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Drivers of heterogeneity in the glioblastoma immune microenvironment

Alina Brosque[1] and Dinorah Friedmann-Morvinski[1,2]

Abstract

Glioblastoma is the most common and aggressive primary brain tumor, characterized by a highly complex and heterogeneous tumor immune microenvironment (TIME). In this review, we discuss the impact of tumor-intrinsic and tumor-extrinsic drivers that contribute to heterogeneity in the adult glioblastoma TIME, focusing on four main factors: genetic drivers, sex, age, and standard of care therapy. We describe recent insights into how each of these factors affects key aspects ranging from TIME composition to therapy response, with an emphasis on the cross-talk between tumor and immune cells. Deciphering these local interactions is fundamental to understanding therapy resistance and identifying novel immunomodulatory strategies.

Addresses

[1] School of Neurobiology, Biochemistry and Biophysics, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel
[2] Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

Corresponding author: Friedmann-Morvinski, Dinorah (dino@tauex.tau.ac.il)
(Brosque A.)
(Friedmann-Morvinski D.)

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Introduction

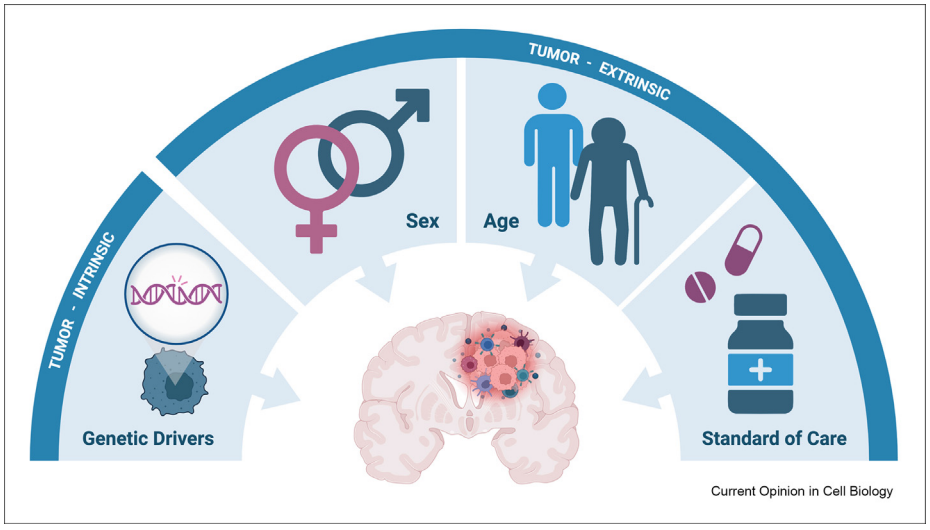
Glioblastoma (GBM) is the most common and aggressive primary brain tumor, with a median overall survival (OS) of approximately 15 months, even with the standard of care (SOC) therapy [1]. One of the hallmarks of GBM is the high degree of heterogeneity, both intertumoral and intratumoral, making it challenging to develop effective treatments [2]. Several promising immunotherapeutic approaches have shown limited and unpredictable success in GBM [3], suggesting that the complexity and heterogeneity of these tumors also extend to the tumor immune microenvironment (TIME).

The immune system in a healthy brain operates within a highly controlled environment to avoid excessive neuroinflammation. Nevertheless, when a tumor develops, a localized struggle arises between immune-suppression, aimed at preserving the brain, and immune-activation targeted at the tumor [4]. The resulting TIME landscape is not the same for all brain tumors, as several studies have shown that primary tumors and brain metastases sculpt the TIME quite differently, from the relative abundance of immune subpopulations, to their localization within the tumor [4–6]. Even though all GBM tumors share some common TIME features, such as being immunologically cold [6], there is still a high degree of heterogeneity determined by various tumor-intrinsic and extrinsic drivers. In this review, we focus on four main factors that contribute to heterogeneity in the adult GBM TIME: tumor genetic drivers, sex, age, and SOC therapy. We will discuss recent findings on how each of these factors affects key aspects such as immune cell infiltration and function, local tumor-immune cell interactions, and response to therapy. Deciphering the role of these factors in shaping the TIME is key to understanding therapy resistance and identifying novel immunomodulatory strategies (Figure 1).

