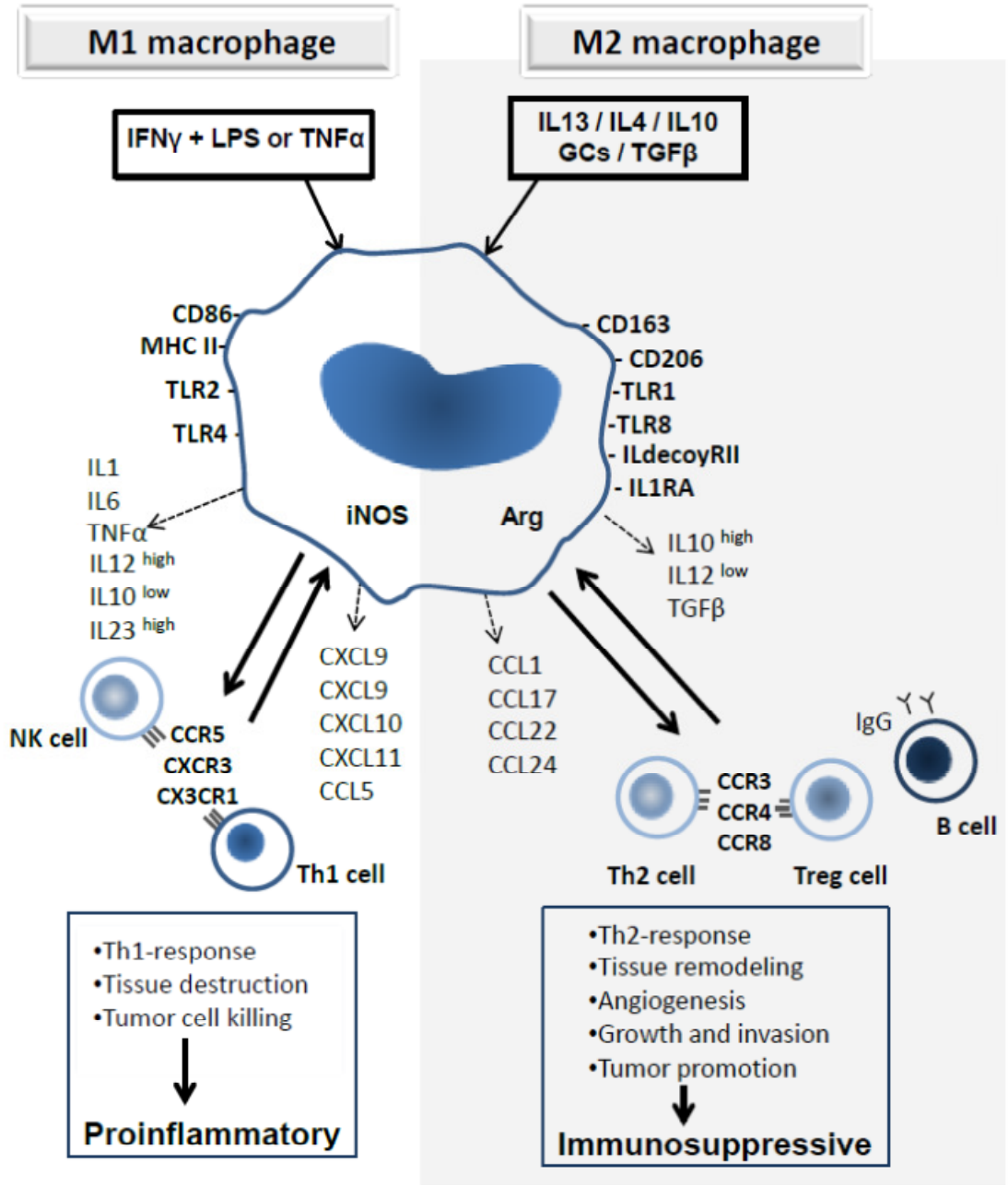

Figure 2. Schematic illustration of the two major populations of polarized tumor-associated macrophages identified in tumor tissues, and their interactions with tumor infiltrating lymphoid cells. Tumor-associated macrophages can have either (beneficial) anti-tumoral or (adverse) pro-tumoral effects, the macrophage functional polarization partially depending on the cellular and tissue microenvironment. The most relevant environment-derived signals and selected functional properties of the two main populations of polarized tissue macrophages, as well as the different cytokines, chemokines, and receptors they produce, are shown. **Abbreviations:** CCL, chemokine

(C-C motif) ligand; EGF, epidermal growth factor; GCSs, glucocorticoid/corticosteroid hormones; IFNγ, interferon-γ; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MMPs, matrix metalloproteases; NK, natural killer; TGFβ, transforming growth factor-β; TLR, Toll-like receptor; TNFα, tumor necrosis factor-α;VEGF, vascular endothelial growth factor.

The designation M2 covers several forms of functionally activated macrophages which differ from the classically activated M1 cells; thus, M2 polarization may be induced through exposure to IL-4, IL-13 and glucocorticoid/corticosteroid hormones(81). M2 macrophages share an IL-12^{low}, IL-23^{low}, IL-10^{high}functional phenotype, in association with variable production of anti-inflammatory cytokines, depending on the signals leading to the activation of these cells (82). In addition, M2 cells have high amounts of scavenger, mannose, and galactose-type receptors (e.g. CD163, CD204 and CD206)(82-84),they show up-regulation of arginase 1 (Arg1) expression and a shift of the arginine metabolism towards production of ornithine and polyamines(77). Furthermore, differential regulation of the production of distinct components of the IL-1 system takes place in polarized macrophages(78), M2 cells being associated with low IL-1α/ IL-1β, high IL-1 receptor antagonist, and high decoy type II receptor levels(78). In general, M2 cells participate in polarized Th2 immune responsesand they are present in established tumors where they promote tumor progression, tissue repair and remodeling(85).Moreover, they are typically associated with lack of cytotoxic activity, through blockade of $CD8^{+}$T-cell infiltration and proliferation, at the same time they display immunoregulatory functions(7, 8, 80). Another major pro-tumoral role of M2 macrophages relates to their effect on promoting angiogenesis through the release of pro-angiogenic growth factors such as

vascular-endothelial growth factor A (VEGF-A), a process which is essential for tumor progression(79).Of note, several products of the polarized TAMs might also affect normal surrounding CNS cells. As an example, IL-1β has been shown to induce production of additional cytokines and growth factors, thereby promoting inflammatory effects in the brain (86).

Despite all the above and the potential utility of the M1/M2 classification, it should be noted that such M1/M2 balance somewhat represents an oversimplification of the functional profiles of TAM, as it does not fully reflect the complexity of macrophage activation, which is often tuned differently in response to distinct tissue microenvironmental conditions(4). In fact, at present it is not entirely clear how macrophages switch phenotypes. Previous studies suggested that hypoxia might be the major factor in mediating the transition from tumor-suppressing to tumor-promoting macrophages(4). Reversion of an M2 back to an M1 phenotype, has also been reported. For example, disruption of nuclear factor kB (NFkB) signaling in an ovarian cancer model resulted in an M2-to-M1 switch, the recruitment of NK cells and subsequent tumor regression(87); similarly, macrophage depolarization from an M2 phenotype by inhibition of colony stimulating factor-1 receptor (CSF-1R) was associated with robust regression of already established high-grade gliomas(88). Altogether, these studies highlight a potential therapeutic opportunity in which re-education of TAM might have a beneficial anti-tumoral effect on the outcome of the disease.

