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# The Molecular and Microenvironmental Landscape of Glioblastomas: Implications for the Novel Treatment Choices

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Glioblastoma (GBM) is the most frequent and aggressive primary central nervous system tumor. Surgery followed by radiotherapy and chemotherapy with alkylating agents constitutes standard first-line treatment of GBM. Complete resection of the GBM tumors is generally not possible given its high invasive features. Although this combination therapy can prolong survival, the prognosis is still poor due to several factors including chemoresistance. In recent years, a comprehensive characterization of the GBM-associated molecular signature has been performed. This has allowed the possibility to introduce a more personalized therapeutic approach for GBM, in which novel targeted therapies, including those employing tyrosine kinase inhibitors (TKIs), could be employed. The GBM tumor microenvironment (TME) exerts a key role in GBM tumor progression, in particular by providing an immunosuppressive state with low numbers of tumor-infiltrating lymphocytes (TILs) and other immune effector cell types that contributes to tumor proliferation and growth. The use of immune checkpoint inhibitors (ICIs) has been successfully introduced in numerous advanced cancers as well as promising results have been shown for the use of these antibodies in untreated brain metastases from melanoma and from non-small cell lung carcinoma (NSCLC). Consequently, the use of PD-1/PD-L1 inhibitors has also been proposed in several clinical trials for the treatment of GBM. In the present review, we will outline the main GBM molecular and TME aspects providing also the grounds for novel targeted therapies and immunotherapies using ICIs for GBM.

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## INTRODUCTION

Glioblastoma (GBM) is the most common and aggressive primary CNS tumor (Stupp et al., 2009; Louis et al., 2016; Mendes et al., 2018; Altmann et al., 2019; Ostrom et al., 2019) and it has been included in the group of diffuse astrocytic and oligodendroglial tumors by the 2016 CNS WHO (Louis et al., 2016). It is thought that genetic alterations affecting neuroglial stem or progenitors cells give origin to GBM. The incidence of this tumor seems to increase with age; in fact, 62 years is

the median age at diagnosis. Males are affected by GBM tumors 1.7 fold more often than females. According to the presence of mutations in the isocitrate dehydrogenase (*IDH*) 1 and *IDH2* genes GBM is subdivided by the WHO into two major types. More than 90% of GBM cases is constituted by GBM with wild type *IDH* (Louis et al., 2016). Clinically, grade IV lesions (namely primary GBM) are presented *de novo* by the majority of patients, while progression from a less aggressive form of WHO grade II diffuse astrocytomas and WHO grade III anaplastic astrocytomas (i.e., secondary GBM) is shown by a small fraction of patients (5–10%; Ohgaki and Kleihues, 2013; Louis et al., 2016). Primary GBM and secondary GBM differ in prognosis and age of onset. As for overall survival (OS) (Doetsch et al., 1999; Louis et al., 2016), primary GBM is typically diagnosed at older age and has a worse prognosis while secondary GBM are less common and affect people under the age of 45; also they develop into low-grade astrocytoma and are associated with better prognosis (Doetsch et al., 1999; Brennan et al., 2013; Ohgaki and Kleihues, 2013; Louis et al., 2016).

Standard of care first-line treatment is constituted by maximal surgical resection (complete resection is performed quite rarely because of the presence of diffuse infiltrations), followed by radiotherapy with concomitant and adjuvant chemotherapy such as the oral alkylating agent, temozolomide (TMZ). Upon this treatment combination GBM show a median OS of about 15 months (Canoll and Goldman, 2008; Stupp et al., 2009; Ohgaki and Kleihues, 2013; Levine et al., 2015).

The increase of patient survival is small and tumors invariably recur after TMZ (Canoll and Goldman, 2008; Stupp et al., 2009; Ohgaki and Kleihues, 2013; Levine et al., 2015). Following the first recurrence, treatment choices can be represented by further surgical resection when possible, or conventional chemotherapy, e.g., TMZ (with different dosing schedules) or nitrosoureas, or treatment with the anti-vascular endothelial growth factor (VEGF) agent, bevacizumab, or the use of the low-intensity alternating electric fields (TTFields). However, these treatments have not achieved significant improvements in survival (Canoll and Goldman, 2008; Stupp et al., 2009; Chamberlain and Johnston, 2010; Stupp et al., 2012; Ohgaki and Kleihues, 2013; Stupp and Hegi, 2013; Chamberlain, 2015). Moreover, the tyrosine kinase inhibitor (TKI) regorafenib has been introduced in the treatment of recurrent GBM (Lombardi et al., 2019).