Genetic drivers

GBM tumors are dynamic ecosystems in which malignant cells exist in plastic cellular states and are in constant cross-talk with other components of the microenvironment. Several studies are shedding light on how specific genetic alterations that determine GBM pathogenesis, can also differentially shape the TIME. Syngeneic mouse models of GBM generated by different oncogenic driver mutations are being used to elucidate the longitudinal interactions that lead to TIME heterogeneity [7–10]. For instance, a recent study identified distinct immune landscapes associated with EGFR wild-type and EGFRvIII-driven GBMs. The latter exhibit an accumulation of granulocytic myeloid-derived suppressor cells (gMDSCs) that leads to immune checkpoint blockade therapy (ICB) resistance [7]. GBM-secreted CXCL1, CXCL2, and CXCL3, and gMDSC-expressed CXCR2 form an axis that promotes the output of gMDSCs from the bone marrow (BM). Interestingly, disruption of this axis reverted ICB resistance and led to prolonged survival in mice with EGFRvIII-driven GBM [7].

Figure 1



Drivers of Heterogeneity in the Glioma Immune Microenvironment. Heterogeneity in the adult GBM TIME is determined by several tumor-intrinsic and extrinsic drivers including: tumor genetic drivers, sex, age, and standard of care (SOC) therapy. Created with Biorender.com

Moreover, syngeneic GBM models driven by PDGFB and RAS oncogenes, resembling the historical proneural and mesenchymal molecular subtypes respectively, have revealed profound immune differences, both in the TIME and systemically [8,9]. A recent study by our group showed how only RAS-induced tumors overexpress cytokines and chemokines that actively recruit BM-derived neutrophils [9]. The recruited neutrophils are pre-committed to a protumorigenic phenotype that promotes angiogenesis, through tumor-derived endothelial cell differentiation, and T-cell immunosuppression via arginase-1 and iNOS [9].

Not only neutrophils, but also glioma-associated macrophages/microglia (GAMs) seem to play different roles in PDGFB and RAS driven tumors. GAMs are the most abundant cells in the GBM TIME, including tissue-resident microglia, recruited monocyte-derived macrophages (MDMs) and border-associated macrophages (BAMs) [10–12]. A study focusing on GAMs showed how targeting these cells inhibited the growth of PDGFB- but not RAS-driven GBM [10]. While GAMs in PDGFB-driven gliomas are mainly composed of microglia and play a pro-tumoral role, GAMs in RAS-driven gliomas exhibit expression profiles of BAMs, enriched in antigen-presenting, pro-inflammatory and angiogenic signaling, which may explain the resistance to treatment [10]. Cell–cell interaction analyses revealed that RAS-driven tumors exhibited more extensive TIME communication and enhanced GAM-to-vascular signaling than PDGFB-driven GBM [10].