Currently, it is well-known that microglial cells and brain macrophages have the potential to exert anti-tumoral effects *in vitro*(89). In this regard, Galarneau et al.(89) reported that macrophage depletion results in an increased volume of glioma, the

tumoral immune infiltrates reflecting type 1 responses and $CD11b^{+}$ cells being the main source of TNFα in the presence of high levels of MCP-1 and IL-1β, but low levels of IL-4 and IL-10. Overall, these results suggest that the brain is either equipped with or it can recruit, cells with potential to act against brain tumors. However, these functions may be overwhelmed by pro-tumoral elements. Indeed, most studies about the microglial cells and brain macrophages in glioma have shown a pro-tumoral phenotype associated with an immunosuppressive microenvironment and promotion of tumor growth and invasion(90). Therefore, microglia/macrophage glioma infiltrates have been mainly associated with an M2 polarization and expression of M2-associated markers such as CD163, CD204 and CD206, as well as IL-10 and arginase 1 production(48, 55, 65) which in turn, might also affect patient symptoms and behavior(91). In line with this, Hussain et al.(49, 50)investigated the functional profile of myeloid cells isolated from malignant gliomas; their results showed that, despite these cells expressed significant levels of TLRs, they did not secrete pro-inflammatory cytokines (e.g. IL-1β, IL-6, TNFα) and they lacked expression of co-stimulatory molecules (e.g. CD86, CD80, and CD40) which are critical for subsequent T-cell activation; furthermore, they showed that STAT3 signaling might also be involved, as STAT3 inhibition was accompanied by an enhancement of immune responses and up-regulation of several key intracellular signaling molecules that regulate T-cell activation(92). Consequently, these results indicate that blockade of microglia/macrophage infiltration and/or their pro-invasive effects could represent a potentially beneficial therapeutic strategy in malignant gliomas. In contrast, recent results from our group suggest that a different profile could exist in meningiomas(59);

thus, significantly increased levels of infiltration of the tumor by TAM was specifically observed among cases with monosomy 22(59), a good-prognosis cytogenetic subgroup of meningiomas; these TAM typically displayed a CD206-negative M1 antitumoral phenotype.

*MDSC.*One of the most prevalent mechanisms of immune evasion in cancer patients is through the immunosuppressive activity of MDSC. MDSC are an heterogeneous group of immature myeloid cells which may be identified in humans by their unique $CD11b^{+}CD14^{+}CD15^{+}HLA\text{-}DR^{-}CD33^{+}$immunophenotype(93). MDSC are mobilized during tumorigenesis and infiltrate developing tumors, where they promote tumor vascularization and disrupt major mechanisms of immunosurveillance, including antigen presentation by DC, T cell activation, M1 macrophage polarization and NK cell cytotoxicity(94). Presence of MDSC in the immune infiltrates of human brain tumors has not yet been described. However, characterization of MDSC infiltrating the GL261 glioma mouse model has been described in some detail(55); in this mouse glioma model, MDSC show a substantial overlap with TAM, they share phenotypic features of both M1 and M2 polarized macrophages, and they display a significant functional and phenotypic plasticity, depending on the surrounding microenvironment(55). Furthermore, circulating $CD33^{+}HLA\text{-}DR^{-}$ MDSC have been detected in the peripheral blood of GBM patients at greater levels than in healthy donors(95), and healthy donor-derived human $CD14^{+}$monocytes exposed to glioma cells may acquire MDSC-like properties, such us an increased production of immunosuppressive factors (e.g. IL-10,

TGF-β and B7-H1) and an increased ability to induce apoptosis of activated lymphocytes(96).

DC. Another subtype of myeloid cells that may be specifically recruited to brain tumors are the DC. Although the brain does not have a standard lymphatic system like other tissues in the body, the perivascular space has been claimed to potentially act as a route for lymph to drain into the cervical lymph nodes, and thus, to act as a flow channel for the adaptive immune system(36). In glioma, DC have been most thoroughly investigated in the GL261 mouse glioma model. In this animal model, infiltrating ($CD11c^+$) DC have been shown to display little or no expression of costimulatory molecules (CD40, B7.1, B7.2) and they are unable to stimulate T cells, whereas they promote the development of Tregs(97). In humans, analysis of circulating myeloid and plasmacytoid DC in the peripheral blood of patients with glioma has shown decreased numbers *vs.* healthy controls(98). Tyrinova et al.(99) investigated monocyte-derived DC from brain glioma patients generated *in vitro* in the presence of IFNα and GM-CSF, and they found functional impairment of the generated DC suggesting they could be potentially involved in the pathogenesis of the tumor. To the best of our knowledge, no study has investigated so far the presence, distribution and/or functionality of DC in meningiomas.

Lymphoid cells. Tumor-infiltrating lymphocytes (TIL) are also a systematic component of the microenvironment of brain tumors(22), suggesting that these cells may be

critically involved in tumor growth, progression and/or control. Of note, myeloid cells engage in complex bidirectional interactions with the lymphoid cells, in order to exert their function in the tumor microenvironment(80). $CD45^{high}$TILhave been found to represent a few percent of all cells in both glioma (e.g. 2.5%)(30) and meningioma (e.g. 1.4±1.5%)(57, 59) and they usually consist of T-cells and to a less extent also, NK cells and B lymphocytes(59).

*T-cells.*T lymphocytes ($CD3^+$)fall into two major broad functional categories: $CD4^+$ T helper (Th) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs) in addition to Tregs. Both $CD4^+$ and $CD8^+$ T cells have been described to infiltrate brain malignancies, such as meningioma(59) and glioma(57). Several studies have further analyzed the relationship between T cell infiltration and patient outcome, with controversial results (100). One potential explanation for the controversial results may be the distinct role played by the different T-cell subsets infiltrating the tumor. Usually, high levels of $CD8^+$ CTLs are related to a greater anti-tumoral activity, whereas high levels of $CD4^+$ Th cells(particularly some subsets of Th cells) are viewed as being associated with a role in favoring tumor development(18). In line with this, Yu et al.(57) reported a high $CD8^+/CD4^+$ T-cell ratio in primary brain tumors to be associated with less aggressive disease, and others have associated greater levels of infiltration by $CD8^+$ T-cells with a longer survival (67, 69, 71). Similarly, we have found increased numbers of activated cytotoxic $CD8^+$ T-cells in meningiomas to be particularly increased in meningiomas with complex karyotypes and a poorer outcome(59).

$CD4^+$ Th cells (e.g. Th1, Th2, Th17 and Tregs) deserve a specific comment since these cells seem to play a very important role in regulating the phenotype of TAM. In this regard, Th1 cells can drive classical M1 polarization of macrophages through production of IFNγ, while Th2 cell-derived IL-4 and IL-13 direct M2 polarization of macrophages. In turn, IL-4-activated macrophages express chemokines such as CCL17, CCL22 and CCL24, whose specific receptors (CCR4 and CCR3) are expressed by Th2 cells (80). Of note, analysis of the activation profile of TIL in malignant glioma has shown predominance of type 2 immune responses in the intratumoral microenvironment, in association with expression of Th2-type cytokines (e.g. IL-4 and IL-10); these findings might contribute to explain the 'immunosuppressive microenvironmental status' of these tumors (101). In order to investigate the Th1/Th2 balance in different types of brain tumors, Kumar et al. (102) analyzed IL-12 (a cytokine related to Th1 responses) and IL-10 (another cytokine related to Th2 responses) serum levels in patients with meningioma, anaplastic astrocytoma and GBM; overall, these authors found a significant reduction in serum IL-12 together with an increase in serum IL-10 in patients *vs.* controls. Of note such balance was much closer to normal values among meningioma (IL-10 levels among meningioma patients were similar to those of the controls), vs glioma patients, suggesting a less predominant type 2 immune response in the former patient group. In line with these observations, Shimato et al. (103) have recently reported on the *in vitro* production of IFNγ (Th1) and IL-5 (Th2) by freshly-isolated, *in vitro* stimulated, peripheral blood mononuclear cells (PBMC) from patients with GBM and meningioma; overall, both patient groups showed a modest decrease in the amount of secreted IFNγ (vs healthy subjects), while a significant elevation of IL-5

levels was found only for recurrent GBM patients. Consequently, when the IFNγ/IL-5 cytokine ratio was considered, no predominant Th1 or Th2 bias was found among meningioma patients, while patients with both primary and recurrent GBM exhibited a significantly decreased IFNγ/IL-5 ratio in favor of the predominance of Th2 immune responses.

