Targeting Tumor Associated Macrophages to Overcome Conventional Treatment Resistance in Glioblastoma

Hélène Grégoire¹, Loris Roncali¹, Audrey Rousseau¹,², Michel Chérel³, Yves Delneste¹,⁴, Pascale Jeannin¹,⁴, François Hindré¹,⁵ and Emmanuel Garcion¹,⁶*

¹ CRCINA, INSERM, Université de Nantes, Université d’Angers, Angers, France, ² Département de Pathologie Cellulaire et Tissulaire, CHU Angers, Angers, France, ³ CRCINA, INSERM, Université d’Angers, Université de Nantes, Nantes, France, ⁴ Laboratoire d’Immunologie et Allergologie, CHU d’Angers, Angers, France, ⁵ PRIMEX, Plateforme de radiobiologie et d’imagerie expérimentale, SFR ICAT, Université d’Angers, Angers, France, ⁶ PACeM, Plateforme d’analyses cellulaires et moléculaires, SFR ICAT, Université d’Angers, Angers, France

Glioblastoma (GB) is the most common and devastating form of brain cancer. Despite conventional treatments, progression or recurrences are systematic. In recent years, immunotherapies have emerged as an effective treatment in a number of cancers, leaving the question of their usefulness also faced with the particular case of brain tumors. The challenge here is major not only because the brain is the seat of our consciousness but also because of its isolation by the blood-brain barrier and the presence of a unique microenvironment that constitutes the central nervous system (CNS) with very specific constituent or patrolling cells. Much of the microenvironment is made up of immune cells or inflammation. Among these, tumor-associated macrophages (TAMs) are of significant interest as they are often involved in facilitating tumor progression as well as the development of resistance to standard therapies. In this review, the ubiquity of TAMs in GB will be discussed while the specific case of microglia resident in the brain will be also emphasized. In addition, the roles of TAMs as accomplices in the progression of GB and resistance to treatment will be presented. Finally, clinical trials targeting TAMs as a means of treating cancer will be discussed.

Keywords: glioblastoma, macrophages, microglia, resistance, radiation, crosstalks, tumor-associated macrophage

INTRODUCTION

Glioblastoma (GB) is the most frequent and malignant form of brain tumors. It is associated with a poor prognosis and the median overall survival of GB patients is about 15 months after standard of care (Stupp et al., 2009). Conventional treatments consist of maximal safe resection followed by external radiotherapy and concomitant chemotherapy based on the use of the alkylating agent temozolomide (TMZ) (Stupp et al., 2005). However, recurrence inevitably occurs. Currently, no therapy can completely cure GB; current treatments can only marginally improve the overall survival of patients. The current...
strategy focuses mostly on targeting the tumor cells, failing to account for other cellular constituents present in the tumor. Hence, to cure and achieve a complete resection of GB tumors, new therapeutic strategies are in great demand.

GB is a highly heterogeneous tumor, with diverse co-existing cell types that include tumor cells, endothelial cells, fibroblasts and different cell types from the immune system (Charles et al., 2011; Quail and Joyce, 2017). A particular emphasis has been placed on the immune system and especially on tumor-associated macrophages (TAMs) as they are the dominant infiltrating immune cell population in GB. These cells interact with tumor cells to promote tumor growth and progression (Feng et al., 2015). The host defense is composed of both innate and adaptive immune cells and they are both involved in cancer immune surveillance in early stages of the disease. However, the tumor is able to escape this immune surveillance during its development. At that point, the tumor can recruit immune cells and change their original function to be one of its accomplices (Brown et al., 2018; Finn, 2018). Tumor cells can inhibit the cytotoxic function of the immune system by secreting immunosuppressive factors or recruiting immunosuppressive inflammatory cells. In relation to this, macrophages appear to be a promising target to improve the effectiveness of actual therapy as more and more information on their physiological and pathological roles in the brain is being uncovered.

Macrophages are the most abundant infiltrating immune cells in GB. Their function is different from their homolog in healthy tissues (Nishie et al., 1999; Hussain et al., 2006). They are able to discriminate the components of the self from the non-self (microbes) but also the altered components of the self. When recognizing the non-self or altered self-components, they can begin their process of elimination. Macrophages located in the tumor microenvironment are called tumor-associated macrophages. Under normal physiological conditions, macrophages are implicated in different processes such as organ development, tissue homeostasis, host defense against infections. These cells can also participate in metabolic disorders, immune diseases and cancer development (Sica et al., 2015). Normally, the myeloid population is the major player of the innate immune system and represents up to 30% of the tumor mass (Rossi et al., 1987; Graeber et al., 2002). Both the activation status and the number of TAMs present in the tumor microenvironment seem to influence GB prognosis (Komohara et al., 2008; Lu-Emerson et al., 2013; Pyonteck et al., 2013).

Macrophages are characterized by their plasticity and heterogeneity. They can be activated by different types of stimuli (growth factors, cytokines, microbial products, nucleotides) which in turn will affect macrophages differently (Poh and Ernst, 2018). In vitro, the stimulation of macrophages by interferon-γ (IFN-γ) and/or lipopolysaccharides (LPS) induces the classical (M1) macrophage polarization (Nielsen and Schmid, 2017). M1 macrophages favor the generation of T helper Type 1 (Th1) lymphocytes. Classically activated macrophages are good effectors to fight malignant tumors and are associated with chronic inflammation (Atri et al., 2018). Those macrophages are characterized by a high expression of IL-12, IL-23, and a low expression of IL-10. They can also produce high levels of pro-inflammatory cytokines IL-1β, tumor necrosis factor α (TNF-α), and IL-6, and increase the expression of inducible nitric oxide synthase (iNOS, NOSII) and reactive oxygen species (ROS). Another known stimulus for M1 macrophages is GM-CSF (Granulocyte Macrophage Colony-Stimulating Factor). It activates STAT5, which leads to the activation of the PI3K-AKT pathway (Jeannin et al., 2018).

On the contrary, macrophages stimulated in vitro by IL-4 and/or IL-13 are called alternatively activated (M2) macrophages (Murray et al., 2014). They are known effectors for promoting Th2 lymphocytes. They are involved in angiogenesis and tumor progression (Martinez and Gordon, 2014). This phenotype is associated with a low expression of IL-12, IL-23, and a high expression of IL-10 and TGF-β. Furthermore, M2 macrophages also have high levels of arginase 1 (Arg1), mannose receptors and scavenger receptors. M-CSF (Macrophage Colony-Stimulating Factor) and IL-34 also induce a M2 phenotype. M-CSF and IL-34 express the same receptor named CD115 and activate the MAP kinases signaling pathway (Jeannin et al., 2018).

Although the traditional M1/M2 dichotomy is useful for understanding the functionality of TAMs, recent analyzes, in particular of single-cell, revealed a spectrum of activation states much more complex than these traditional polarizations (Locati et al., 2020). Hence, macrophages in cancer are double-edged swords exerting pro- and antitumor functions. More than a real opposition, the M1/M2 signature crystallize a continuum of two extremes capable of specific adaptations (eg., chromatin remodeling, epigenetic marks, trained immunity, metabolic reprogramming,...) to various loco-regional cues (eg., cytokines, chemokines, miRNA, or immune checkpoints). In addition, proliferating monocytes could persist in a state of self-renewal within tumor tissues, rather than immediately differentiate into macrophages indicating a much higher complexity (Lin et al., 2019). It should again be emphasized that the M1 and M2 markers are distinct across species and in particular between humans and mice (eg., in human NOSII and Arg1 do not account for M1 and M2 macrophages, respectively) (Thomas and Mattila, 2014). In this regard, there are no specific surface markers in humans except a privileged panel of produced cytokines.

TAMs that are described in the tumor have in most cases protumorigenic functions that promote tumor growth, invasion, angiogenesis, and tumor metastasis. In the GB microenvironment, both TAMs derive from blood monocytes; some originate from resident macrophages called microglia. Hence, macrophages appear to be an attractive target for new therapeutic strategies (Noy and Pollard, 2014).

The goal of this review is to discuss whether macrophages are worth considering as therapeutic targets in GB and to summarize the existing drugs targeting macrophages. In the second part of this review, the presence of microglia in brain tumor will be discussed. Then, the roles of TAMs in regulating the tumor
development, progression, and the response to conventional therapy will be reviewed. Finally, a survey of clinical trials testing drugs against macrophages in cancer will be presented.

THE PRESENCE OF TAMS IN GB: REALITY OR NOT?

The World Health Organization (WHO) classification of Central Nervous System (CNS) tumors was restructured in 2016. Diagnoses are based on both molecular alterations and histopathologic features (integrated diagnosis) in contrast to the 2007 WHO classification that only included histopathologic features (Louis et al., 2007; Louis et al., 2016). The tumor is essentially defined by the characteristics of the tumor cells that compose it, independently of the ecosystem in which they evolve and which they could themselves modify. GB also consists of many different noncancerous cells. The following cells are known to define the tumor microenvironment: endothelial cells, pericytes, fibroblasts, and immune cells in addition to cancer cells (Quail and Joyce, 2013).