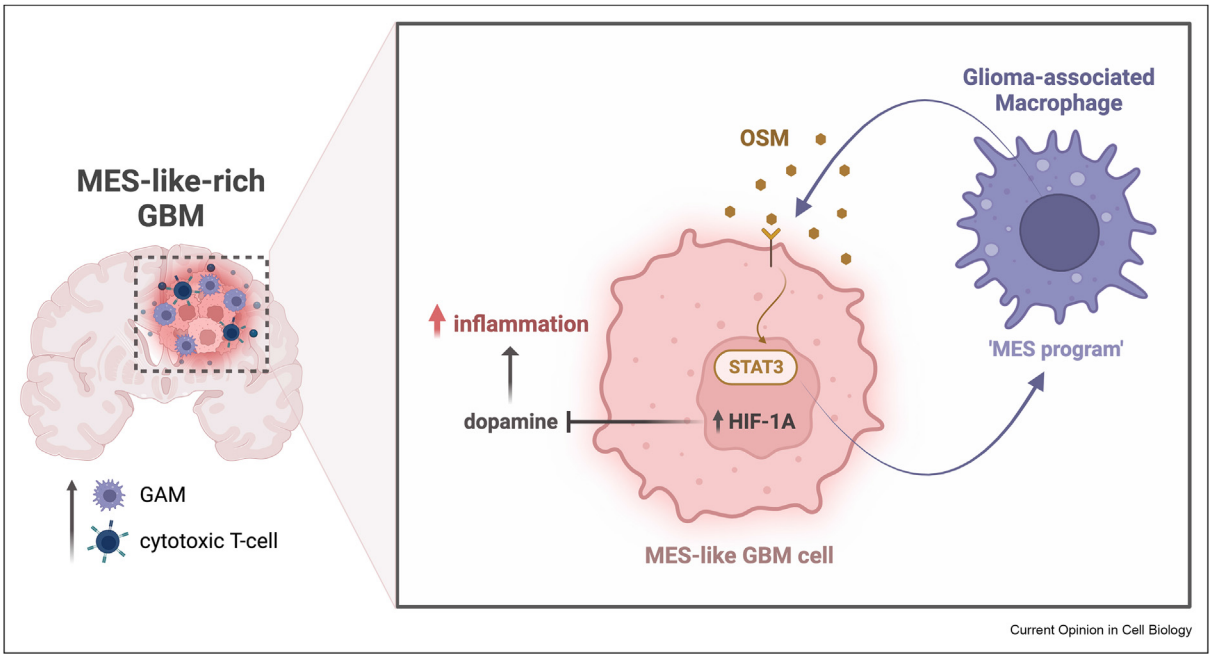
Recently, based on single-cell RNA sequencing (scRNA-seq) data, GBM cells were classified into four plastic states including neural progenitor-like (NPC-like), oligodendrocyte progenitor-like (OPC-like), astrocyte-like (AC-like), and mesenchymal-like (MES-like), which can coexist within the same tumor [13]. Several studies have shed light on how a particular cellular state can distinctly shape the TIME. For example, gliomas enriched with MES-like cells exhibit a more inflammatory TIME. Further research uncovered that increased expression of HIF-1A in MES-like cells may enhance the dopamine degradation process and decrease the expression of dopamine receptor D1, leading to increased inflammasome-mediated inflammation (Figure 2) [14]. A different study described how the environment of MES-like GBM cells contains increased numbers of cytotoxic T-cells and is enriched in GAMs (Figure 2) [15]. Using a GBM mouse model, the authors show how GAMs actually induce the MES-like state through GAM-derived oncostatin M, which binds to its receptors in GBM cells triggering STAT3 signaling. Interestingly, through this interaction, GAMs change their expression profile to a 'MES program', suggesting a reciprocal loop (Figure 2) [15]. The MES-like state has since been subdivided into three variants: hypoxia-related (MES-Hyp), astrocyte-related (MES-Ast), and an intermediate state [16]. As for the TIME, MES-Hyp is associated with GAM abundance, a high MDM/microglia ratio, and overall immunosuppression. In contrast, MES-Ast is associated with a high number of cytotoxic T-cells and general immune activation [16].

Overall, these studies demonstrate how particular genetic alterations and specific transcriptomic signatures, beyond tumor-intrinsic prognostic implications, result in unique interactions within the TME that can be exploited therapeutically.

Sex

Epidemiological data indicates sex-specific differences in GBM incidence and survival, with increased prevalence and worse survival in men [17]. These differences

Figure 2



Interactions in the Tumor Immune Microenvironment of MES-like enriched Glioblastoma. Glioblastomas (GBM) enriched with mesenchymal-like (MES-like) cells have increased numbers of cytotoxic T-cells and glioma-associated macrophages (GAMs) in their tumor immune microenvironment (TIME) [15]. GAMs induce the MES-like state through GAM-derived Oncostatin M (OSM), which binds to its receptors in GBM cells triggering STAT3 signaling. Through this interaction, GAMs change their expression profile to a 'MES program', indicating a reciprocal loop [15]. MES-like cells overexpress HIF-1A, leading to a decrease in dopamine which, in turn, enhances inflammation [14]. Created with BioRender.com.

extend to therapeutic responses, with female patients responding better to SOC [17], while males benefit more from ICB therapy [18]. This supports the idea that sex-specific GBM outcomes are determined not only by tumor-intrinsic factors [17], but also by differences in the TIME. A pan-cancer study analyzing sex differences in immune-related features, classified GBM as a 'strong sex-biased' tumor, with significant differences in immune cells, immune checkpoint genes, and functional pathways according to sex [19]. In addition, recent studies have shown differences in the relative abundance and phenotypes of both myeloid [11,20] and lymphocyte [18] cell subpopulations.