A detailed characterization of the GBM-associated molecular signatures has made possible the development of novel therapies, including the use of TKIs (Friedman et al., 2009; Quant et al., 2009; Brennan et al., 2013; Wang et al., 2016; Lombardi et al., 2019). On the other hand, based on the results obtained in the context of other tumors (Brahmer et al., 2010; Eder and Kalman, 2014; Larkin et al., 2015; Weber et al., 2015; Kessler et al., 2018; Stathias et al., 2018), the use of programmed cell death protein (PD-1) receptor/programmed death ligand 1 (PD-L1) inhibitors has been suggested for gliomas, including GBM (Motzer et al., 2015; Goldberg et al., 2016; Reck et al., 2016; Schwartz et al., 2016; Reiss et al., 2017; Reardon et al., 2018; Cloughesy et al., 2019; Schalper et al., 2019).

In the present review, we will outline the principal GBM molecular and tumor microenvironment (TME) aspects

providing also the grounds for novel targeted therapies and immunotherapy approaches using ICIs for the treatment of GBM affected patients.

# GENOMIC LANDSCAPE OF GBM

Specific molecular signatures of GBM have been identified through the introduction of next generation sequencing methods, in particular in untreated GBM tumors. It has been found mutations of several genes in GBM including phosphatase and tensin homolog (*PTEN*), tumor suppressor P53 (*TP53*), epidermal growth factor receptor (*EGFR*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), phosphatidylinositol 3-kinase regulatory subunit alpha (*PIK3R1*), platelet derived growth factor receptor alpha (*PDGFRA*), retinoblastoma 1 (*RB1*), neurofibromin 1 (*NF1*), gamma-aminobutyric acid receptor subunit alpha-6 (*GABRA6*), *IDH1*, mutS homolog 2 (*MSH2*), mutS homolog 6 (*MSH6*), mutL homolog 1 (*MLH1*), and PMS1 homolog 2 (*PMS2*). Furthermore, several hotspot mutations have been found, like the *IDH1* R132H mutation, the B-Raf proto-oncogene (*BRAF*) V600E mutation (Zhao et al., 2009; Kloosterhof et al., 2011; Kannan et al., 2012; Schwartzentruber et al., 2012; Brennan et al., 2013; Eder and Kalman, 2014; Ceccarelli et al., 2016; Wang et al., 2016; Kessler et al., 2018; Stathias et al., 2018; D'Angelo et al., 2019).

Glioblastoma cases characterized by the presence of mutations in DNA mismatch repair (MMR) genes, e.g., *MSH2*, *MSH6*, *MLH1*, and *PMS2* have been suggested to be defined as having a hypermutated profile (Hunter et al., 2006; Cahill et al., 2007; Greenman et al., 2007; Tcga, 2008; Yip et al., 2009; Brennan et al., 2013; Daniel et al., 2019).

Frequent amplification events found in GBM concern chromosome 7 [*EGFR*/ MET proto-oncogene (*MET*)/ cyclin dependent kinase 6 (*CDK6*)], chromosome 12 [cyclin dependent kinase 4 (*CDK4*)/, mouse double minute 2 homolog (*MDM2*)], and chromosome 4 (*PDGFRA*). Gains of the genes SRY-box transcription factor 2 (*SOX2*), MYCN proto-oncogene (*MYCN*), cyclin D1 (*CCND1*), and cyclin E2 (*CCNE2*) have also been found (Hunter et al., 2006; Cahill et al., 2007; Kuttler and Mai, 2007; Parsons et al., 2008; Tcga, 2008; Yip et al., 2009; Brennan et al., 2013; Sanborn et al., 2013; Zheng et al., 2013; Furgason et al., 2015). Frequent deletions in GBM include deletions in cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*), deletions of 6q26 in which the minimal deleted region seems to include the QKI, KH domain containing RNA binding (*QKI*) gene, and single gene deletions of low-density lipoprotein receptor-related protein 1B (*LRP1B*), neuronal PAS domain protein 3 (*NPAS3*), limbic system associated membrane protein (*LSAMP*), SET and MYND domain-containing protein 3 (*SMYD3*) genes (Kamiryo et al., 2002; Hunter et al., 2006; Cahill et al., 2007; Tcga, 2008; Yip et al., 2009; Moreira et al., 2011; Chen et al., 2012; Mizoguchi et al., 2012; Brennan et al., 2013; Nobusawa et al., 2014; Tabouret et al., 2015; Yang et al., 2016).