The tumor microenvironment is now emerging as an important regulator of cancer progression (Quail and Joyce, 2017). Data from the literature seem to suggest that distinct molecular profiles in GB are correlated with differences in their microenvironment (Zhemakova et al., 2018). Even if the WHO classification now includes molecular data, no information on the tumor microenvironment has been integrated so far. Despite the fact that a solid tumor has never been seen without infiltrating immune cells, current diagnostic guidelines often forget voluntarily to take this into account. Although this does not necessarily modify the diagnosis as it is perceived today, it could be useful as regards the consideration of patient management and escape or not to new well identified therapies. The presence of TAMs has already been well described in GB (Saha et al., 2017; Séhédic et al., 2017; Roesch et al., 2018). In a mouse model, TAMs were observed in perivascular areas in the tumor and seem to be implicated in gliomagenesis Feng et al., 2015. Interestingly, their localization in the tumor appears to depend on their phenotypes Schiffer et al., 2018. In 2012, a meta-analysis showed that a high density of TAMs appeared to be associated with a poor prognosis in head and neck, ovarian and breast cancer and with a better prognosis in colorectal cancer (Zhang et al., 2012; Yuan et al., 2017; Zhao et al., 2017). Further evidence revealed that human GB-induced polarization of resident microglia (Walentynowicz et al., 2018). Although many studies tried to decipher the origin of TAMs in the tumor, no clear answer has yet been obtained.

Resident microglia are described to be involved in many processes including tumor growth and progression (Bryukhoverstkyi et al., 2016; Matias et al., 2018). Microglia were shown to contribute to the invasiveness of GB by upregulating serpin family A member 3 (SERPINA3) expression in GB stem cells (GSCs), that is implicated in the remodeling of the extracellular matrix (Li et al., 2018). Resident microglia were also shown to mediate GB progression and stemness through the activation of interferon regulatory factor 7 (IRF7) that generates an inflammatory environment (Li Z. et al., 2017). Resident microglia are also involved in antitumor immunity processes through the expression of toll-like receptor 2 (TLR2) that down regulates their major histocompatibility complex class II (MHCII) expression (Qian et al., 2018). In a murine model, enhancer of zeste homolog 2 (EZH2) expression in GB was shown to be involved in the polarization of TAMs toward the M2 phenotype, creating an immune deficient environment (Yin et al., 2017). A 6 cytokine-related gene signature in resident microglia was shown to be sufficient to predict survival and identify M2 cells in GB (Cai et al., 2015).

Resident and peripheral macrophages are uniquely involved

MICROGLIA: THE RESIDENT MACROPHAGES OF THE CNS

Microglia are the resident macrophages of the CNS and a healthy CNS macrophage population consists only of resident microglia. The blood brain barrier is impaired in neuropathological diseases, thus allowing an infiltration of monocytes form peripheral blood. In GB, both resident microglia and peripheral macrophages can be detected (Lisi et al., 2017). It is crucial to understand their molecular differences and their specific roles in the tumor. Resident microglia and newly recruited macrophages, hereafter referred to as peripheral macrophages have a distinct origin, as microglia arise from the yolk sac primitive macrophages (Ginhoux et al., 2013; Ginhoux and Guilliams, 2016). Although their origin differs, they share common histologic characteristics. Differentiating between microglia and peripheral macrophages is a difficult task, since they share common surface markers. The name TAM may very well include both resident microglia and monocyte-derived macrophages (Szulzewsky et al., 2015; Kloepper et al., 2016). In order to separate macrophages of hematopoietic origin from resident microglia, CD45 was used in flow cytometry analysis (Badie et al., 2000). However, resident microglia can upregulate their CD45 expression, making them indistinguishable from peripheral macrophages (Müller et al., 2015). Using a genetically engineered mouse, it was demonstrated that peripheral macrophages represent the majority of TAMs in the tumor, and resident microglia form a minor TAM population (Chen et al., 2017). Moreover, resident microglia and peripheral macrophages have different preferential localizations. Peripheral macrophages mostly appear in perivascular areas while resident macrophages are usually located in the peritumoral zone. A recent study showed that only a small batch of common genes toward species (rat, mice, human) differentiates GB-induced polarization of resident microglia (Walentynowicz et al., 2018). Although many studies tried to decipher the origin of TAMs in the tumor, no clear answer has yet been obtained.

Resident microglia are described to be involved in many processes including tumor growth and progression (Bryukhoverstkyi et al., 2016; Matias et al., 2018). Microglia were shown to contribute to the invasiveness of GB by upregulating serpin family A member 3 (SERPINA3) expression in GB stem cells (GSCs), that is implicated in the remodeling of the extracellular matrix (Li et al., 2018). Resident microglia were also shown to mediate GB progression and stemness through the activation of interferon regulatory factor 7 (IRF7) that generates an inflammatory environment (Li et al., 2017). Resident microglia are also involved in antitumor immunity processes through the expression of toll-like receptor 2 (TLR2) that down regulates their major histocompatibility complex class II (MHCII) expression (Qian et al., 2018). In a murine model, enhancer of zeste homolog 2 (EZH2) expression in GB was shown to be involved in the polarization of TAMs toward the M2 phenotype, creating an immune deficient environment (Yin et al., 2017). A 6 cytokine-related gene signature in resident microglia was shown to be sufficient to predict survival and identify M2 cells in GB (Cai et al., 2015).
in supporting GB growth and progression. Hence, if we wish to target TAMs as a mean to treat GB, we must first characterize this population as peripheral macrophages and/or resident microglia and counter their exact roles in GB initiation and maintenance.

**TUMOR-ASSOCIATED MACROPHAGES: A PARTNER IN CRIME FOR TUMOR CELLS**

A tumor can influence its microenvironment, and inversely. Thus, the interactions between the tumor cells and the nearby non-tumor cells are crucial to promote tumor angiogenesis, peripheral immune tolerance, and tumor growth. As previously said, TAMs are highly represented inside the tumor microenvironment. They are known for their heterogeneous phenotype, which by simplification can be with either anti-tumor (M1-like) or pro-tumor functions (M2-like). As TAMs are highly plastic cells, they can program themselves into both subpopulations. This gives them the ability to have different functions in different tumor areas and at different times during the tumor development.

**Biology of the Tumor**

**Tumor Cells**

The effect of TAMs on tumor cells is dependent on their type of activation. The reprogrammed M1 TAMs suppress the growth of GB cells (Li T. et al., 2017) meanwhile the M2 macrophages are described to favor tumor growth and resistance to therapy (Xue et al., 2017).

A macrophage with pro-tumor function in the tumor microenvironment is a macrophage that enhances tumor initiation and growth. TAMs and tumor cells actively communicate with each other leading to tumor progression. Their communication is mediated by interleukins IL-6 and IL-10 and transforming growth factor-β1 (TGF-β1) (Wagner et al., 1999; Ye et al., 2012). These cytokines activate signaling pathways in the tumor cells that boost processes such as proliferation, invasion and vascularization (Figure 1). TGF-β1 secretion by TAMs is responsible for the recruitment of cancer stem-like cells (CSCs) expressing CD133. Another consequence of TGF-β1 secretion is the production of metalloproteinase 9 (MMP-9) by CSCs rendering them highly invasive (Ye et al., 2012). TAMs are able to secrete pleiotrophin (PTN); CSCs express the PTN receptor PTPRZ1 on their cell surface. Once PTN is recognized by its receptor, it stimulates CSCs maintenance and tumorigenic potential, and therefore promotes GB growth (Shi et al., 2017). PTN-expressing TAMs also express CD163 which is an M2 lineage marker. Wang et al. showed that macrophages support GB invasiveness through the CCL4-CCR5 axis that enhances MMP-9 expression (Wang et al., 2016). Hypoxia was also shown to positively contribute to this mechanism by enhancing CCL4 and CCR5 expression. An increase of TAMs in a mouse model was shown to decrease the survival of the mice associated with a reduction of CD8+ T cells (Chae et al., 2015). On top of that, EGFR activation level correlates with TAM infiltration. Consequently, EGF can induce an upregulation of vascular cell adhesion molecule-1 (VCAM-1) that favors the interaction between TAMs and tumor cells, which in turn promoted tumor cell invasion (Zheng et al., 2013). MerTK (Myeloid-Epithelial-Reproductive Tyrosine Kinase) is a tyrosine kinase expressed by macrophages that suppresses the innate immune response. Its expression was shown to be higher in tumor recurrences. TAMs that express MerTK are also associated with tumor growth and resistance to treatment, making MerTK a potential therapeutic target (Wu et al., 2018). The molecular crosstalk between tumor cells and macrophages appears to be important for tumor growth and malignant progression. Therefore, modulating the exchange between those two cell populations may be therapeutically relevant.