A strong sexually dimorphic phenotype has been described in GBM mouse models for MDSCs subsets [21]. While males display higher levels of monocytic MDSCs in their TIME, females have more gMDSCs in circulation [21]. These differences also imply differential sensitivity to drugs, demonstrating the fundamental clinical importance of understanding how sex shapes the TIME [21]. Another study revealed sex-biased transcriptomic differences in microglia [11]. By performing scRNA-seq of myeloid cells in GBM-bearing mice, they found a more proinflammatory gene signature in female microglia, but higher expression of MHC II-related genes in microglia from males [11]. This was validated in cocultures of human microglia with GBM patient-derived cells of different sex, where female microglia exhibited a more proinflammatory phenotype than male microglia in all cases [20]. JAM-A, a cell-adhesion protein highly overexpressed in glioma stem cells, was described to play a key role in the proinflammatory phenotype of female microglia [22]. Microglia from JAM-A-deficient female mice adopted an anti-inflammatory and pro-tumorigenic profile, which led to increased tumor proliferation [22].

As for MDMs, it was reported that during tumor progression infiltrating monocytes undergo a monocyte-to-macrophage transition in the TIME, characterized by downregulation of IFN-response genes and the acquisition of a tumor-supportive signature with upregulation of MHC II and PD-L1 expression [20]. This process was found to be more pronounced in males, which have higher overall levels of immunosuppressive GAMs, in both mice and humans [20]. Although lymphocytes are much less abundant than GAMs in the TIME, T-cells also appear to be a critical driver of sex differences in GBM. A recent study showed that males have a lower infiltration of T-cells, which are more prone to exhaustion than female T-cells [18]. In this context, GBM-bearing males responded better to anti-PD1 therapy than females. Importantly, the sex differences observed

in mouse T-cells were recapitulated in human GBM samples [18].

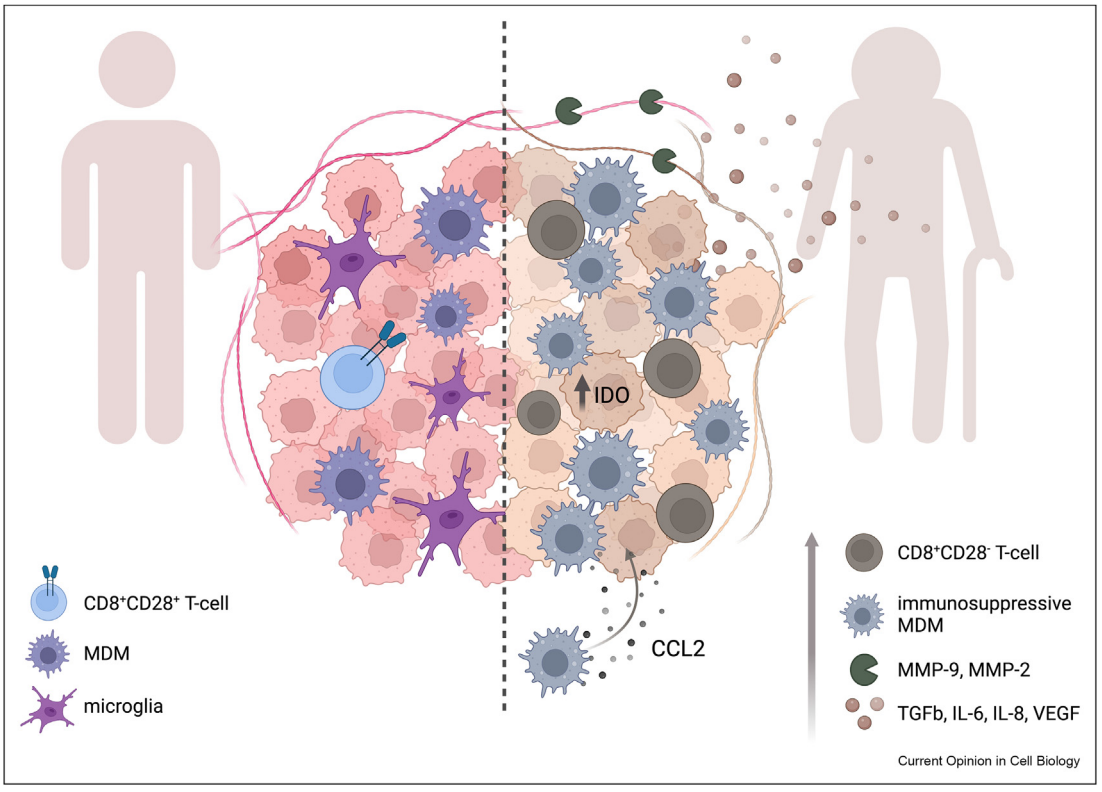
The growing evidence on sex as a diver of heterogeneity in the glioma TIME underscores the need for sex-specific evaluation of therapeutic strategies in both preclinical and clinical settings and discourages the generalization of findings from single-sex studies [11,18].