Repeatedly, *EGFR* mutations have been found associated with regional gene amplification (Ekstrand et al., 1991; Jaros et al., 1992; Schlegel et al., 1994; Dunn et al., 2012; Brennan et al.,

2013; Cominelli et al., 2015). Remarkably, the aberrant exon 1–8 junction of epidermal growth factor receptor variant III (*EGFRvIII*) was found expressed in a relevant proportion of cases. Additional recurrent non-canonical *EGFR* transcript forms were also observed (Ekstrand et al., 1991; Jaros et al., 1992; Nishikawa et al., 1994; Tcga, 2008; Brennan et al., 2013; Cominelli et al., 2015). The O-6-methylguanine-DNA methyltransferase (*MGMT*) locus has been found methylated in about 50% of GBM cases (Esteller et al., 2000; Paz et al., 2004; Hegi et al., 2005; Tcga, 2008; Zawlik et al., 2009; Malmstrom et al., 2012; Reifenberger et al., 2012; Armstrong et al., 2013; Brennan et al., 2013; Wiestler et al., 2013; Wick et al., 2014, 2018; Cominelli et al., 2015).

Recent studies have defined the evolution of tumor cells in GBM cases undergoing therapy as a process of clonal replacement where a fraction of tumor cells is eliminated by the treatment while clones of resistant cells are positively selected (Tcga, 2008; Brennan et al., 2013; Wang et al., 2016). Specifically, intratumoral heterogeneity, with the presence of resistant subclones, both in low grade and high grade glioma is frequently associated with treatment failure. Although a clearly defined pattern of tumor evolution has not yet been described in GBM, *TP53* gene mutations have been recently proposed as a marker of subclonal heterogeneity (Tcga, 2008; Brennan et al., 2013; Wang et al., 2016).

Glioblastoma evolution is highly branched, specific alterations and evolutionary patterns frequently occurring depending on the treatment. There is no linear link between the dominant clone at diagnosis and the dominant clone at relapse (Tcga, 2008; Brennan et al., 2013; Wang et al., 2016). Remarkably, genes such as *TP53, EGFR, PDGFRA* are frequently subjected to a process of mutational switching where a mutated version of a gene, found at diagnosis, is replaced by another mutated version of the same gene at relapse (Tcga, 2008; Brennan et al., 2013; Wang et al., 2016). Hypermutated tumors, which are highly enriched for mutations at CpG dinucleotides, generally harbor mutations in *MMR* pathway genes, most commonly in *MSH6*. These *MMR* alterations have been thought to be associated with putative mutagenic mechanisms of TMZ treatment (Hunter et al., 2006; Cahill et al., 2007; Tcga, 2008; Yip et al., 2009; Brennan et al., 2013; Wang et al., 2016).

A gene expression-based molecular classification has been proposed to integrate somatic mutation and DNA copy number data (Verhaak et al., 2010; Behnan et al., 2019). According to this classification, GBM cases were subdivided in proneural, neural, classical and mesenchymal subtypes. These different subtypes have been associated with gene signatures of normal brain cell types of different neural lineages. Moreover, GBM cases included in the different subtypes have also been associated with a different pathogenesis with GBM clones developing as the result of different causes and/or from different cell type of origin. However, further studies, also investigating glioma stem cells, have been able to identify three subtypes: proneural, mesenchymal and classical subtypes (Verhaak et al., 2010; Behnan et al., 2019).