**Angiogenesis**

GB is a highly hypoxic tumor with prominent necrotic regions due to the rapid proliferation of GB cells. The cell composition of the tumor core is quite different from that of the peritumoral area. The tumor core is more hypoxic, contains more CD163+ TAMs and has a higher expression of VEGF-A (Tamura et al., 2018) (a major factor for vascularization). A downstream effect of hypoxia and necrosis is an increase in vascular proliferation. In the tumor microenvironment, TAMs are located near blood vessels. In mice, endothelial cells produce IL-6 that induces the expression of Arg1 and thus the alternative phenotype in TAMs (Wang et al., 2018). This alternative activation is mediated by the hypoxia-inducible factor-2α (HIF-2α). Wang et al. targeted IL-6 expression in a mouse model and improved the survival of GB-bearing mice. VEGF was shown to be implicated in promoting pro-angiogenic functions of TAMs in a GB rodent model (Turkowski et al., 2018). Gliomas overexpressing VEGF were correlated with an increase in the expression of MHC1 and MHCII on macrophages. Endothelial cells and TAMs interaction leads to angiogenesis through the expression of TGF-β1 and integrin αvβ3, which induces the activation of the SRC-PI3K-YAP signaling (Cui et al., 2018) (Figure 1). The pro-angiogenic properties of TAMs are mediated by the protein CRCR1. This protein activates the PDGFB–PDGFRβ pathways and promotes pericytes recruitment, migration, and tumor angiogenesis (Zhu C. et al., 2017). In sum, TAMs have a proangiogenic function in GB. Thus, targeting macrophages may improve the response to anti-angiogenic therapies (Deng et al., 2017; Gagner et al., 2017). Indeed, blocking the macrophages recruitment by combining the chemokine SDF-1 and VEGF inhibitors was more effective and decreased tumor invasiveness and vascular density.

**Immune Environment**

Each tumor is characterized by an immune suppressive environment that forms one hallmark of cancer (Hanahan et al., 2011). This is in part due to the presence of TAMs in tumors but also to a complex regulation of the expression of immune and inflammatory genes by the global tumor ecosystem. It was found that IKKβ levels were reduced in GB; consequently, the NF-κB expression was decreased leading to defective immune and inflammatory gene expression in macrophages (Mieczkowski et al., 2015). NF-κB signaling is required for macrophage polarization and immune suppression in GB, making NF-κB a suitable target to improve overall survival in
GB (Achyut et al., 2017). TAMs strongly inhibit the proliferation of antitumor T cells in the tumor microenvironment (Kumar et al., 2017). It was shown that an inhibition of transcription factors such as NF-κB, a mediator of M2 macrophage polarization, led to slower tumor growth and prolonged survival in a mouse model. It also decreased T cell induction which made the tumor less immunosuppressive (Barberi et al., 2018). Targeting NF-κB may improve the effectiveness of the current standard therapies.

TAMs express IL-4Rα that promotes immunosuppression. In mice, they also express Arg1 that is critical for T cell inhibition (Kohanbash et al., 2013). Chemokine ligand 22 (CCL22) is produced by TAMs and its expression is associated with a low survival rate and CD4+ T cell activation (Zhou et al., 2015). One of the key regulators of the immunosuppressive environment in GB is fibrinogen-like protein 2 (FGL2). Its expression was correlated with a higher number of CD4+ T cells and M2 macrophages (Latha et al., 2018). The colony stimulating factor receptor (CSF1R) is required for the recruitment of TAMs in the tumor microenvironment. It is also involved in promoting the polarization of macrophages toward the M2 phenotype. Inhibition of CSF1R attenuates the recruitment of TAMs and also increases the CD8+ T cell infiltration (Strachan et al., 2013) (Figure 1). Another regulator of the immune microenvironment is the receptor tyrosine kinase AXL that is expressed in TAMs (Sadahiro et al., 2018). Its inhibition in a GB mouse model was
associated with prolonged survival. Furthermore, myeloid derived suppressor cells (MDSC) such as TAMs have been described to be activated by GB CSCs through MIF expression, having then an immunosuppressive activity on CD8+ T cells, notably through the Arg1 expression in mice models (Flavahan et al., 2016). Overall, targeting TAMs may disturb the immunosuppressive environment of the tumor, allowing the immune cells to function more effectively.

**Loco-Regional Cues for Metabolic Reprogramming**

A peculiarity of GB is that it affects the seat of our consciousness, the CNS, whose immune status remains privileged due notably to the presence of the blood-brain barrier (BBB) and of unique resident cells (microglia, astrocytes, endothelial cells) (cf. **Box 1**). Although a precise control of the inflammatory or immune infiltrate is realized, the physiological and anatomical characteristics of the CNS is fed by the field of new recent knowledge, such as the identification of direct vascular channels connecting skull bone marrow to the brain surface enabling myeloid cell migration (Herisson et al., 2018), and make evolve our representation of its immune status. It should be stressed, however, that depending on the therapeutic strategy envisaged, the drug used can have a distinct impact when used according to a peripheral or loco-regional mode of administration (cf. **Tables 1–3**). Hence, if TAMs influence immune and adaptive signaling, reciprocally, loco-regional metabolic signals produced in tumor environments (glucose, glutamine, cysteine, lactate, IDO, adenosine, itaconic acid, acidic pH) impacted the polarization fate and immunosuppressive functions of TAMs, thus possibly resulting in immune tolerance and treatment resistance in GB (for review, see Won et al., 2019). Hence, tolerance can be reversed at both the promoter and enhancers of tolerized genes involved in metabolism and lipid biosynthesis, leading to transcriptional programs that rewired the intracellular signaling of innate immune cells thus increasing the capability of macrophages to respond to stimulation (for review see, Locati et al., 2020). In line with this, it has been observed that inhibition of fatty acid synthase (FAS), which catalyzes the synthesis of long-chain fatty acids, prevents the pro-inflammatory response in macrophages (Carroll et al., 2018).

Interestingly, using metabolic profiling, it was found that exposure to β-amyloid triggers acute reactive microglial inflammation accompanied by metabolic reprogramming from oxidative phosphorylation to glycolysis while metabolic boosting with recombinant interferon-γ treatment reversed the defective glycolytic metabolism and inflammatory functions of microglia (Baik et al., 2019). Such microglial metabolic switch may also have a strong impact on GB development.

**TAMs and Therapeutics**

**TAMs and Surgical Resection**

Surgical resection is the current standard treatment for GB. However, limited data on the biological consequences of surgical resection have been published so far. It was reported that surgical resection increases proliferation and angiogenesis (Kong et al., 2010). After surgical resection, TAMs were shown to express higher levels of CD163, a M2 macrophage marker, and their localization was close to the site of recurrence (Zhu H. et al., 2017). Both TAMs and oligodendrocyte progenitor cells are localized near the tumor periphery. They enhance the stemness and chemoresistance in GB cells (Hide et al., 2018). It was shown that tumor phenotypes associated with telomerase overexpression and TAMs infiltration were more complicated to resect, probably due to improvement of GB cell migratory capabilities (Hung et al., 2016). The inability to surgically remove the whole tumor contributes to the poor prognosis and recurrence of GB.

**TAMs and Radiotherapy**

Macrophages inside the tumor mass are involved in multiple phenomena that include radiation resistance. Radiation therapy itself induces changes in the tumor microenvironment and renders the tumor more aggressive. In fact, recurrence mostly appears near the irradiated area (Gupta and Burns, 2018). Radiotherapy induces a rapid inflammatory response leading to TAMs recruitment. This inflammatory response is correlated with a short survival time (Tabatabaei et al., 2017). TAMs participate in the induction of GB cell differentiation to a mesenchymal state through NF-κB production, an event that correlated with radiation resistance (Bhat et al., 2013). Recently, Leblond et al. showed that M1 macrophages are more sensitive to radiation than M2 macrophages (Leblond et al., 2017). The proportion of M2 macrophages in irradiated tissues is thus increased. Moreover, M2 macrophages were described to contribute to relapses in oral cancer by promoting vascularization after radiation treatment (Okubo

**Box 1** | Non-cancerous brain cells alter macrophages polarization and functions.

Tumor cells cooperate with its surroundings such as the tumor microenvironment. The brain is also the home of specific cell types with their own characteristics and functions; although those cells are not part of the tumor, they can also interact with it. The interaction between cells residing in the brain and TAMs are very poorly understood in cancer but has been studied in depth in other pathologies, which will be quickly reviewed in this box. Both neurons and astrocytes can produce CX3CL1R, the receptor for CX3CL1 found on microglia Matias et al., 2018. CX3CL1 promotes TAM recruitment and increases the expression of MMPs and thus invasive properties. When an ischaemic stroke happens, ischaemic neurons are able to prime microglia toward an M1 phenotype during an injury Hu et al., 2012. Another cell type is oligodendrocyte which accounts for the formation of the myelin sheath in the CNS. It was found that macrophages and oligodendrocyte progenitor cells colocalized near the tumor border. At this site of colocalization, those cells induced stemness and resistance to therapy in GB cells Hide et al., 2018. In the peripheral nervous system, Schwann cells are the cells responsible for myelin sheath formation. Schwann cells were shown to promote cancer invasion by direct contact with tumor cells Debrode et al., 2016. The mechanism involved in this process remains unclear. In neurofibromas (peripheral nerve sheath tumors due to NF1 loss in Schwann cells), macrophages were shown to be abundant Stratton et al., 2018. In this case, Schwann cells and macrophages communicate with each other and are involved in the regulation of inflammatory gene expression. As Schwann cells and oligodendrocytes share a common function in normal tissue, it may be interesting to further study the involvement of oligodendrocytes in GB. Non-cancerous cells of the CNS and peripheral nervous system interact with macrophages and lead them to polarize toward a specific phenotype.
et al., 2016). In a radioresistant GB model, the total RNA was sequenced and it was found that there was a positive regulation of macrophage chemotaxis following radiation (Doan et al., 2018). Also, in a murine glioma model, an increase in SDF-1 at the tumor invasion front after radiotherapy was correlated with the recruitment of TAMs and radioresistance (Wang et al., 2013).