In recent years, several reports have specifically investigated the presence of $CD4^{+}CD25^{high}FOXP3^{+}CD127^{low}$ regulatory T cells (Tregs) in brain tumors, since these cells have been shown to play an important role in the regulation of immune responses via suppression of proliferation of other T cells present in the tumor microenvironment, through either direct cell-to-cell contact-dependent mechanisms or indirectly by IL-10 and TGFβ secretion(18). Thus, Tregs have been shown to infiltrate both primary and metastatic brain tumors(73). Overall, results from our group and other research groups showed almost no accumulation of Tregs in meningiomas, while GBM and metastatic brain tumors displayed massive infiltration by regulatory T-cells(56, 59, 73). However, Waziri et al.(56)found infiltration by total $CD3^{+}$Tcells and Tregs in meningiomas to be of 1.7% ± 0.7% and 0.6% ± 0.2%, respectively, with a unique cytokine production profile associated with high IFNγ and low IL-4/IL-13 and IL-10 cytokine expression levels. Despite these findings, Tregs infiltrating brain tumors have been shown to be fully activated and to strongly suppress proliferation and cytokine production by TIL, thereby contributing to a more aggressive clinical behavior of high-grade brain tumors (73). In this regard, a strong correlation has been reported in GBM between immunosuppression and presence of Tregs in the tumor microenvironment, and tumor infiltration by Tregs has also been shown to correlate

with tumor grade(58); in addition, depletion of Tregs has been associated in animal models with prolonged survival and infiltration by non-immunosuppressive myeloid cells(104).In the tumor microenvironment, production of specific chemokines (e.g. CCL22) and cytokines (e.g. TGFβ) appears to be associated with preferential recruitment of Tregs and promotion of tumorigenesis(7, 80). In this regard, tumor infiltrating Tregs can also affect the function of TAM in the tumor microenvironment by favoring polarization towards an M2 suppressive phenotype (80).

Natural killer (NK) cells. NK cells consist of cytotoxic effector lymphocytes that play an important role in anti-tumoral innate immune responses through e.g. apoptotic killing of tumor cells(18). NK cells exert their effects via two major cytotoxic pathways. On one side, NK-cells are rich in perforin- and granzyme-containing granules, that once released, lead to the damage of the cytoplasmic membrane of targeted cells, entry of NK-cell released proteins in such cells and their subsequent death by apoptosis; on the other hand, they constitutively express the CD95-ligand and TNFα on the cell surface which bind to apoptotic receptors on the target cells, also leading to their death by apoptosis. In addition, NK cells secrete a variety of cytokines and chemokines (e.g. IFNγ), which exert immunomodulatory effects such as priming of Th1-biased T-cell responses and classical M1 polarization of macrophages(80). Thus, NK cell infiltration into tumors has been associated with a more pronounced anti-tumor effect, more favorable cytogenetics and a better patient outcome(3, 105). However, it should be noted that in many studies NK cells have been identified using CD57 or CD56 and both phenotypic markers despite being characteristic of NK cells, are not specific NK-cell

markers; in fact, NK cells should be better characterized as $CD3^-CD56^+$ and/or $CD3^-$ $CD57^+$ cells, after excluding $CD56^+/CD3^+$ and $CD57^+/CD3^+$ T-cells(59, 106). In brain tumors, the tumor-suppressing role of NK cells has been demonstrated both *in vitro*(107) and *in vivo*(108). Moreover, these cells have been identified in both primary (e.g. meningiomas and gliomas) and metastatic brain neoplasms(59, 64, 69). However, the level of tumor infiltration by NK cells tends to remain low and the functionality of such cells is often affected by factors released by the tumor and/or other immunosuppressive cells(106). As an example, TGF-β secreted locally by tumor cells and other infiltrating cells down-regulates the expression of the NKG2D activating receptor on NK cells isolated from glioblastoma patients, at significantly more pronounced levels than in NK cells from meningioma patients(109).

*B cells***.** The specific role of B-lymphocytes in the development of brain tumors remains unclear. Some reports identified B cell infiltration in meningiomas(59, 72, 110)and gliomas(64). However, such B cells only represented a minor fraction of the immune cell infiltrates and they were restricted to a subset of these tumors(59). In other types of cancer, tumor-infiltrating B cells have been associated with the recognition of a wide variety of tumor antigens, and they have been claimed to closely interact with T cells and other immune cells, in association with a more favorable outcome(111). In this regard, B cells can indeed act as APC and therefore, they may be relevant for inducing $CD4^+$T cell-dependent $CD8^+$memory T cells that help to control tumor invasion, spread and metastasis(111). In a GBM model, Candolfi et al.(112) showed that B cells can act as APC for T-cells and potentially play a critical role in T-cell-mediated

antitumor immunity and T cell-dependent tumor regression within the CNS. Similarly, a recent report on meningioma-infiltrating B cells provided clear evidence for the presence of antigen-experienced B-lymphocytes in the tumor microenvironment(72). However, presence of tumor infiltrating B cells may have a paradoxical effect, as some reports also found B cells to suppress the development of immune responses in some tumors, and to directly regulate macrophage effector functions through IL-10 production, which may activate an M2 macrophage phenotype and promote tumorigenesis(80).

The major overall profiles of cellular immune infiltrates that have been reported in glioma vs meningioma tumor samples are summarized in Table 4.

Table 4. Different cellular immune infiltrate profiles reported in glioma and meningioma tumors.

Tumor type	Myeloid cell infiltrate cell numbers		Lymphoid cell infiltrate cell numbers			
	TAM/microglia	Dendritic cells	T cells		B cells	NK cells
			$CD8^+$ T cells	$CD4^+$ T cells		
Glioma	low to high	low	low to high	low to high	low	intermediate
Meningioma	high	-	low to Intermediate	-	low	low

low: 0-1% of cells in the tumor; intermediate: 1-20% of cells in the tumor; high: 20-30% of cells in the tumor

Immunotherapeutic approaches for CNS tumors

The invasive nature of the tumor, together with its capacity to infiltrate into adjacent normal brain tissue in most gliomas and also a small fraction of meningiomas

makes it virtually impossible to completely resect the tumor in every patient. Therefore, usage of complementary/alternative therapies including immunotherapy protocols is being evaluated in addition to surgery, for the treatment of human CNS tumors. Immunotherapy typically takes advantage of the immune system's ability to specifically recognize tumor cell markers and to respond against the tumor cells, while leaving the normal brain tissue intact. At present, several clinical trials are ongoing in which the safety and efficacy of immunotherapy is evaluated in the clinical setting of CNS glioma, and to a less extent also, meningioma (Table 5).