According to the first proposed classification, GBM cases belonging to the classical subtype show in about the 100% of cases the chromosome 7 amplification paired with chromosome 10 loss. This event is also very frequent in the totality of GBM cases. High-level of *EGFR* amplification has been observed in 97% of cases belonging to the classical subtype, whereas this alteration has been infrequently found in the other GBM subtypes. Moreover, in association with frequent EGFR alteration, a lack of *TP53* mutations has been found in a subset of the classical subtype (Tcga, 2008; Verhaak et al., 2010; Brennan et al., 2013; Wang et al., 2016). The focal 9p21.3 homozygous deletion, targeting the *CDKN2A* gene, has been also frequently found in the classical subtype, in the 94% of cases belonging to this subtype found associated with *EGFR* amplification. The homozygous 9p21.3 deletion has been also found mutually exclusive with aberrations in genes belonging to the RB1 pathway such as *RB1*, *CDK4* and cyclin-D2 (*CCDN2)*, thus suggesting that in the cases with focal *EGFR* amplification the *CDKN2A* deletion is the sole alteration affecting the RB1 pathway. GBM cases belonging to the classical subtype are also characterized by the high expression of genes belonging to the notch homolog 1, translocation-associated (NOTCH) pathway such as neurogenic locus notch homolog-3 (*NOTCH3*), jagged-1 (*JAG1*) and lunatic fringe (*LFNG*), sonic hedgehog pathway such as smoothened (*SMO*), growth arrest-specific protein 1 (*GAS1*) and zinc finger protein GLI1 (*GLI1*) and the neural precursor and stem cell marker nestin (NES) pathway (Tcga, 2008; Verhaak et al., 2010; Brennan et al., 2013; Wang et al., 2016).

Glioblastoma cases belonging to the proneural subtype were found to be mainly characterized by alterations of *PDGFRA* and point mutations of *IDH1*. The focal amplification at the locus 4q12 harboring *PDGFRA* was associated with high levels of *PDGFRA* gene expression and the frequent presence of mutations in the *PDGFRA* gene. The great majority of *IDH1* mutations has been found in GBM cases belonging to the proneural subtype. Of note, they have been found to be generally mutually exclusive to *PDGFRA* alterations. Loss of heterozygosity and mutations of the *TP53* gene have been found to be frequent events in the proneural subtype. *PIK3CA/PIK3R1* mutations have also been found in the proneural subtypes in cases without *PDGFRA* abnormalities. The proneural group has been found to be characterized also by the high expression of genes other than *PDGFRA* that characterize the oligodendrocytic development such as oligodendrocyte transcription factor (*OLIG2*) and homeobox protein nkx-2.2 (*NKX2-2*). This group has been found also characterized by the expression of proneural development genes such as *SOX* family genes and achaete-scute homolog 1 (*ASCL1*), doublecortin (*DCX*), delta-like 3 (*DLL3*), transcription factor 4 (*TCF4*; Tcga, 2008; Verhaak et al., 2010; Brennan et al., 2013; Wang et al., 2016).

Glioblastoma cases belonging to the neural subtype were characterized by the expression of genes well-known as neuron markers such as *GABRA1*, neurofilament light chain (*NEFL*), synaptotagmin-1 (*SYT1*) and solute carrier family 12 member 5 (*SLC12A5*). GBM cases belonging to the neural subtype show an enrichment in genes involved in neuron protection and in axon and synaptic transmission (Tcga, 2008; Verhaak et al., 2010; Brennan et al., 2013; Wang et al., 2016).

Glioblastoma cases belonging to the mesenchymal subtype are frequently characterized by the presence of focal hemizygous deletions at 17q11.2 region encompassing the *NF1* gene. This

has been frequently associated with low *NF1* expression levels. Moreover, mutations at the *NF1* gene have been found in GBM cases belonging to the mesenchymal subgroup. Concomitant *PTEN* mutations have also been found in mesenchymal subgroup cases carrying *NF1* mutations (Tcga, 2008; Verhaak et al., 2010; Brennan et al., 2013; Wang et al., 2016). GBM cases belonging to the mesenchymal subtype are also characterized by the expression of mesenchymal markers such as chitinase-3-like protein 1 (*CHI3L1*) and *MET*. It has been thought that the higher activity of mesenchymal and astrocytic markers such as *CD44* and *MERKT* is linked to an epithelial-to-mesenchymal transition proper of dedifferentiated and transdifferentiated tumors. Finally, GBM cases belonging to the mesenchymal subtype are also characterized by the high expression of genes belonging to the TNF superfamily pathway and NF-kB pathway such as tumor necrosis factor receptor type 1-associated death domain (*TRADD*), *RELB* and TNF receptor superfamily member 1A (*TNFRSF1A*) (Tcga, 2008; Verhaak et al., 2010; Brennan et al., 2013; Wang et al., 2016).