Irradiation of the tumor leads to the alteration of multiple pathways. In particular, it modifies the macrophage activation type, rendering them more supportive of tumor growth.

TAMs and Chemotherapy

The standard treatment of GB affects the molecular profiles of the tumor. Temozolomide (TMZ) is commonly used to treat GB. TAMs that express CD74 were described to be involved in TMZ resistance by inducing AKT and Erk1/2 activation in tumor cells (Kitange et al., 2010). Gene expression profiling showed that the tumor that recurred after treatment did not match the primary treatment-naive tumor. After treatment, the polarization toward the M2 phenotype was upregulated (Hudson et al., 2018). Tumor protein 53 (p53) is involved in promoting the development of the tumor. GB with the p53 isoform Δ133p53β had increased CD163+ macrophages (Kazantseva et al., 2018). Moreover, Δ133p53β supports cancer stemness (Arsic et al., 2015). In addition, it is correlated with resistance to TMZ (Kazantseva et al., 2018). GB is able to evade the toxic effects of chemotherapy, but it can equally evade the action of the immune system. Hence, a cocktail of multiple drugs targeting different pathways may provide the most effective therapy for GB and improve overall survival.

**CURRENT THERAPIES TARGETING TUMOR-ASSOCIATED MACROPHAGES IN CANCER**

**Targeting the Recruitment of TAMs**

One strategy to target TAMs is to block their recruitment to the tumor site. It can be achieved by targeting the chemokine ligand 2 (CCL2) - chemokine receptor 2 (CCR2) axis. CCL2 is an inflammatory chemokine that can recruit macrophages and Treg lymphocytes leading to an immunosuppressive environment (Chang et al., 2016). To achieve this, a human IgG1k mAb called Carlumab was developed. A survey of clinical trials involving the CCL2-CCR2 axis is provided in Table 1.

| Target | Drugs | Inhibitor type | Clinical trial | Tumor type | Benefit |
|--------|-------|----------------|----------------|------------|---------|
| CCL2-CCR2 axis | Carlumab mAb | NCT00992186 (2009) (completed, has results) NCT01204996 (2010) (Completed) NCT00537368 (2007) (Completed) NCT02792938 (2016) (Terminated) | Metastatic Castrate-Resistant Prostate Cancer Solid Tumors Solid Tumors Metastatic Pancreatic Cancer | Information about the disease’s progression |
| PF-04136309 Small molecule | Small molecule | NCT01015560 (2009) (Completed with results) | Bone Metastases | Well tolerated |
| MLN1202 mAb | MLN1202 mAb | NCT03778879 (2018) (Not yet recruiting) | Pancreatic Ductal Adenocarcinoma (PDAC) | Unknown |
| CCX872-B Small molecule | Small molecule | NCT03496662 (2018) (Recruiting) | Unknown |
| BMS-813160 Small molecule | Small molecule | NCT02653500 (2016) (Recruiting) NCT00052479 (2017) (Recruiting) NCT02211649 (2014) (Active, not recruiting) NCT02678338 (2016) (Recruiting) NCT02963782 (2016) (Recruiting) NCT03503683 (2018) (Recruiting) NCT02663518 (2016) (Recruiting) | B-cell Non-Hodgkin’s Lymphoma Haematological Malignancies Haematological Malignancies Haematological Malignancies Haematological Malignancies Colorectal Cancer Refractory Lymphoma, Myeloma Hematologic Malignancies and Selected Solid Tumors | Unknown |
| CD47 Hu5F9-G4 mAb | Hu5F9-G4 mAb | NCT003013218 (2017) (Recruiting) | Solid Tumors and Lymphoma | Unknown |
| TTI-621 Small molecule | Small molecule | NCT00351233 (2018) (Recruiting) NCT02267196 (2015) (Recruiting) NCT03763149 (2018) (Not yet recruiting) NCT03717103 (2018) (Recruiting) | Solid and Hematologic Cancers Solid and Hematologic Cancers Malignant Tumors and Lymphomas Advanced Malignancies | Unknown |
| ALX148 Small molecule | Small molecule | NCT03013218 (2017) (Recruiting) | Solid Tumors and Lymphoma | Unknown |
| SRF231 mAb | SRF231 mAb | NCT03512340 (2018) (Recruiting) | Solid and Hematologic Cancers Solid and Hematologic Cancers | Unknown |
| CC-90002 mAb | CC-90002 mAb | NCT03761178 (2018) (Recruiting) | Malignant Tumors and Lymphomas | Unknown |
| IBI188 mAb | IBI188 mAb | NCT03717103 (2018) (Recruiting) | Malignant Tumors and Lymphomas | Advanced Malignancies |
chemotherapy was also shown to be well-tolerated and led to a tumor response (Nywening et al., 2016).

### Reprogramming of TAMs Toward an Antitumoral Phenotype

As mentioned previously, TAMs can exist in different functional states between the M1 and M2 phenotypes, making them highly heterogeneous and plastic cells (Biswas and Mantovani, 2010). Thus, they can be either pro- or anti-tumoral (Wynn et al., 2013). Reprogramming the TAMs toward a tumoricidal or a tumor-inhibition state may be a plausible therapeutic strategy. Different strategies are being studied in the clinic. These are reported in **Table 2** (please refer also to **Box 2**).

#### Inhibition of CD47

Inhibition of CD47 is a strategy that can facilitate phagocytosis of tumor cells by macrophages. Indeed, CD47 expressed by cancer cells inhibits phagocytosis through its interaction with signal

---

**Table 2 | Clinical trials with toll-like receptor (TLR) agonists for macrophages reprogramming.**

| Target | Drugs | Inhibitor type | Clinical trial | Tumor type | Benefit |
|--------|-------|----------------|----------------|------------|---------|
| CD40   | APX005M mAb | NCT03502330 (2018) (Recruiting) | Non-small Cell Lung Cancer, Renal Cell Carcinoma | Unknown |
|        |       | NCT02482168 (2015) (Active, not recruiting) | Solid tumors | Unknown |
|        |       | NCT03123783 (2017) (Recruiting) | Non-small Cell Lung Cancer or Metastatic Melanoma | Unknown |
|        |       | NCT03389802 (2016) (Recruiting) | Pediatric CNS Tumors | Unknown |
|        |       | NCT03165994 (2017) (Recruiting) | Resectable Esophageal and Gastroesophageal Junction Cancers | Unknown |
|        | Selicrelumab mAb | NCT02304393 (2014) (Recruiting) | Locally Advanced and/or Metastatic Solid Tumors | Unknown |
|        | ChILob 7/4 mAb | NCT01561911 (2012) (Completed) | Non-Hodgkin Lymphoma | Unknown |
|        | CP-870,893 mAb | NCT00607048 (Completed) | Non-Hodgkin Lymphoma | Unknown |
|        | CDX-1140 Small molecule | NCT03329950 (Recruiting) | Advanced Malignancies | Unknown |
| TLR7   | LHC165 Small molecule | NCT03301896 (2017) (Recruiting) | Advanced Malignancies | Unknown |
|        | Imiquimod Small molecule | NCT01421017 (2011) (Completed) | Breast Cancer With Skin Metastases | Well tolerated. |
|        |       | NCT00895674 (2009) (Completed with results) | Chest Wall Recurrence or Skin Metastases | Partial response: tumor regression and immune response |
|        | NKTR-262 Small molecule | NCT03435640 (2018) (Recruiting) | Locally Advanced or Metastatic Solid Tumor Malignancies | Unknown |
|        | IMO-8400 Small molecule | NCT02252146, (Completed with results) | Diffuse Large B Cell Lymphoma (DLBCL) | Lack of efficacy |
|        | Resiquimod Small molecule | NCT00821652 (2009) (Completed) | Surgically resected Stage IIIB, IIC, Stage III or Stage IV (AJCC criteria) Melanoma | Unknown |
|        | DSP-0509 Small molecule | NCT03416335 (2018) (Recruiting) | Advanced Solid Tumors | Unknown |
| TLR8   | VTX-2337 Small molecule | NCT02431559 (2015) (Completed) | Platinum-Resistant Ovarian Cancer | Unknown |
|        |       | NCT01294293, (Completed) | Ovarian Epithelial, Fallopian Tube, or Peritoneal Cavity Cancer | Unknown |
|        |       | NCT01334177, (Completed) | Ovarian Epithelial, Fallopian Tube, or Peritoneal Cavity Cancer | Unknown |
|        |       | NCT02452697 (2015) (Recruiting) | Myeloid and Lymphoid Malignancies | Unknown |
| TLR9   | EMD 1201081 Small molecule | NCT01408382 (2009) (Completed with results) | Recurrent or Metastatic Squamous Cell Carcinoma of the Head and Neck | EMD 1201081 was well tolerated in combination with cetuximab, but no clinical efficacy was observed Ruzsa et al., 2014 |
|        |       | NCT02452697 (2015) (Recruiting) | Myeloid and Lymphoid Malignancies | Unknown |
|        | DUK-CPG-001 Small molecule | NCT02452697 (2015) (Recruiting) | Metastatic Colorectal Cancer | Unknown |
|        | IMO-2055 Small molecule | NCT00719199 (2008) (Completed) | Colorectal Cancer | NSCLC |
|        |       | NCT00635529 (2008) (Completed) | NSCLC | Unknown |
|        | CMP-001 Small molecule | NCT03619641 (2015) (Recruiting) | Stage IIIB/C/D Melanoma Patients With Clinically Apparent Lymph Node Disease | Unknown |
|        | SD-101 Small molecule | NCT03507699 (2018) (Recruiting) | Hormone-Naïve Oligometastatic Prostate Cancer | Well tolerated |
|        |       | NCT03007732 (2017) (Recruiting) | Low-Grade B-Cell Non-Hodgkin Lymphoma | but progression of the tumor was observed |
|        |       | NCT03410901 (Recruiting) | Refractory Grade 1-3A Follicular Lymphoma | |
|        |       | NCT02929764 (2016) (Recruiting) | Recurrent Low-Grade B-Cell Lymphoma | |
|        |       | NCT02254772 (2014) (Completed with results) | | |