*Monoclonal antibody-based therapy***.** Despite being protected by both the blood–brain and blood–tumor barriers, a significant fraction of CNS tumors are actively infiltrated by immune cells. In recent years, an increasing number of mAb directed against surface receptors expressed by immune cells, which mediate T cell inhibition upon binding to its ligand have been developed and evaluated particularly in high-grade gliomas such as anti-PD1 and anti-CTLA4 antibodies. PD-1 (CD279), a member of the CD28 signaling receptors, is expressed on activated T cells, B cells, DC, and macrophages. Signaling through PD-1 induces functional or inhibition of immune cells through two distinct ligands: B7-H1 (CD274 or PDL1) and B7-DC (CD273 or PDL2). The B7-H1/PD1 interactions induces a negative regulation of T cell activity and tunes down inflammatory immune responses (113). This represents the basis for the anti-PD1 signalling pathway immunotherapy. In line with this, indoleamine 2, 3 dioxygenase 1 (IDO, a tryptophan catabolic enzyme) is not usually expressed at relevant levels in the CNS parenchyma. However, higher IDO expression is observed in GBM, suggesting that

this metabolite may play a role in suppressing the antitumor immune response via e.g. programmed death 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4 mediated signaling to cytotoxic T-cells and other immune effector cells. The accumulation of $CD4^{+}CD25^{+}FoxP3^{+}$ Tregs, as well as the interaction between T-cells expressing CTLA-4 and $CD80^{+}$ DC and between PDL1 expressing cells and/or macrophages and PD1 T-cells may lead to the escape of tumor cells from the immune response(114).

Other monoclonal antibody-based therapies which are currently under evaluation include usage of antibodies directed against: 1)the anti-epidermal growth factor receptor (EGFR) and/or its variant III (EGFRvIII) such as cetuximab (C225, IMC-C225, Erbitux^{TM}), and the 806, 528, nimotuzumab and panitumumab (Vectibix, Amgen, Thousand Oaks, CA, USA) antibodies, and; 2)anti-VEGF (bevacizumab)antibodies which have also been tested in gliomas (115-118). Cetuximab binds to EGFR on the tumor cell; in vitro binding of the antibody is followed by internalization of the receptor, reduction of EGFRvIII phosphorylation and inhibition of cell proliferation(117). Of note, the ligand-independent EGFRvIII mutant is present in a substantial fraction of all GBM; in recent years several monoclonal antibodies (MAb) have been produced which target the EGFRvIII mutated protein in the absence of cross-reactivity with the wild type EGFR protein (e.g. the Y10 and L8A4 antibodies); such MAb have been shown to induce an increased survival of mice with intracranial glioma tumors (119, 120). Another antibody clone with promising results that binds to EGFRvIII expressing cells, as well as to a small proportion of wild type-EGFR expressing tumor cells that show dysregulated signaling due to overexpression of this receptor(120)or to the presence of an autocrine

loop due to increased production of EGF and EGFR, is the 806MAb clone (121). Finally, Herceptin, an anti-HER2 antibody used for the treatment of other tumors such as breast cancer, has also been shown to mediate cell death of HER2–expressing glioblastoma cell lines in vitro; however, the in vivo effects of Herceptin against intra-CNS tumors is almost null, probably due to an impaired uptake of the drug in the CNS (122).

Anti-tumoral vaccination. In parallel to the development of MAb-based therapies, a peptide vaccine (rindopepimut) based on a the peptide sequence that encompasses the mutated segment of the EGFRvIII mutant protein, has also been developed and demonstrated to induce cytotoxic responses against malignant gliomas in preclinical models and phase I/II clinical trials (123, 124); based on these results, a phase II/III trial is currently ongoing in GBM patients, in which vaccination against EGFRvIII is used in combination with radiotherapyand temozolomide therapy. In addition, for patients with newly-diagnosed tumors that contain this specific EGFR mutation, a peptide vaccine that encompasses the mutated segment of EGFRvIII plus heat shock protein(HSP)-tumor peptides that display immunogenic properties and act as potent activators of antigen-presenting cells (e.g. Gp96 and HSP70 tumor-associated peptides), has also been recently produced(125). The development of DC-based antitumor vaccines as a way to promote the immune system to recognize and eliminate malignant cells has also emerged as a promising therapeutic strategy. Preclinical studies demonstrate that vaccination with DC pulsed with glioma antigens (glioblastoma lysates) can prime a tumor specific cytotoxic lymphocyte response;

encouraging results have emerged from phase I and II clinical trials using DC immunotherapeutic strategies and multiple other clinical trials are under way (126).

Cytokine-based therapy. Cytokines are potential therapeutic tools for malignant gliomas because of their immunomodulatory effects. Overall, two main study subtypes have been developed in which cytokine-based therapies are used. Thus, while some studies focused on supplementing immuno-activating cytokines such as interleukin-2 (IL2) and interleukin-4(IL-4), others have attempted to target the production, or to interfere with the effects, of immunosuppressive cytokines such as TGFβ.

Intratumoral injection of IL-2 in combination with a herpes simplex virus tyrosine kinase inserted in a retroviral vector, or IL-2 infusion in combination with cytotoxic T cells, have both emerged as potentially beneficial strategies for the treatment of recurrent glioma patients. Despite this, it should be noted that therapeutic levels of intravenously administrated IL-2 have shown a variable grade of toxicity including fever, headache and transient neurologic irritation; furthermore, patients receiving cytokines, mainly IL-2 and interferon-alpha (IFN-α), for the treatment of cancer frequently develop depressive symptoms(127). In this regard, several studies have demonstrated a strong relationship between decreases in the peripheral levels of tryptophan, the amino acid precursor of serotonin, and the development and intensity of the depressive symptoms observed in cancer patients receiving such therapies, including neurovegetative somatic symptoms, depressed mood, anxiety and cognitive impairment(128). Of note, such symptoms are mainly related to immunotherapy rather than to the disease being treated, and they are

usually alleviated by antidepressant treatment(129).In contrast, the incidence and severity of these adverse events after local administration of IL2 directly in the tumor region, is significantly lower; practical guidelines for safe and effective IL-2 administration and management of toxicity have been agreed upon and they are currently available(130).

Despite all the above, usage of an IL-4-based cytokine therapy to specifically target tumor cells, currently appears to represent the most (clinically) viable cytokine-based therapy. Thus, such IL-4 cytotoxin-based therapy [a recombinant fusion protein consisting of human IL-4 and a truncated *Pseudomonas* exotoxin termed IL4(38-37)-PE38KDEL, or IL-4 cytotoxin] has been demonstrated to cause tumor necrosis without damage to the normal brain parenchyma (131). Similarly, a chimeric fusion protein composed of human interleukin-13(IL-13) fused to a truncated, mutated form of *Pseudomonasaeruginosa* exotoxin A (PE38QQR) [IL13-PE38QQR, cintredekin besudotox) has also been developed and shown to have a specific cytotoxic effect on glioma cell lines (132). However, recent studies have failed to demonstrate a survival benefit after administration of this cytokine conjugate; further clinical studies are required to understand the in vivo mechanisms involved in the failure of this therapy(133-135). Other cytokine/cytokine receptor targets for the treatment of gliomas include IL-13Rα2. IL-13Rα2 is a cytokine receptor which is highly expressed in glioma cells and that has been investigated as a potential target for immune cell activation since it contains an immunogenic peptide that induces IFNγ secretion and activation of $CD8^+$ T cells directed against IL-13R$\alpha 2^+$ tumor cells(136). Despite this, the

role of IL-13Rα2 in glioma cells remains to be defined and the potential benefit of an in vivo IL-13Rα2-based therapy still deserves further investigations.