Several clinical features have been associated with the four subtypes. In particular, an association between proneural subtype and age as well as between this subtype and a trend for a longer survival. However, GBM belonging to the proneural subtype have not shown a survival advantage from aggressive treatment protocols. On the other hand, a clear treatment effect has been observed among GBM cases belonging to the classical and mesenchymal subtypes (Tcga, 2008; Verhaak et al., 2010; Brennan et al., 2013; Wang et al., 2016).
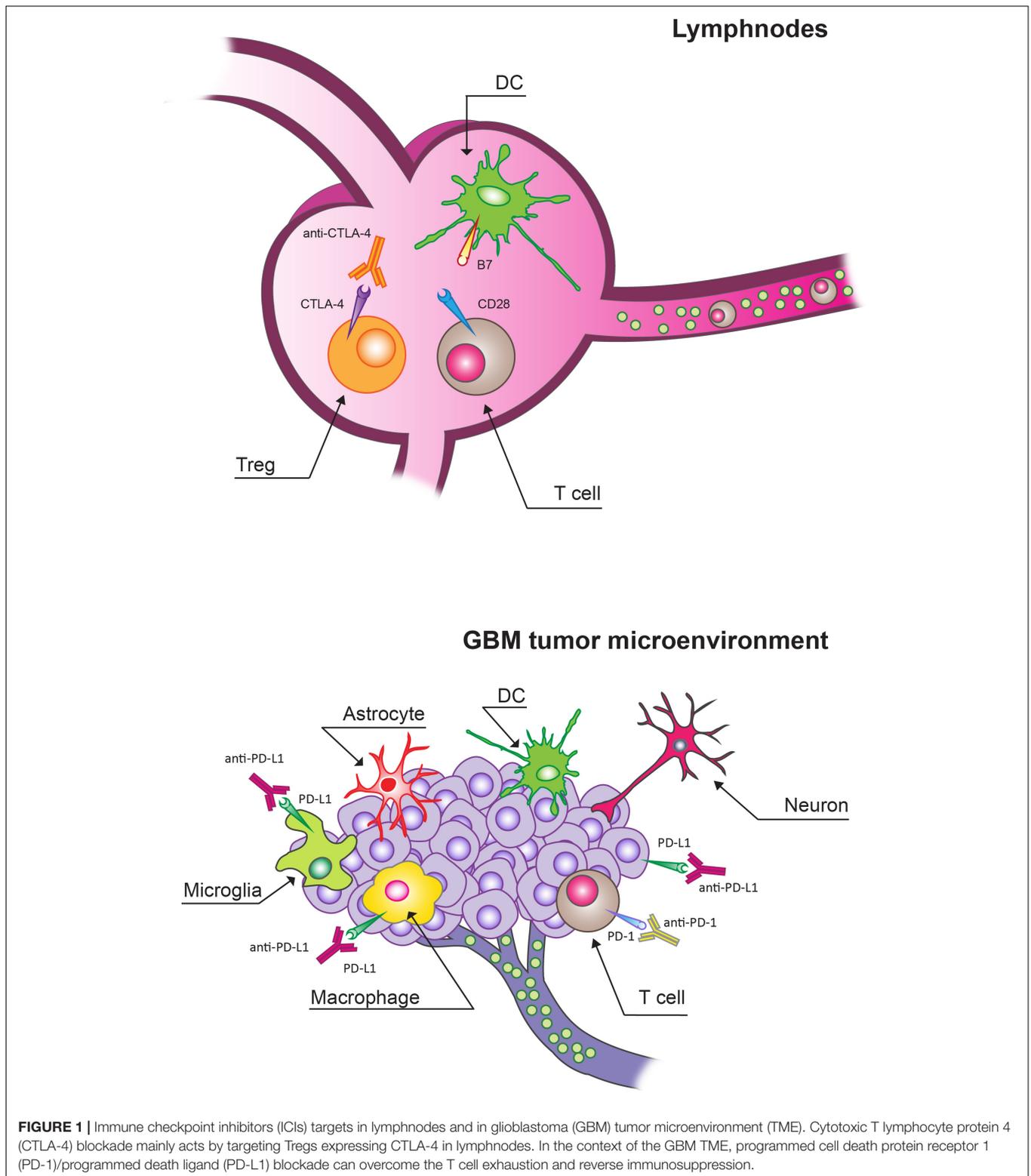
The proneural-to-mesenchymal transition upon tumor recurrence has been proposed as a mechanism of treatment resistance for GBM to radiotherapy and/or chemotherapy. GBM patients belonging to the mesenchymal subtype have been associated with survival shorter than the other subtypes, particularly when cases with low transcriptional heterogeneity are considered. Although in the context of poor prognosis patients, GBM cases belonging to the mesenchymal subtype have been found to show favorable response to immunotherapy and intensive radiotherapy and chemotherapy (Verhaak et al., 2010; Behnan et al., 2019).