---

Grégoire et al. Targeting Macrophages in Glioblastoma

Frontiers in Pharmacology | www.frontiersin.org April 2020 | Volume 11 | Article 368
regulatory protein-α (SIRPα) expressed by macrophages thus sending out a “do not eat me” signal. Alternatively, CD47 can serve as a receptor for thrombospondin 1 (TSP1) to trigger specific signaling. Many tumors are described to overexpress CD47 (Zhang et al., 2015; Zhao et al., 2016). Inhibition of CD47 in a preclinical model showed a modification of microglia phenotypes in GB that was correlated with better survival (Hutter et al., 2019). Furthermore, in vivo, the anti-CD47 treatment is able to shift the macrophage phenotype toward an M1 type (Zhang et al., 2016) and induces anti-tumor effects (Li F. et al., 2017). The preclinical study of Hu5F9-G4 in pediatric malignant primary brain model demonstrated that this CD47 inhibitor is a safe and effective therapeutic agent (Gholamini et al., 2017). Hu5F9-G4 was also shown to be well tolerated in a clinical trial (Sikic et al., 2018) (NCT02216409, Table 2). TTI-621, a small molecule inhibiting CD47, is being investigated in an ongoing clinical trial. Interestingly, however, it has recently been observed that CD47 inhibition may result in cancer cell resistance to chemotherapy through escape to senescence (Guillon et al., 2019).

Activation of CD40

CD40 is expressed on monocytes, macrophages, dendritic cells, and B cells. It is a receptor that belongs to the TNF receptor superfamily. Many clinical trials targeting CD40 notably through agonistic or activating antibodies are ongoing (Table 3). In a mouse model, targeting CD40 was useful in producing antitumor effects that greatly improved the overall survival (Shoji et al.,

### Table 3 | Clinical trials using drugs to deplete macrophages from the tumor’s microenvironment.

| Target | Drugs | Inhibitor type | Clinical trial | Benefit |
|--------|-------|----------------|----------------|---------|
| CSF1R  | Pexidartinib | Small molecule | NCT02777710 (2016) (Recruiting) | Metastatic/Advanced Pancreatic or Colorectal Cancers | Unknown |
|        | DCC-3014 | Small molecule | NCT03089469 (2017) (Recruiting) | Advanced Malignancies | Unknown |
|        | LY30228655 | mAb | NCT03153410 (2017) (Recruiting) | Pancreas Adenocarcinoma | Unknown |
|        | | | NCT02718911 (2016) (Completed) | Advanced Solid Tumors | Melanoma |
|        | | | NCT03101254 (2017) (Recruiting) | | |
|        | PLX3397 | Small molecule | NCT01004861 (2009) (Completed) | Solid Tumors | Unknown |
|        | | | NCT02452424 (2015) (Completed) | Melanoma and Other Solid Tumors | Unknown |
|        | | | NCT01349036 (2011) (Completed) | Recurrent Glioblastoma | Unknown |
|        | | | NCT02371369 (2015) (Active, not recruiting) | Pigmented Villonodular Synovitis (PVNS) or Giant Cell Tumor of the Tendon Sheath (GCT-TS) | Unknown |
|        | MCS110 | Small molecule | NCT08689477 (2018) (Not yet recruiting) | Gastric Cancer | Unknown |
|        | IMC-CS4 | Small molecule | NCT01346558 (2011) (Completed) | Advanced Solid Tumors | Unknown |
|        | Cabiralizumab | mAb | NCT03697564 (2018) (Not yet recruiting) | Stage IV Pancreatic Cancer | Unknown |
|        | | | NCT02526017 (2015) (Active, not recruiting) | | |
|        | SNDX-6352 | mAb | NCT03238027 (2017) (Recruiting) | Solid Tumors | Unknown |
|        | JNJ-45246527 | Small molecule | NCT03557970 (2018) (Not yet recruiting) | Acute Myeloid Leukemia | Unknown |
|        | ARRY-382 | Small molecule | NCT02881371, (Recruiting) | Acute Myeloid Leukemia | Unknown |
|        | | | NCT01316822 (2011) (Completed) | Advanced or Metastatic Cancers | Unknown |
|        | BLZ945 | Small molecule | NCT02829723 (2016) (Recruiting) | Advanced Solid Tumors | Unknown |
|        | RO5509554 | Small molecule | NCT01494688 (2011) (Completed) | Advanced Solid Tumors | Unknown |
|        | NA | Clodronate | Bisphosphonate | NCT01198457 (2010) (Completed) | Breast Neoplasms, Prostatic Neoplasms, Multiple Myeloma | Treatment with clodronate suggests a benefit in recurrence rates for postmenopausal women with breast cancer (Paterson et al., 2012) |
|        | | | NCT00009945 (2010) (2003) (Completed with results) | Stage I or Stage II Breast Cancer | Unknown |
|        | | | NCT00009942 (2009) (Completed) | Bone neoplasms | Unknown |
|        | | | NCT00009232 (2004) (Completed) | Hormone Refractory Metastatic Prostate Cancer | Unknown |
|        | | | NCT00127205 (2005) (Active, not recruiting) | Primary Breast Cancer | Unknown |
|        | Zoledronate | Bisphosphonate | NCT00301873 (2006) (Completed, has results) | Primary Malignant Glioma | Unknown |
|        | | | NCT00885326 (2009) (Active, not recruiting) | High-Risk Neuroblastoma | Unknown |
|        | | | NCT01346019 (2011), (Active, not recruiting) | Multiple Myeloma | Unknown |
responses after treatment (Tap et al., 2015) (NCT01004861). PLX3397 was also well tolerated and showed anti-tumor activity associated with tumor regression and increased lymphocytic infiltration. Imiquimod has been tested. It was well tolerated and showed possible antitumor activity when combined with erlotinib and bevacizumab (Smith et al., 2014) (NCT00633529).

In glioma (Pyonteck et al., 2013), another small molecule inhibitor of CSF1R, can alter the polarization of TAMs (Butowski et al., 2016) (NCT01349036). BLZ945, another small molecule agonist 852A was also well tolerated with reversible side effects in glioma (Pyonteck et al., 2013). It is currently being assessed in clinical trials.

Table 2

For example, the TLR7 agonist 2C11 expression in TAMs associated with a decrease in c-Myc mRNA levels. GB-derived exosomes were shown to modify the expression of cell surface proteins and cytokines (IL-6 and VEGF), and to increase phagocytic activity in macrophages (De Vrij et al., 2015). Also, blood samples from patients with GB were analyzed and shown to harbor GB-derived exosomes containing immunoglobulin (Ig) G2 and IgG4 antibody isotypes (Harshyne et al., 2016). Those exosomes were able to induce the expression of CD163, associated with the M2 phenotype. Exosomes appear to be important for the communication between tumor cells and TAMs in GB. As key players from the tumor ecosystem, targeting them may impair the regulatory effects of GB cells on TAM immunosuppressive properties.

### Depletion of TAMs

The activation of TAMs is dependent on the CSF1R signaling pathway. Therefore, CSF1R may be a way to target macrophages specifically. Many small molecules and antibodies were developed against CSF1R, and numerous clinical trials have been completed or are ongoing (Table 3). PLX3397 is a small molecule targeting CSF1R, it reduced the number of TAMs in a preclinical GB model and showed an antimetastic activity (Coniglio and Segall, 2013; Yan et al., 2017). In clinical studies, PLX3397 was also well tolerated and showed anti-tumor responses after treatment (Tap et al., 2015) (NCT01004861). PLX3397 was also well tolerated but showed no efficacy in GB (Butowski et al., 2016) (NCT01349036). BLZ945, another small molecule inhibitor of CSF1R, can alter the polarization of TAMs in glioma (Pyonteck et al., 2013). It is currently being assessed in a clinical trial.