Age

Advanced age is associated with higher incidence and decreased OS of patients with GBM [23,24]. Nevertheless, older patients with brain metastases do not have a decreased OS compared to their younger counterparts [24], suggesting that there are some unique features of the aging GBM TIME that may be responsible for the poor disease outcomes. Recent evidence supporting the notion that GBM exacerbates the effects of aging on immune dysfunction described how GBM enhances telomere shortening in infiltrating $CD8^+$ T-cells by inducing overexpression of the telomerase-inhibitory protein PIF1 [25]. The same study showed that a senescent subpopulation of $CD8^+$ T-cells, characterized by loss of CD28, was enriched in the blood and TIME of elderly GBM patients when compared with healthy, age-matched adults as well as younger patients (Figure 3) [25]. The $CD8^+CD28^-$ T-cell population is resistant to apoptosis and phenotypically distinct from exhausted $CD8^+$ T-cells since they do not express the checkpoint molecules PD-1 and TIM3, posing a challenge for successful ICB [25].

The association between advanced age and decreased OS in GBM patients and mice treated with ICB was also noted in another study. In this case, immunosuppression was attributed to the age-related increase in the expression of the enzyme indoleamine 2,3 dioxygenase (IDO) in the brain (Figure 3) [24]. In general, IDO suppresses immune responses through both enzymatic function, which converts the essential amino acid tryptophan to kynurenines, and through non-enzymatic intracellular signaling. This leads to increased apoptosis of cytotoxic T-cells and differentiation of naive T-cells into immunosuppressive Treg cells [26]. In GBM in particular, IDO appears to exert immunosuppression independent of its enzymatic function, as pharmacological inhibition failed to improve immunotherapeutic efficacy [24]. In addition to IDO, there is an accumulation

Figure 3



Effects of Aging on the Tumor Immune Microenvironment of Glioblastoma. The TIME of elderly GBM patients becomes more immunosuppressive through several mechanisms, including: (i) the enrichment in a senescent subpopulation of CD8 T-cells characterized by loss of CD28 ($CD8^+CD28^-$) [25], (ii) the age-related increase in the expression of indoleamine 2,3 dioxygenase (IDO) in the brain [24], (iii) the overexpression of CCL2, leading to hyper-accumulation of immunosuppressive monocyte-derived macrophages (MDMs) [27], and (iv) the increase in several other immunosuppressive and pro-tumorigenic factors such as TGFβ, IL-6, IL-8, VEGF and matrix metalloproteinases MMP-2 and MMP-9 [23]. Created with BioRender.com.

of several other immunosuppressive and pro-tumorigenic factors associated with age, termed 'secretory-associated senescent phenotype (SASP) factors', including TGF-β, IL-6, IL-8, VEGF and matrix metalloproteinases MMP-2 and MMP-9 (Figure 3) [23]. In the aged TIME, there is also increased expression of CCL2, leading to hyper-accumulation of immunosuppressive MDMs (Figure 3) [27].

Overall, these studies highlight the importance of considering age as a driver of heterogeneity in the GBM TIME. In general, current preclinical studies do not use age-relevant animal models and thus do not mimic the differential effects of age across the lifespan. Ultimately, a better understanding of age-related changes in the TIME could lead to the development of personalized therapeutic approaches that will also benefit older patients [23].