Other, cytokines such as IFNα have also been evaluated for the treatment of high-grade glioma patients; however, either no clear benefit or inconclusive results have been obtained with them (e.g. INFα in combination with BCNU), as regards both tumor response and patient survival (137, 138). In turn, TGF-β2, a cytokine that promotes glioma invasion, angiogenesis, and immunosuppression, has also been evaluated as a potential target for GBM therapy. SB-431542, a novel small-molecule that inhibits the TGFβ receptor, has been shown to prevent glioma growth in preclinical trials (139). Similarly, the AP12009, TGF-β2 antisense oligonucleotide, has also been associated with some antitumoral activity, its administration providing to be safe in early clinical trials(140).

Table 5. Immunotherapeutic strategies in human glioma and meningioma.

Tumor	**Therapy**	**Agent**	**Target**	**Clinical trial phase**
Glioma	MAbs	Anti-PD-1	PD-1	III
		Ipilimumab	CTLA-4	III
		Nimotuzumab	EGFR	II, III
		Panitumumab	EGFR	II
		Cetuximab	EGFR, EGFRvIII	I, II
		mAb 806	EGFR, EGFRvIII	I
		Trastuzumab	Her2	I, II
		Bevacizumab	VEGF	I, II, III
		Ramucirumab	VEGFR	II
	Vaccination	DC	Tumor cells	I, II
		Rindopepimut	Tumor cells	I, II, III
	Cytokines	IL-2	T cells	I, II

		IL-4(38-37)-PE38KDEL	Tumor cells	I
		IL13-PE38QQR	Tumor cells	I, II, III
		IL-13Rα2	$TCD8^+$ / Tumor cells	I
		AP12009	TGF-β	II, III
		SB-431542	TGF-βR	Preclinical*
		INF-α	Tumor cells	III
Meningioma	MAbs	Bevacizumab	VEGF	II
	Cytokines	IFN-α-2B	Tumor cells	II

MAb: monoclonal antibody. *: Hjelmeland et al 2004.

Concluding remarks

At present it is well-established that tumor-infiltrating immune cells play a very important role in tumor development and control. Current efforts focused on the identification and characterization of those immune cells present within the tumor have brought significant insight into the understanding of their effects on tumor behavior, at the same time they have opened new therapeutic pathways(141, 142). However, individual tumors may show unique immune profiles which can only be fully understood based on the functional assessment of the infiltrating cells and their numbers. Thus, several reports have shown the presence of tumor infiltrates by immune cells in both gliomas and meningiomas. Although such infiltrates may vary substantially in their numbers, macrophages/microglial cells typically predominate over TIL; in turn, among the TIL, T-cells and particularly $CD8^+$ T-cells most frequently predominate, in association or not with NK-cells, and to a less extent also, B-lymphocytes. Of note, while the presence of immune infiltrates was initially viewed as being a sign of immune control, today it is well known that infiltration by M2-polarized

macrophages and Tregs is associated with recurrence and a poorer prognosis, as well as failure of anti-tumor vaccination after surgery(143). Thus, there is still a long way to go until we have a clear view of all the different functional subsets of cells present in the tumor microenvironment, their precise functions, and how they interact with each other, as well as with the tumor cells and the tumor stroma itself and the surrounding CNS-tissue cells, potentially contributing also to modulate the neuropsychiatric symptoms of the disease and the changes in patient behavior.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

This work was partially supported by the following grants: PIC/IC/83108/2007 from the Fundação para a Ciência e Tecnologia (FCT, Portugal);RD12/0036/0048 from the Instituto de Salud Carlos III (ISCIII/FEDER), Ministerio de Sanidad y Consumo, Madrid, (Spain);GRS689/A/11 & GRS909A14 from the Consejeria Sanidad, Junta de Castilla y León (Spain) and;IB1405 from the Instituto Biosanitario de Salamanca (IBSAL), Salamanca(Spain).Patrícia Domingues was supported by a grant (SFRH/BD/64799/2009) from FCT and Maria Dolores Tabernero is supported by the Instituto de Estudios Ciencias de la Salud de Castilla y León (IECSCYL-IBSAL), Soria (Spain).

References

1. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene 2008;27:5904-12.

2. Taphoorn MJ, Klein M. Cognitive deficits in adult patients with brain tumours. Lancet Neurol 2004;3:159-68.

3. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 2012;12:298-306.

4. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med 2013;19:1423-37.

5. Pages F, Kirilovsky A, Mlecnik B*, et al.* In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. J Clin Oncol 2009;27:5944-51.

6. Mahmoud SM, Paish EC, Powe DG*, et al.* Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 2011;29:1949-55.

7. Shiao SL, Ganesan AP, Rugo HS, Coussens LM. Immune microenvironments in solid tumors: new targets for therapy. Genes Dev 2011;25:2559-72.

8. Mantovani A, Romero P, Palucka AK, Marincola FM. Tumour immunity: effector response to tumour and role of the microenvironment. Lancet 2008;371:771-83.

9. Yuile P, Dent O, Cook R, Biggs M, Little N. Survival of glioblastoma patients related to presenting symptoms, brain site and treatment variables. J Clin Neurosci 2006;13:747-51.

10. Cheema FA, Badr A, Iqbal J. Glioblastoma multiforme presenting as treatment-resistant depression. The Journal of neuropsychiatry and clinical neurosciences 2010;22:123 E26.

11. Bunevicius A, Deltuva VP, Deltuviene D, Tamasauskas A, Bunevicius R. Brain lesions manifesting as psychiatric disorders: eight cases. CNS spectrums 2008;13:950-8.

12. Gehring K, Sitskoorn MM, Gundy CM*, et al.* Cognitive rehabilitation in patients with gliomas: a randomized, controlled trial. J Clin Oncol 2009;27:3712-22.

13. Louis DN, Ohgaki H, Wiestler OD*, et al.* The 2007 WHO classification of tumours of the central nervous system. Acta neuropathologica 2007;114:97-109.

14. Fathi AR, Roelcke U. Meningioma. Current neurology and neuroscience reports 2013;13:337.

15. Domingues PH, Sousa P, Otero A*, et al.*Proposal for a new risk stratification classification for meningioma based on patient age, WHO tumor grade, size, localization, and karyotype. Neuro-oncology 2014;16:735-47.

16. Huang Y, Hoffman C, Rajappa P*, et al.* Oligodendrocyte progenitor cells promote neovascularization in glioma by disrupting the blood-brain barrier. Cancer research 2014;74:1011-21.

17. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. Neurobiology of disease 2010;37:13-25.

18. Hamilton A, Sibson NR. Role of the systemic immune system in brain metastasis. Mol Cell Neurosci 2013;53:42-51.

19. Wainwright DA, Nigam P, Thaci B, Dey M, Lesniak MS. Recent developments on immunotherapy for brain cancer. Expert Opin Emerg Drugs 2012;17:181-202.

20. Louveau A, Smirnov I, Keyes TJ*, et al.* Structural and functional features of central nervous system lymphatic vessels. Nature 2015.

21. Guillemin GJ, Brew BJ. Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. J Leukoc Biol 2004;75:388-97.

22. Johnson TS, Munn DH, Maria BL. Modulation of tumor tolerance in primary central nervous system malignancies. Clinical & developmental immunology 2012;2012:937253.

23. Ousman SS, Kubes P. Immune surveillance in the central nervous system. Nat Neurosci 2012;15:1096-101.

24. Lossinsky AS, Shivers RR. Structural pathways for macromolecular and cellular transport across the blood-brain barrier during inflammatory conditions. . Histol Histopathol 2004;19:535-64.

25. Serrats J, Schiltz JC, Garcia-Bueno B*, et al.* Dual roles for perivascular macrophages in immune-to-brain signaling. Neuron 2010;65:94-106.