Another way to deplete the number of TAMs in the tumor is to use bisphosphonates. They are described for both direct and indirect anti-tumor effects such as induction of tumor apoptosis and inhibition of cell adhesion. More importantly, they alter the behavior of TAMs (Van Acker et al., 2016). Bisphosphonates are divided in two classes depending on their structure and mechanism of action. Cladronate belongs to the first group while zoledronate belongs to the second group. Both zoledronate and clodronate are still being assessed in clinical trials (Table 3).

### CONCLUSION

In GB microenvironment, both resident and peripheral macrophages are present and there is an urgent need to understand their specific roles in tumor progression and resistance to treatment. It is obvious that macrophages may be a useful target to improve the outcome of cancer. Currently, many drugs targeting macrophages are being tested in the clinic. However, only a few are tested specifically in GB. The immune landscape in GB, and in cancer in general, has to be investigated further as there is a lack of efficacy in the clinic when only TAMs are targeted. The targeting of TAMs must be implemented hand in hand with the standard treatment to potentially improve the overall effect. In summary, TAMs seem to be a promising target to overcome resistance that arises in GB.

### AUTHOR CONTRIBUTIONS

HG, LR and EG wrote the manuscript. FH and EG contributed to the conception and design of the work. HG, LR, AR, MC, YD, PJ, FH, and EG contributed to manuscript amendments and revisions. All authors read and approved the submitted version.

### FUNDING

This work was supported by the French national research agency (ANR) through the LabEx IRON "Innovative Radiopharmaceuticals in Oncology and Neurology" as part of the French government “Investissements d’Avenir” program (ANR-11-LABX-0018). It was also supported by the ANR under the frame of EuroNanoMed III (project GLIOSILK). The work was additionally funded by the "Institut National de la Santé et de la Recherche Médicale" (INSLRM) and by the University of Angers (Angers, France). It was also related to: (i) the PL-BIO 2014-2020 INCa (Institut National du Cancer) consortium MARENGO <<: MicroRNA agonist and antagonist Nanomedicines for..."
Glioblastoma treatment: from molecular programmation to preclinical validation>>, (ii) to the MuMoFRAIT project <<Multi-scale Modeling & simulation of the response to hypo-Fractionated Radiotherapy or repeated molecular radiation Therapies>> supported by “La Région Pays-de-la-Loire” and by the Cancéropôle Grand-Ouest (Vectorization, imaging and radiotherapies network), (iii) the LabEx IGO and the ANR through the investment of the future program ANR-11-LABX-0016-01, (iv) the SIRIC ILLIAD program supported by INCa, and (v) the Ministry of Health and the Institute for Health and Medical Research (Inserm) (contract INCa-DGOS-Inserm_12558). HG and LR were PhD fellows funded by the LabEx IRON and by the LabEx IRON-2 and the University of Angers, respectively.

REFERENCES

Achut, B. R., Angara, K., Jain, M., Borin, T. F., Rashid, M. H., Iskander, A. S. M., et al. (2017). Canonical NFkB signaling in myeloid cells is required for the glioblastoma growth. Sci. Rep. 7, 1–12. doi: 10.1038/s41598-017-14079-4

Adams, S., Kozhaya, L., Martinuik, F., Meng, T., Chiriboga, L., Liebes, L., et al. (2013). Rejection of Skin Metastases in Patients With Breast Cancer. Clin. Cancer Res. 18, 6748–6757. doi: 10.1158/1078-0432.CCR-12-1149

Arsic, N., Gadea, G., Lagerqvist, E. L., Busson, M., Caluzac, N., Brock, C., et al. (2015). The p53 isoform Δ133p53β promotes cancer stem cell potential. Stem Cell Rep. 4, 331–540. doi: 10.1016/j.stemc.2015.02.001

Atri, C., Guerfali, F. Z., and Laouini, D. (2018). Role of Human Macrophage Polarization in Inflammation during Infectious Diseases. Int. J. Mol. Sci. 19, 1801. doi: 10.3390/ijms19061801

Badie, B., Schartner, J., Vorpahl, J., and Preston, K. (2000). Interferon-β in Cerebral Tumors. J. Neuroonc. 45, 35655–35665. doi: 10.18632/oncotarget.26273

Carroll, R. G., Zaslona, Z., Galván-Peña, S., Koppe, E. L., Sévin, D. C., Angiari, S., et al. (2018). An unexpected link between fatty acid synthase and cholesterol synthesis in proinflammatory macrophage activation. J. Biol. Chem. doi: 10.1074/jbc.RA118.001921

Chae, M., Peterson, T. E., Balgeman, A., Chen, S., Zhang, L., Renner, D. N., et al. (2015). Increasing glioma-associated monocytes leads to increased intratumoral and systemic myeloid-derived suppressor cells in a murine model. Neuro. Oncol. 17, 978–991. doi: 10.1093/neuonc/nou343

Chang, A. L., Misra, J., Wainwright, D. A., Dey, M., Rivetta, C. V., Yu, D., et al. (2016). CCL2 Produced by the Glioma Microenvironment Is Essential for the Recruitment of Regulatory T Cells and Myeloid-Derived Suppressor Cells. Cancer Res. 76, 5671–5682. doi: 10.1158/0008-5472.CAN-16-0144

Charles, N. A., Holland, E. C., Gilbertson, R., Glass, R., and Kettenmann, H. (2011). The brain tumor microenvironment. Glia 59, 1169–1180. doi: 10.1002/glia.21136

Chen, Z., and Hambardzumyan, D. (2018). Immune Microenvironment in Glioblastoma Subtypes. Front. Immunol. 9, 1004. doi: 10.3389/fimmu.2018.01004

Chen, Z., Feng, X., Herting, C. J., Garcia, V. A., Nie, K., Pong, W. W., et al. (2017). Cellular and Molecular Identity of Tumor-Associated Macrophages. Cancer Cell 37, 2266–2278. doi: 10.1016/j.ccell.2017.12.021

Coniglio, S. J., and Segall, J. E. (2013). Review: Molecular mechanism of microglia stimulated glioblastoma invasion. Matrix Biol. 32, 372–380. doi: 10.1016/j.matbio.2013.07.008

Cui, X., Morales, R. T. T., Qian, W., Wang, H., Gagner, J. P., Dolgalev, I., et al. (2018). Hacking macrophage-associated immunosuppression for regulating glioblastoma angiogenesis. Biomaterials 161, 164–178. doi: 10.1016/j.biomaterials.2018.01.053

De Vrij, J., Niek Maas, S. L., Kwappenberg, K. M. C., Schnoor, K., Kleinj, A., Dekker, L., et al. (2015). Glioblastoma-derived extracellular vesicles modify the phenotype of monocytic cells. Int. J. Cancer 137, 1630–1642. doi: 10.1002/ijc.29524

Deborde, S., Hall, A., Wong, R. J., Deborde, S., Omelchenko, T., Lyubchik, A., et al. (2016). Schwann cells induce cancer cell dispersion and invasion Find the latest version : Schwann cells induce cancer cell dispersion and invasion. J. Clin. Invest. 126, 1538–1554. doi: 10.1172/JCI82658

Deng, L., Stafford, J. H., Liu, S. C., Chernikova, S. B., Merchant, M., Recht, L., et al. (2017). SDF-1 Blockade Enhances Anti-VEGF Therapy of Glioblastoma and Can Be Monitored by MRI. Neoplasia (United States) 19, 1–7. doi: 10.1080/15280040.2016.110110

Doan, N. B., Nguyen, H. S., Albajada, H. S., Jaber, B., Al-Gazwiy, M. M., Ahn, E.-Y. E., et al. (2018). Identification of radiation responsive genes and transcriptome profiling via complete RNA sequencing in a stable radiosensitive U87 glioblastoma model. Oncotarget 9, 23532–23542. doi: 10.18632/oncotarget.25247

Dudek, A. Z., Yunis, C., Harrison, L. I., Kumar, S., Hawkinson, R., Cooley, S., et al. (2007). First in human phase I trial of 852A, a novel systemic toll-like receptor 7 agonist, to activate innate immune responses in patients with advanced cancer. Clin. Cancer Res. 13, 7119–7125. doi: 10.1158/1078-0432.CCR-07-1443

Feng, X., Zulewkszy, F., Verevanian, A., Chen, Z., Heinzmann, D., Rasmussen, R. D., et al. (2015). Loss of CX3CR1 increases accumulation of inflammatory monocytes and promotes gliomagenesis. Oncotarget 6, 15077–15094. doi: 10.18632/oncotarget.3730

Feng, Y., Mu, R., Wang, Z., Xing, P., Zhang, J., Dong, L., et al. (2019). A toll-like receptor agonist mimicking microbial signal to generate tumor-suppressive macrophages. Nat. Commun. 10, 2272. doi: 10.1038/s41467-019-10354-2

Finn, O. J. A. (2018). Believer’s Overview of Cancer Immunosurveillance and Immunotherapy. J. Immunol. 200, 385–391. doi: 10.4049/jimmunol.1701302