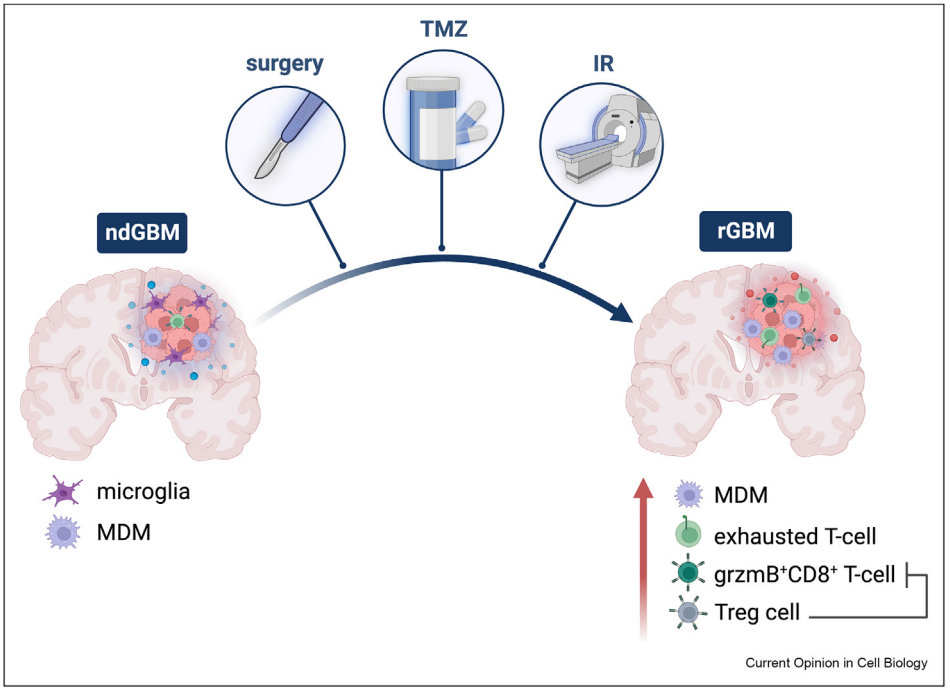
Standard of care therapy

Newly diagnosed (ndGBM) untreated tumors exhibit unique immune cell compositions and activation states compared with recurrent GBM (rGBM), i.e., tumors that reappear after initial treatment. SOC therapy for GBM consists of surgical resection, ionizing radiation (IR), and chemotherapy with temozolomide (TMZ). The extent to which SOC therapy shapes the TIME is still not fully understood, although recent work has shed light on this issue [28].

A closer look into the TIME through mouse and human studies revealed that microglia-derived GAMs were predominant in ndGBM, but were outnumbered by MDMs in rGBM (Figure 4) [28–30], especially in hypoxic tumor regions [29]. This could be a direct consequence of the inflammation induced by therapy, which increases recruitment of monocytes into the tumor. In parallel, the presence of MDMs in hypoxic regions could be attributed to the higher plasticity of these cells, which can adapt to such environments [29]. Regarding lymphocytes, analysis of longitudinal transcriptomic data from the GLASS cohort revealed that T-cells, B lineage cells, and PD-1 expression were significantly enriched in rGBM [34]. Another study also found a significant increase in the proportion of exhausted T-cells (Figure 4) [28].

The mechanisms by which chemoradiotherapy affects the GBM TIME are complex, with both positive and negative effects on anti-tumor immunity. For example, chemoradiotherapy has been reported to induce lymphopenia in nearly one third of GBM patients, which can be exacerbated by the use of high-dose

Figure 4



Changes in the Tumor Immune Microenvironment of Glioblastoma after Standard of Care (SOC) Therapy. Newly diagnosed GBM (ndGBM), exhibit unique immune cell compositions compared with recurrent GBM (rGBM), i.e., tumors that reappear after SOC therapy, which consists of surgical resection, chemotherapy with temozolomide (TMZ), and ionizing radiation (IR). Microglia-derived macrophages are predominant in ndGBM, but are outnumbered by monocyte-derived macrophages (MDMs) in rGBM [28–30]. In rGBM there is also a significant increase in exhausted T-cells [28], granzyme B$^+$ (grzmB$^+$) CD8 T-cells and regulatory T-cells (Tregs) [12]. Created with BioRender.com.