26. Ford E, Catt S, Chalmers A, Fallowfield L. Systematic review of supportive care needs in patients with primary malignant brain tumors. Neuro-oncology 2012;14:392-404.

27. Gregg N, Arber A, Ashkan K*, et al.* Neurobehavioural changes in patients following brain tumour: patients and relatives perspective. Support Care Cancer 2014;22:2965-72.

28. Derecki NC, Katzmarski N, Kipnis J, Meyer-Luehmann M. Microglia as a critical player in both developmental and late-life CNS pathologies. Acta neuropathologica 2014;128:333-45.

29. Prinz M, Tay TL, Wolf Y, Jung S. Microglia: unique and common features with other tissue macrophages. Acta neuropathologica 2014;128:319-31.

30. Parney IF, Waldron JS, Parsa AT. Flow cytometry and in vitro analysis of human glioma-associated macrophages. Laboratory investigation. J Neurosurg 2009;110:572-82.

31. Audoy-Remus J, Richard JF, Soulet D*, et al.* Rod-Shaped monocytes patrol the brain vasculature and give rise to perivascular macrophages under the influence of proinflammatory cytokines and angiopoietin-2. J Neurosci 2008;28:10187-99.

32. Italiani P, Boraschi D. From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. Frontiers in immunology 2014;5:514.

33. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. Physiol Rev 2011;91:461-553.

34. Yang I, Han SJ, Kaur G, Crane C, Parsa AT. The role of microglia in central nervous system immunity and glioma immunology. J Clin Neurosci 2010;17:6-10.

35. Romo-Gonzalez T, Chavarria A, Perez HJ. Central nervous system: a modified immune surveillance circuit? Brain, behavior, and immunity 2012;26:823-9.

36. D'Agostino PM, Gottfried-Blackmore A, Anandasabapathy N, Bulloch K. Brain dendritic cells: biology and pathology. Acta neuropathologica 2012;124:599-614.

37. Kowarik MC, Grummel V, Wemlinger S*, et al.* Immune cell subtyping in the cerebrospinal fluid of patients with neurological diseases. Journal of neurology 2014;261:130-43.

38. Quijano S, Lopez A, Manuel Sancho J*, et al.* Identification of leptomeningeal disease in aggressive B-cell non-Hodgkin's lymphoma: improved sensitivity of flow cytometry. J Clin Oncol 2009;27:1462-9.

39. van Dongen JJ, Lhermitte L, Bottcher S*, et al.* EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012;26:1908-75.

40. de Graaf MT, de Jongste AH, Kraan J*, et al.*Flow cytometric characterization of cerebrospinal fluid cells. Cytometry B Clin Cytom 2011;80:271-81.

41. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008;9:46-56.

42. Backer-Grondahl T, Moen BH, Arnli MB, Torseth K, Torp SH. Immunohistochemical characterization of brain-invasive meningiomas. International journal of clinical and experimental pathology 2014;7:7206-19.

43. Matos LL, Trufelli DC, de Matos MG, da Silva Pinhal MA. Immunohistochemistry as an important tool in biomarkers detection and clinical practice. Biomarker insights 2010;5:9-20.

44. Kakar S, Puangsuvan N, Stevens JM*, et al.* HER-2/neu assessment in breast cancer by immunohistochemistry and fluorescence in situ hybridization: comparison of results and correlation with survival. Mol Diagn 2000;5:199-207.

45. Edin S, Wikberg ML, Dahlin AM*, et al.* The distribution of macrophages with a M1 or M2 phenotype in relation to prognosis and the molecular characteristics of colorectal cancer. PloS one 2012;7:e47045.

46. Chang Q, Hedley D. Emerging applications of flow cytometry in solid tumor biology. Methods 2012;57:359-67.

47. Legres LG, Janin A, Masselon C, Bertheau P. Beyond laser microdissection technology: follow the yellow brick road for cancer research. Am J Cancer Res 2014;4:1-28.

48. Gabrusiewicz K, Ellert-Miklaszewska A, Lipko M*, et al.* Characteristics of the alternative phenotype of microglia/macrophages and its modulation in experimental gliomas. PloS one 2011;6:e23902.

49. Hussain SF, Yang D, Suki D*, et al.* The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. Neuro-oncology 2006;8:261-79.

50. Hussain SF, Yang D, Suki D, Grimm E, Heimberger AB. Innate immune functions of microglia isolated from human glioma patients. J Transl Med 2006;4:15.

51. Badie B, Schartner JM. Flow cytometric characterization of tumor-associated macrophages in experimental gliomas. Neurosurgery 2000;46:957-61; discussion 61-2.

52. Schartner JM, Hagar AR, Van Handel M*, et al.* Impaired capacity for upregulation of MHC class II in tumor-associated microglia. Glia 2005;51:279-85.

53. Bloch O, Crane CA, Kaur R*, et al.* Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. Clin Cancer Res 2013.

54. Badie B, Schartner J, Prabakaran S, Paul J, Vorpahl J. Expression of Fas ligand by microglia: possible role in glioma immune evasion. J Neuroimmunol 2001;120:19-24.

55. Umemura N, Saio M, Suwa T*, et al.*Tumor-infiltrating myeloid-derived suppressor cells are pleiotropic-inflamed monocytes/macrophages that bear M1- and M2-type characteristics. J Leukoc Biol 2008;83:1136-44.

56. Waziri A, Killory B, Ogden AT*, et al.* Preferential in situ CD4+CD56+ T cell activation and expansion within human glioblastoma. J Immunol 2008;180:7673-80.

57. Yu JS, Lee PK, Ehtesham M*, et al.* Intratumoral T cell subset ratios and Fas ligand expression on brain tumor endothelium. Journal of neuro-oncology 2003;64:55-61.

58. El Andaloussi A, Lesniak MS. CD4+ CD25+ FoxP3+ T-cell infiltration and heme oxygenase-1 expression correlate with tumor grade in human gliomas. Journal of neuro-oncology 2007;83:145-52.

59. Domingues PH, Teodosio C, Ortiz J*, et al.* Immunophenotypic identification and characterization of tumor cells and infiltrating cell populations in meningiomas. Am J Pathol 2012;181:1749-61.

60. Grund S, Schittenhelm J, Roser F*, et al.* The microglial/macrophagic response at the tumour-brain border of invasive meningiomas. Neuropathology and applied neurobiology 2009;35:82-8.

61. Strik HM, Stoll M, Meyermann R. Immune cell infiltration of intrinsic and metastatic intracranial tumours. Anticancer research 2004;24:37-42.

62. Asai J, Suzuki R, Fujimoto T*, et al.* Fluorescence automatic cell sorter and immunohistochemical investigation of CD68-positive cells in meningioma. Clin Neurol Neurosurg 1999;101:229-34.

63. Okada M, Saio M, Kito Y*, et al.*Tumor-associated macrophage/microglia infiltration in human gliomas is correlated with MCP-3, but not MCP-1. International journal of oncology 2009;34:1621-7.

64. Yang I, Han SJ, Sughrue ME, Tihan T, Parsa AT. Immune cell infiltrate differences in pilocytic astrocytoma and glioblastoma: evidence of distinct immunological microenvironments that reflect tumor biology. J Neurosurg 2011;115:505-11.

65. Komohara Y, Ohnishi K, Kuratsu J, Takeya M. Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas. J Pathol 2008;216:15-24.

66. El Andaloussi A, Lesniak MS. An increase in CD4+CD25+FOXP3+ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. Neuro-oncology 2006;8:234-43.

67. Lohr J, Ratliff T, Huppertz A*, et al.* Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-beta. Clin Cancer Res 2011;17:4296-308.