Flavahan, W. A., Nakano, I., Rich, J. N., Otvos, B., Silver, D. J., Snyuk, M., et al. (2016). Cancer Stem Cell-Secreted Macrophage Migration Inhibitory Factor
Stimulates Myeloid Derived Suppressor Cell Function and Facilitates Glioblastoma Immune Evasion. *Stem Cells* 34, 2026–2039. doi: 10.1002/stem.2393

Gabrusiewicz, K., Li, X., Wei, J., Hashimoto, Y., Marietta, A. L., Ott, M., et al. (2018). Glioblastoma stem cell-derived exosomes mediate M2 macrophages and PD-L1 expression on human monocytes. *Oncoimmunology* 7 (e1412909), 1–10. doi: 10.21263/2017.1412909

Gagner, J. P., Sarfraz, Y., Ortenzi, V., Alotabi, F. M., Chiriboga, L. A., Tayyib, A. T., et al. (2017). Multifaceted C-C-X Chemokine Receptor 4 (CXCR4) Inhibition Interferes with Anti–Vascular Endothelial Growth Factor Therapy–Induced Glioma Dissemination. *Am. J. Pathol.* 187, 2080–2094. doi: 10.1016/j.ajpath.2017.04.020

Gholamin, S., Mitra, S. S., Feroze, A. H., Liu, J., Kahn, S. A., Zhang, M., et al. (2017). Disrupting the CD47-SIRPα anti-phagocytic axis by a humanized anti–CD47 antibody is an efficacious treatment for malignant pediatric brain tumors. *Sci. Transl. Med.* 9, 1–14. doi: 10.1126/scitranslmed.aaf2968

Ginhous, F., and Guillon, J. (2018). Tissue-Resident Macrophage Ontogeny and Homeostasis. *Immunity* 44, 439–449. doi: 10.1016/j.immuni.2016.02.024

Ginhous, F., Lim, S., Hoevel, G., Low, D., and Huber, T. (2013). Origin and differentiation of microglia. *Front. Cell. Neurosci.* 7, 45. doi: 10.3389/fncel.2013.00045

Graeber, M. B., Scheithauer, B. W., and Kreutzberg, G. W. (2002). Microglia in brain tumors. *Glia* 40, 252–259. doi: 10.1002/glia.10147

Guillon, J., Petit, C., Moreau, M., Toutain, B., Henry, C., Roché, H., et al. (2019). Regulation of senescence escape by TSP1 and CD47 following chemotherapy treatment. *Cell Death Dis.* doi: 10.1038/s41419-019-1406-7

Gupta, K., and Burns, T. C. (2018). Radiation-Induced Alterations in the Recurrent Glioblastoma Microenvironment: Therapeutic Implications. *Front. Oncol.* 8, 503. doi: 10.3389/fonc.2018.00503

Hambardzumyan, D., Gutmann, D. H., and Kettenmann, H. (2015). The role of microglia and macrophages in glioma maintenance and progression. *Nat. Neurosci.* 19, 20. doi: 10.1038/nn.4185

Hanahan, D., Weinberg, R. A., Adams, J. M., Cory, S., Aguirre-Ghiso, J. A., Lonning, S. M., et al. (2012). Resistance of glioblastoma-initiating cells to temozolomide resistance. *J. Neurooncol.* 100, 177–186. doi: 10.1007/s11060-011-0787-8

Klopper, J., Riedemann, L., Amoorgaz, Z., Seano, G., Susek, K., Yu, V., et al. (2016). Ang-2/VEGF bispacific antibody reprograms macrophages and resident microglia to anti-tumor phenotype and prolongs glioblastoma survival. *Nat. Proc. Nat. Acad. Sci.* 113, 4476 LP–4481. doi: 10.1073/pnas.1523630113

Kohanbash, G., McKaveney, K., Sakaki, M., Ueda, R., Mintz, A. H., Amanokur, N., et al. (2013). GM-CSF promotes the immunosuppressive activity of glioma-infiltrating myeloid cells through interleukin-4 receptorα. *Cancer Res.* 73, 6413–6423. doi: 10.1158/0008-5472.CAN-12-4124

Komohara, Y., Ohnishii, K., Kuratsu, J., and Takeya, M. (2008). Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas. *J. Pathol.* 216, 15–24. doi: 10.1002/path.2370

Kong, B., Michalski, C. W., Friess, H., and Kleeff, J. (2010). Surgical procedure as an inducer of tumor angiogenesis. *Exp. Oncol.* 32, 186–189.

Kumar, R., De Mooij, T., Peterson, T. E., Kaptzan, T., Johnson, A. J., Daniels, D. J., et al. (2017). Modulating glioma-mediated myeloid-derived suppressor cell development with sulforaphane. *PloS One* 12, 1–26. doi: 10.1371/journal.pone.0179012

Latha, K., Yan, J., Yang, Y., Gressot, L. V., Kong, L.-Y., Manym, G., et al. (2018). The Role of Fibrinogen-Like Protein 2 on Immunosuppression and Malignant Progression in Glioma. *JNCI J. Nat. Cancer Inst.* 111, 1–9. doi: 10.1093/jnci/djy070

Leblond, M. M., Persé, E. A., Helaine, C., Gérault, A. N., Moulin, D., Anfray, C., et al. (2017). M2 macrophages are more resistant than M1 macrophages following radiation therapy in the context of glioblastoma. *Oncotarget* 8, 72597–72612. doi: 10.18632/oncotarget.19994

Lee, J., Dang, X., Borbo, A., Coimbra, R., Baird, A., and Ethier, B. P. (2015). Thrombin-processed Egr4 recruits myeloid cells and induces antitumorigenic inflammation. *Neuro Oncol.* 17, 685–696. doi: 10.1093/neo/nuu302

Li, Z., Huang, Q., Chen, H., Lin, Z., Zhao, M., and Jiang, Z. (2017). Interferon Regulatory Factor 7 Promoted Glioblastoma Progression and Stemness by Modulating IL-6 Expression in Microgliosis. *J. Cancer* 8, 207–219. doi: 10.7150/jca.16415

Li, T.-F., Li, K., Wang, C., Liu, X., Wen, Y., Xu, Y.-H., et al. (2017). Harnessing the cross-talk between tumor cells and tumor-associated macrophages with a nanodrug for modulation of glioblastoma immune microenvironment. *J. Control. Release* 268, 128–146. doi: 10.1016/j.jconrel.2017.10.024

Li, F., Lv, B., Liu, Y., Hua, T., Han, J., Sun, C., et al. (2017). Blocking the CD47–SIRPα axis by delivery of anti–CD47 antibody induces antitumor effects in glioma and glioma stem cells. *Oncoimmunology* 7, e1391973–e1391973. doi: 10.1080/2162402X.2017.1391973