corticosteroids [29]. This correlates with recent findings on TMZ treatment, demonstrating the coexistence of two circuits, one exerting a deleterious influence on peripheral immunity and the other undermining local immunosuppressive mechanisms [32]. In support of the latter, a recent study described how treatment of GBM-bearing mice with high doses of TMZ resulted in a decrease in immunosuppressive MDMs and gMDSCs in the TIME [12]. In the same study, the authors treated GBM-bearing mice with IR alone or in combination with TMZ and found that in both cases treatment promoted the recruitment of granzymeB^{+} CD8^{+} T-cells. However, IR also caused an increase in Treg cells (Figure 4), providing a possible explanation for the transient therapeutic benefit of SOC therapy [12].

A possible mechanism for the IR-induced increase in Treg cells was proposed in a different study [33]. Here the authors describe how IR promotes exosome biogenesis in GBM cells, as well as an enrichment in B7−H4 receptor expression in these exosomes. Exosomal B7−H4, in turn, impairs Th1 differentiation of naive T-cells via inactivation of STAT1 pathway and induction of FoxP3 expression, thus inhibiting T-cell antitumor response [33]. In a different study based on human scRNAseq data, IR therapy was also reported to induce the activator protein 1 (AP1) transcription factor in rGBM [31], which positively regulates genes related to IR resistance (TLK1), invasiveness (TNC, FN1) and inflammation (NFKB1, FYN and IL1B) [28].

Understanding the changes induced by SOC therapy, that lead to TIME heterogeneity between ndGBM and rGBM tumors, can help to guide personalized treatment strategies for rGBM patients and promote the development of novel therapies targeting specific components of the TIME.

Discussion

In recent years, many promising immunotherapies have been assessed in GBM with disappointing outcomes, with therapeutic benefit observed in only a small proportion of patients [3]. This indicates that the heterogeneity of GBM is not limited to tumor cells, but also extends to the TIME. With the advent of scRNA-seq technologies, many studies are working on elucidating the drivers of TIME heterogeneity as well as the mechanisms leading to differential therapeutic response. Recent efforts have focused on classifying gliomas into TIME-specific subtypes that correlate with disease prognosis and predict response to immunotherapy [31,34,35]. One of these studies characterized TME^{high}, TME^{Med}, and TME^{low} subtypes, where TME^{High} GBM tumors displayed elevated transcripts of lymphocytes and immune checkpoint receptors and showed increased overall survival after neoadjuvant administration of anti-PD-1 [31]. Longitudinal assessment of these TIME subtypes also revealed a dynamic nature, with switching of subtypes observed upon tumor recurrence [31]. This type of classification represents a promising starting point for precision immunotherapy approaches, but there is still a need for more in-depth characterization.

One of the limitations of multi-omic strategies based on tumor samples from patients is that they do not allow longitudinal studies from early time points. Since early-stage gliomas are asymptomatic, patients are almost always diagnosed at advanced stages [7], making it difficult to decipher the local interactions that determine TIME heterogeneity. This highlights the importance of using immunocompetent mouse models in preclinical GBM studies, as well as having the right tools for longitudinal assessment of TIME evolution [36−39]. Nevertheless, there is still a need for a more widespread use of sophisticated models to assess the influence of sex and age in particular. Some examples include the 'four core genotypes' (FCG) model [40], which allows to distinguish the effects of chromosomal and gonadal sex, bone marrow chimeras with donors from mismatched sex [18] or age, and accelerated aging models [41].

Animal studies are still limited by the number of variables that can be evaluated simultaneously, and most of the work discussed in this review focuses on a single driver of heterogeneity at a time. In reality, all these variables overlap and create multiple layers of complexity that should be considered as much as possible in order to improve translational outcomes of preclinical studies. For instance, assessment of the interplay between genetic drivers and other factors, such as sex and age, could provide a better reflection of how the TIME is actually shaped. Lastly, we emphasize the importance of taking these aspects into account in the design and evaluation of clinical trials.

Author contribution

Conceptualization and writing: AB and DFM; visualization: AB; supervision and funding acquisition: DFM.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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