68. Roessler K, Dietrich W, Kitz K. Expression of BCL-2 oncoprotein on tumor cells and tumor-infiltrating lymphocytes (TIL) in meningiomas. Neurosurg Rev 1999;22:205-9.

69. Kmiecik J, Poli A, Brons NH*, et al.* Elevated CD3+ and CD8+ tumor-infiltrating immune cells correlate with prolonged survival in glioblastoma patients despite integrated immunosuppressive mechanisms in the tumor microenvironment and at the systemic level. J Neuroimmunol 2013;264:71-83.

70. Heimberger AB, Abou-Ghazal M, Reina-Ortiz C*, et al.* Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas. Clin Cancer Res 2008;14:5166-72.

71. Kim YH, Jung TY, Jung S*, et al.* Tumour-infiltrating T-cell subpopulations in glioblastomas. Br J Neurosurg 2012;26:21-7.

72. Fang L, Lowther DE, Meizlish ML*, et al.* The immune cell infiltrate populating meningiomas is composed of mature, antigen-experienced T and B cells. Neuro-oncology 2013;15:1479-90.

73. Jacobs JF, Idema AJ, Bol KF*, et al.* Regulatory T cells and the PD-L1/PD-1 pathway mediate immune suppression in malignant human brain tumors. Neuro-oncology 2009;11:394-402.

74. Sielska M, Przanowski P, Wylot B*, et al.* Distinct roles of CSF family cytokines in macrophage infiltration and activation in glioma progression and injury response. J Pathol 2013;230:310-21.

75. Braun B, Lange M, Oeckler R, Mueller MM. Expression of G-CSF and GM-CSF in human meningiomas correlates with increased tumor proliferation and vascularization. Journal of neuro-oncology 2004;68:131-40.

76. Wang X, Li C, Chen Y*, et al.* Hypoxia enhances CXCR4 expression favoring microglia migration via HIF-1alpha activation. Biochem Biophys Res Commun 2008;371:283-8.

77. Sharda DR, Yu S, Ray M*, et al.* Regulation of macrophage arginase expression and tumor growth by the Ron receptor tyrosine kinase. J Immunol 2011;187:2181-92.

78. Dinarello CA. Blocking IL-1 in systemic inflammation. The Journal of experimental medicine 2005;201:1355-9.

79. Hao NB, Lu MH, Fan YH*, et al.* Macrophages in tumor microenvironments and the progression of tumors. Clinical & developmental immunology 2012;2012:948098.

80. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nature immunology 2010;11:889-96.

81. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature 2013;496:445-55.

82. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. Immunity 2005;23:344-6.

83. Davies LC, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. Nature immunology 2013;14:986-95.

84. Barros MH, Hauck F, Dreyer JH, Kempkes B, Niedobitek G. Macrophage polarisation: an immunohistochemical approach for identifying M1 and M2 macrophages. PloS one 2013;8:e80908.

85. Muraille E, Leo O, Moser M. TH1/TH2 paradigm extended: macrophage polarization as an unappreciated pathogen-driven escape mechanism? Frontiers in immunology 2014;5:603.

86. Sun W, Depping R, Jelkmann W. Interleukin-1beta promotes hypoxia-induced apoptosis of glioblastoma cells by inhibiting hypoxia-inducible factor-1 mediated adrenomedullin production. Cell death & disease 2014;5:e1020.

87. Hagemann T, Lawrence T, McNeish I*, et al.* "Re-educating" tumor-associated macrophages by targeting NF-kappaB. The Journal of experimental medicine 2008;205:1261-8.

88. Pyonteck SM, Akkari L, Schuhmacher AJ*, et al.* CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med 2013;19:1264-72.

89. Galarneau H, Villeneuve J, Gowing G, Julien JP, Vallieres L. Increased glioma growth in mice depleted of macrophages. Cancer research 2007;67:8874-81.

90. Szulzewsky F, Pelz A, Feng X*, et al.* Glioma-associated microglia/macrophages display an expression profile different from m1 and m2 polarization and highly express gpnmb and spp1. PloS one 2015;10:e0116644.

91. Xin J, Wainwright DA, Mesnard NA*, et al.* IL-10 within the CNS is necessary for CD4+ T cells to mediate neuroprotection. Brain, behavior, and immunity 2011;25:820-9.

92. Hussain SF, Kong LY, Jordan J*, et al.* A novel small molecule inhibitor of signal transducers and activators of transcription 3 reverses immune tolerance in malignant glioma patients. Cancer research 2007;67:9630-6.

93. Khaled YS, Ammori BJ, Elkord E. Myeloid-derived suppressor cells in cancer: recent progress and prospects. Immunol Cell Biol 2013;91:493-502.

94. Kohanbash G, Okada H. Myeloid-derived suppressor cells (MDSCs) in gliomas and glioma-development. Immunol Invest 2012;41:658-79.

95. Raychaudhuri B, Rayman P, Ireland J*, et al.* Myeloid-derived suppressor cell accumulation and function in patients with newly diagnosed glioblastoma. Neuro-oncology 2011;13:591-9.

96. Rodrigues JC, Gonzalez GC, Zhang L*, et al.*Normal human monocytes exposed to glioma cells acquire myeloid-derived suppressor cell-like properties. Neuro-oncology 2010;12:351-65.

97. Biollaz G, Bernasconi L, Cretton C*, et al.* Site-specific anti-tumor immunity: differences in DC function, TGF-beta production and numbers of intratumoral Foxp3+ Treg. Eur J Immunol 2009;39:1323-33.

98. Gousias K, von Ruecker A, Voulgari P, Simon M. Phenotypical analysis, relation to malignancy and prognostic relevance of ICOS+T regulatory and dendritic cells in patients with gliomas. J Neuroimmunol 2013;264:84-90.

99. Tyrinova TV, Leplina OY, Mishinov SV*, et al.* Cytotoxic activity of ex-vivo generated IFNalpha-induced monocyte-derived dendritic cells in brain glioma patients. Cell Immunol 2013;284:146-53.

100. Dunn GP, Dunn IF, Curry WT. Focus on TILs: Prognostic significance of tumor infiltrating lymphocytes in human glioma. Cancer Immun 2007;7:12.

101. Zisakis A, Piperi C, Themistocleous MS*, et al.* Comparative analysis of peripheral and localised cytokine secretion in glioblastoma patients. Cytokine 2007;39:99-105.

102. Kumar R, Kamdar D, Madden L*, et al.* Th1/Th2 cytokine imbalance in meningioma, anaplastic astrocytoma and glioblastoma multiforme patients. Oncol Rep 2006;15:1513-6.

103. Shimato S, Maier LM, Maier R*, et al.* Profound tumor-specific Th2 bias in patients with malignant glioma. BMC Cancer 2012;12:561.

104. Maes W, Verschuere T, Van Hoylandt A, Boon L, Van Gool S. Depletion of regulatory T cells in a mouse experimental glioma model through anti-CD25 treatment results in the infiltration of non-immunosuppressive myeloid cells in the brain. Clinical & developmental immunology 2013;2013:952469.

105. Domingues PH, Teodosio C, Otero A*, et al.* The protein expression profile of meningioma cells is associated with distinct cytogenetic tumour subgroups. Neuropathology and applied neurobiology 2014.

106. Kmiecik J, Zimmer J, Chekenya M. Natural killer cells in intracranial neoplasms: presence and therapeutic efficacy against brain tumours. Journal of neuro-oncology 2014;116:1-9.

107. Castriconi R, Daga A, Dondero A*, et al.* NK cells recognize and kill human glioblastoma cells with stem cell-like properties. J Immunol 2009;182:3530-9.