Li, Y., Dong, X., Cai, J., Yin, S., Sun, Y., Yang, D., et al. (2018). SERPINA3 induced by astrogliosis/microglia co-culture facilitates glioblastoma stem-like cell invasion. *Oncol. Lett.* 15, 285–291. doi: 10.3892/ol.2017.7275
NCT02367196 (2015). A Phase 1, Dose Finding Study of CC-90002 in Subjects
Grégoire et al. Targeting Macrophages in Glioblastoma
NCT02452424 (2015). A Combination Clinical Study of PLX3397 and
NCT02502330 (2018). APX005M With Nivolumab and Cabiralizumab in
NCT02451091 (2017). TLR9 Agonist SD-101, Ibrutinib, and Radiation Therapy in
NCT02431559 (2015). A Phase 1/2 Study of Motolimod (VTX-2337) and
NCT02431559 (2015). A Phase 1 Study of Molotomod (VTX-2337) and
NCT02482168 (2015). Study of the CD40 Agonistic Monoclonal Antibody
NCT02452424 (2015). A Combination Clinical Study of PLX3397 and
NCT02718911 (2016). A Study of LY3022855 in Patients With Advanced Malignancies and Selected Solid Tumors. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02723938
NCT02732938 (2016). Phase 2 study of PF-04136309 in combination with gem
NCT02777710 (2016). Evaluation of Safety and Activity of an Anti-PD1 Antibody (DURVALUMAB) Combined With CSF-1R TKI (PEXIDARTINIB) in Patients With Metastatic/Advanced Pancreatic or Colorectal Cancers (MEDIPLEX). ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02526017
NCT02663518 (2015). A Trial of TT1-621 for Patients With Hematologic Malignancies and Selected Solid Tumors. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02663518
NCT02678338 (2016). CAMELLIA: Anti-CD47 Antibody Therapy in Haematological Malignancies. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02678338
NCT02718911 (2016). A Study of LY3022855 in combination with durvalumab or tremelimumab in participants with advanced solid tumors. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02718911
NCT02723938 (2016). Phb/2 study of PF-04136309 in combination with gem
NCT02953509 (2016). Trial of HuSF9-G4 in combination with rituximab in relapsed/refractory B-cell non-Hodgkin’s lymphoma. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02953509
NCT02953782 (2016). Trial of HuSF9-G4 in combination with cetuximab in patients with solid tumors and advanced colorectal cancer. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT02953782
NCT03007732 (2017). Pembrolizumab in combination with intratumoral sd-101 therapy. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03007732
NCT03013218 (2017). A Study of ALX148 in Patients With Advanced Solid Tumors and Lymphoma. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03013218
NCT03069469 (2017). Study of dcc-3014 in patients with advanced malignancies. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03069469
NCT03101254 (2017). LY3022855 with BRAF/MEK inhibition in patients with melanoma. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03101254
NCT03123783 (2017). CD40 Agonistic Antibody APX005M in combination with nivolumab. ClinicalTrials.gov.
NCT03153410 (2017). Pilot Study With CY, Pembrolizumab, GVAX, and IMC-CS4 (LY3022855) in Patients With Borderline Resectable Adenocarcinoma of the Pancreas. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03153410
NCT03165994 (2017). APX005M With Concurrent Chemoradiation for Resectable Esophageal and gastroesophageal junction cancers. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03165994
NCT03389802 (2018). Phase I Study of APX005M in pediatric CNS tumors. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03389802
NCT03410901 (2018). TLR9 Agonist SD-101, Anti-OX40 Antibody BMS 986178, and Radiation Therapy in treating patients with low-grade B-cell non-Hodgkin lymphomas. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03410901
NCT03416335 (2018). A study of DSP-0509 in patients with advanced solid tumors to determine the safety and the pharmacokinetic profile. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03416335
NCT03435640 (2018). A study of NKTR-262 in combination with NKTR-214 and nivolumab in patients with locally advanced or metastatic solid tumor malignancies (REVEAL). ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03435640
NCT03496662 (2018). BMS-813160 with nivolumab and gemcitabine and nab-paclitaxel in borderline resectable and locally advanced pancreatic ductal adenocarcinoma (PDAC). ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03496662
NCT03502330 (2018). APX005M With nivolumab and cabiralizumab in advanced melanoma, non-small cell lung cancer or renal cell carcinoma. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03502330
NCT03507699 (2018). Combined immunotherapy and radiosurgery for metastatic colorectal cancer. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03507699
NCT03512340 (2018). Study of SRF231 in patients with advanced solid and hematologic cancers. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03512340
NCT03530683 (2018). A trial of tt1-622 in patients with advanced relapsed or refractory lymphoma or myeloma (tt1-622-01). ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03530683
NCT03557970 and NCT02880371 (2018). CSF1R inhibitor JNJ-40346527 in treating patients with cytogenetically defined CML. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03557970 and NCT02880371
NCT03618641 (2018). CMP-001 in combo with nivolumab in stage IIIB/C/D melanoma patients with clinically apparent lymph node disease. ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03618641
non-small cell lung cancer patients who have progressed following chemotherapy. Cancer Immunol. Immunother. 63, 787–796. doi: 10.1007/s00262-014-1547-6

Strachan, D. C., Ruffell, B., Oei, Y., Büssel, M. J., Cousens, L. M., Pryer, N., et al. (2013). CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8+T cells. Oncoimmunology 2, 1–12. doi: 10.4161/onci.26968

Stratton, J. A., Holmes, A., Rosin, N. L., Sinha, S., Vohra, M., Burma, N. E., et al. (2018). Vascular niche IL-6 induces alternative macrophage activation in glioblastoma through HIF-2α. Nat. Commun. 9, 559. doi: 10.1038/s41467-018-03050-0

Wang, Q., He, Z., Huang, M., Liu, T., Wang, Y., Xu, H., et al. (2018). Metallo- and functional reprogramming of myeloid-derived suppressor cells and their therapeutic control in glioblastoma. Cell Stress. 3, 47–65 doi: 10.15698/cst2019.02.176

Wu, J., Frady, L. N., Bash, R. E., Cohen, S. M., Schorzonan, A. N., Yu, Y.-T., et al. (2018). MerTK as a therapeutic target in glioblastoma. Neuro. Oncol. 20, 92–102. doi: 10.1093/neuonc/nox111

Wynn, T. A., Chawla, A., and Pollard, J. W. (2013). Origins and Hallmarks of Macrophages: Development, Homeostasis, and Disease. Nature 496, 445–455. doi: 10.1038/nature12034

Xue, N., Zhou, Q., Ji, M., Jin, J., Lai, F., Chen, J., et al. (2017). Chlorogenic acid inhibits glioblastoma growth through repolarizing macrophage from M2 to M1 phenotype. Sci. Rep. 7, 70021. doi: 10.1038/srep42031

Yin, Y., Qiu, S., Li, X., Huang, B., Xu, Y., and Peng, Y. (2017). EZH2 suppression in glioblastoma shifts microglia toward M1 phenotype in tumor microenvironment. J. Neuroinflammation 14, 220. doi: 10.1186/s12974-017-0993-4

Yuan, D., Zhao, Y., Banks, W. A., Bullock, K. M., Haney, M., Batrakova, E., et al. (2017). Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. Biomaterials 142, 1–12. doi: 10.1016/j.biomaterials.2017.07.011

Zeiner, P. S., Preuss, C., Golebiowska, A., Zinke, J., Iriondo, A., Muller, A., et al. (2018). Distribution and prognostic impact of microglia/macrophage subpopulations in gliomas. Brain Pathol. 29, 513–529. doi: 10.1111/bpa.12690

Zhang, Q., et al. (2012). Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. PloS One 7, e59946–e59946. doi: 10.1371/journal.pone.0059046

Zheng, G., Hu, X., Lian, X., Li, H., Zhang, C., and Samanta, D., et al. (2015). HIF-1 regulates CD47 expression in breast cancer cells to promote evasion of phagocytosis and maintenance of cancer stem cells. Proc. Natl. Acad. Sci. 112, E6215–E6223. doi: 10.1073/pnas.1520032112

Zhang, M., Hutter, G., Kahn, S. A., Azad, T. D., Gholamin, S., Xu, C. Y., et al. (2016). Anti-CD47 Treatment Stimulates Phagocytosis of Glioblastoma by M1 and M2 Polarized Macrophages and Promotes M1 Polarized Macrophages In Vivo. J. Immunol. 197, e0153550. doi: 10.1152/jimmunol.0153550

Zhang, G., Zhang, Y., Cheng, S., Wu, Z., Liu, F., and Zhang, J. (2017). CD133 positive U87 glioblastoma cells-derived exosomal microRNAs in hypoxia-versus normoxia-microenvironment. J. Neurooncol. 135, 37–46. doi: 10.1007/s11060-017-2426-x

Zhang, H., Hu, L., Xiang, L., Bullen, J. W., Zhang, C., and Samanta, D., et al. (2015). CD47 promotes tumor invasion and metastasis in non-small cell lung cancer. Sci. Rep. 6 (29719), 1–11. doi: 10.1038/srep29719

Zhao, X., Qu, J., Sun, Y., Wang, J., Liu, X., Wang, F., et al. (2017). Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature. Oncotarget 8, 30576–30586. doi: 10.18632/oncotarget.15736

Zhang, Y., Yang, W., Adlake, K., He, J., and Lu, Z. (2013). Epidermal growth factor (EGF)-enhanced vascular cell adhesion molecule-1 (VCAM-1) expression promotes macrophage and glioblastoma cell interaction and tumor cell invasion. J. Biol. Chem. 288, 31488–31495. doi: 10.1074/jbc.M113.499020

Zhermakova, A., Garmaeva, S., Fu, J., Chen, L., and Wijemena, C. (2018). A system biology perspective on environment–host–microbe interactions. Hum. Mol. Genet. 27, R187–R194. doi: 10.1093/hmg/ddy137

Zhou, M., Bracci, P. M., McCoy, I. L., Hsuang, G., Wiemels, J. L., Rice, T., et al. (2015). Serum macrophage-derived chemokine/CCL2 levels are associated with glioma risk, CD4 T cell lymphopenia and survival time. Int. J. Cancer 137, 826–836. doi: 10.1002/ijc.29441
Zhou, W., Ke, S. Q., Huang, Z., Flavahan, W., Fang, X., Paul, J., et al. (2015). Periostin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. Nat. Cell Biol. 17, 170–182. doi: 10.1038/ncb3090

Zhou, J., Reddy, M. V., Wilson, B. K. J., Blair, D. A., Taha, A., Frampton, C. M., et al. (2018). MR Imaging Characteristics Associate with Tumor-Associated Macrophages in Glioblastoma and Provide an Improved Signature for Survival Prognostication. Am. J. Neuroradiol. 39, 252 LP–25259. doi: 10.3174/ajnr.A5441

Zhu, C., Chriﬁ, I., Mustafa, D., Van Der Weiden, M., Leenen, P. J. M., Duncker, D. J., et al. (2017). CECR1-mediated cross talk between macrophages and vascular mural cells promotes neovascularization in malignant glioma. Oncogene 36, 5356–5368. doi: 10.1038/onc.2017.145

Zhu, H., Leiss, L., Yang, N., Rygh, C. B., Mitra, S. S., Cheshier, S. H., et al. (2017). Surgical debulking promotes recruitment of macrophages and triggers glioblastoma phagocytosis in combination with CD47 blocking immunotherapy. Oncotarget 8, 12145–12157. doi: 10.18632/oncotarget.14553

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Grégoire, Roncali, Rousseau, Chérel, Delneste, Jeannin, Hindré and Garcion. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.