108. Alizadeh D, Zhang L, Brown CE*, et al.* Induction of anti-glioma natural killer cell response following multiple low-dose intracerebral CpG therapy. Clin Cancer Res 2010;16:3399-408.

109. Crane CA, Han SJ, Barry JJ*, et al.* TGF-beta downregulates the activating receptor NKG2D on NK cells and CD8+ T cells in glioma patients. Neuro-oncology 2010;12:7-13.

110. Epari S, Sharma MC, Sarkar C*, et al.* Chordoid meningioma, an uncommon variant of meningioma: a clinicopathologic study of 12 cases. Journal of neuro-oncology 2006;78:263-9.

111. Nelson BH. CD20+ B cells: the other tumor-infiltrating lymphocytes. J Immunol 2010;185:4977-82.

112. Candolfi M, Curtin JF, Yagiz K*, et al.* B cells are critical to T-cell-mediated antitumor immunity induced by a combined immune-stimulatory/conditionally cytotoxic therapy for glioblastoma. Neoplasia 2011;13:947-60.

113. Yao S, Zhu Y, Zhu G*, et al.* B7-h2 is a costimulatory ligand for CD28 in human. Immunity 2011;34:729-40.

114. Wainwright DA, Chang AL, Dey M*, et al.* Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors. Clin Cancer Res 2014;20:5290-301.

115. Wu G, Yang W, Barth RF*, et al.* Molecular targeting and treatment of an epidermal growth factor receptor-positive glioma using boronated cetuximab. Clin Cancer Res 2007;13:1260-8.

116. Patel D, Lahiji A, Patel S*, et al.* Monoclonal antibody cetuximab binds to and down-regulates constitutively activated epidermal growth factor receptor vIII on the cell surface. Anticancer research 2007;27:3355-66.

117. Perera RM, Narita Y, Furnari FB*, et al.* Treatment of human tumor xenografts with monoclonal antibody 806 in combination with a prototypical epidermal growth factor receptor-specific antibody generates enhanced antitumor activity. Clin Cancer Res 2005;11:6390-9.

118. Scott AM, Lee FT, Tebbutt N*, et al.* A phase I clinical trial with monoclonal antibody ch806 targeting transitional state and mutant epidermal growth factor receptors. Proceedings of the National Academy of Sciences of the United States of America 2007;104:4071-6.

119. Sampson JH, Crotty LE, Lee S*, et al.* Unarmed, tumor-specific monoclonal antibody effectively treats brain tumors. Proceedings of the National Academy of Sciences of the United States of America 2000;97:7503-8.

120. Yang W, Barth RF, Wu G*, et al.* Molecular targeting and treatment of EGFRvIII-positive gliomas using boronated monoclonal antibody L8A4. Clin Cancer Res 2006;12:3792-802.

121. Johns TG, Luwor RB, Murone C*, et al.* Antitumor efficacy of cytotoxic drugs and the monoclonal antibody 806 is enhanced by the EGF receptor inhibitor AG1478. Proceedings of the National Academy of Sciences of the United States of America 2003;100:15871-6.

122. Mineo JF, Bordron A, Quintin-Roue I*, et al.* Recombinant humanised anti-HER2/neu antibody (Herceptin) induces cellular death of glioblastomas. British journal of cancer 2004;91:1195-9.

123. Schuster J, Lai RK, Recht LD*, et al.* A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. Neuro-oncology 2015;17:854-61.

124. Sampson JH, Heimberger AB, Archer GE*, et al.* Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J Clin Oncol 2010;28:4722-9.

125. Bethke K, Staib F, Distler M*, et al.* Different efficiency of heat shock proteins (HSP) to activate human monocytes and dendritic cells: superiority of HSP60. J Immunol 2002;169:6141-8.

126. Anguille S, Smits EL, Lion E, van Tendeloo VF, Berneman ZN. Clinical use of dendritic cells for cancer therapy. The Lancet 2014;15:e257-67.

127. Capuron L, Ravaud A, Dantzer R. Early depressive symptoms in cancer patients receiving interleukin 2 and/or interferon alfa-2b therapy. J Clin Oncol 2000;18:2143-51.

128. Capuron L, Ravaud A, Neveu PJ*, et al.* Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Molecular psychiatry 2002;7:468-73.

129. Dantzer R. Cytokine-induced sickness behavior: mechanisms and implications. Annals of the New York Academy of Sciences 2001;933:222-34.

130. Hayes RL, Arbit E, Odaimi M*, et al.* Adoptive cellular immunotherapy for the treatment of malignant gliomas. Critical reviews in oncology/hematology 2001;39:31-42.

131. Rand RW, Kreitman RJ, Patronas N*, et al.* Intratumoral administration of recombinant circularly permuted interleukin-4-Pseudomonas exotoxin in patients with high-grade glioma. Clin Cancer Res 2000;6:2157-65.

132. Debinski W, Obiri NI, Powers SK, Pastan I, Puri RK. Human glioma cells overexpress receptors for interleukin 13 and are extremely sensitive to a novel chimeric protein composed of interleukin 13 and pseudomonas exotoxin. Clin Cancer Res 1995;1:1253-8.

133. Kunwar S, Prados MD, Chang SM*, et al.* Direct intracerebral delivery of cintredekin besudotox (IL13-PE38QQR) in recurrent malignant glioma: a report by the Cintredekin Besudotox Intraparenchymal Study Group. J Clin Oncol 2007;25:837-44.

134. Jarboe JS, Johnson KR, Choi Y, Lonser RR, Park JK. Expression of interleukin-13 receptor alpha2 in glioblastoma multiforme: implications for targeted therapies. Cancer research 2007;67:7983-6.

135. Kunwar S, Chang S, Westphal M*, et al.* Phase III randomized trial of CED of IL13-PE38QQR vs Gliadel wafers for recurrent glioblastoma. Neuro-oncology 2010;12:871-81.

136. Okano F, Storkus WJ, Chambers WH, Pollack IF, Okada H. Identification of a novel HLA-A*0201-restricted, cytotoxic T lymphocyte epitope in a human glioma-associated antigen, interleukin 13 receptor alpha2 chain. Clin Cancer Res 2002;8:2851-5.

137. Buckner JC, Schomberg PJ, McGinnis WL*, et al.* A phase III study of radiation therapy plus carmustine with or without recombinant interferon-alpha in the treatment of patients with newly diagnosed high-grade glioma. Cancer 2001;92:420-33.

138. Olson JJ, McKenzie E, Skurski-Martin M*, et al.* Phase I analysis of BCNU-impregnated biodegradable polymer wafers followed by systemic interferon alfa-2b in adults with recurrent glioblastoma multiforme. Journal of neuro-oncology 2008;90:293-9.

139. Hjelmeland MD, Hjelmeland AB, Sathornsumetee S*, et al.* SB-431542, a small molecule transforming growth factor-beta-receptor antagonist, inhibits human glioma cell line proliferation and motility. Molecular cancer therapeutics 2004;3:737-45.

140. Hau P, Jachimczak P, Schlingensiepen R*, et al.* Inhibition of TGF-beta2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies. Oligonucleotides 2007;17:201-12.

141. Okada H, Kalinski P, Ueda R*, et al.* Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. J Clin Oncol 2011;29:330-6.

142. Hoepner S, Loh JM, Riccadonna C*, et al.* Synergy between CD8 T cells and Th1 or Th2 polarised CD4 T cells for adoptive immunotherapy of brain tumours. PloS one 2013;8:e63933.

143. Predina J, Eruslanov E, Judy B*, et al.*Changes in the local tumor microenvironment in recurrent cancers may explain the failure of vaccines after surgery. Proceedings of the National Academy of Sciences of the United States of America 2013;110:E415-24.