Long non-coding RNAs (LncRNAs) are RNA transcripts longer than 200 base pairs which do not code for proteins. Although the human genome harbors more than 50,000 LncRNA genes, they are still poor characterized. However, LncRNAs have been found to play key roles in various cell activities related to regulation of gene expression, protein synthesis, stemness, immunity (Schlackow et al., 2017). Moreover, LncRNAs have been found to exert relevant roles in pathogenesis and progression of various cancers including GBM. In particular, a large number of LncRNAs has been found associated with deregulated gene expression and imbalanced biological processes in GBM (Zeng et al., 2018). In this context, the expression of the LncRNA P73 antisense RNA 1T (*TP73-AS1*) has been associated with poor outcome in GBM patients. GBM patients belonging to less aggressive subgroups have been found to be characterized by hypermethylation and low expression of *TP73-AS1*. Moreover, it has been found that *TP73-AS1* downregulation is associated with the loss of aldehyde dehydrogenase 1 family

member A1 (*ALDH1A1*) expression and the re-sensitivity of the GBM stem cell (GSC) population to TMZ treatment (Mazor et al., 2019). Expression of the LncRNA HOX transcript antisense intergenic RNA (*HOTAIR*) in GBM has been demonstrated to be significantly higher than in normal tissues and low grade gliomas. Moreover, *HOTAIR* has been demonstrated to be an independent prognostic factor in GBM associated with proliferation and tumorigenic potential of GBM cells (Zhang et al., 2015; Zhang et al., 2018). LncRNA colorectal neoplasia differentially expressed (*CRNDE*) has been found highly expressed in GBM and other brain cancers such as astrocytomas. It has also been explained that its overexpression is associated with promotion of tumor cell growth and migration (Ellis et al., 2014; Wang et al., 2016). LncRNA nuclear enriched abundant transcript 1 (*NEAT1*) has been shown to be a key regulator of nuclear domains implicated in mRNA nuclear retention and splicing. *NEAT1* has been found upregulated in human GBM tissues and GBM cell line models and a high *NEAT1* expression has been associated with larger tumor size, higher WHO grade, higher recurrence rate and unfavorable overall survival (He et al., 2016). The LncRNA X-inactive specific transcript (*XIST*) has been found highly expressed in glioma tissues and GSCc. The knockdown of *XIST* has been shown to suppress proliferation, migration, invasion and tumorigenic potential of GSCs by upregulating *miR152* (Yao et al., 2015). The LncRNA SOX2 overlapping transcript (*SOX2OT*) is characterized by the fact that its transcription genomic region includes the *SOX2* gene; a *SOX2OT* involvement in the transcriptional regulation of *SOX2* has also been observed. *SOX2OT* has been shown to be involved in the proliferation, migration, invasion of GSCs (Su et al., 2017). The LncRNA *H19* has been shown to be upregulated in glioma tissues and associated with poor outcome. Moreover, invasion, angiogenesis, stemness and tumorigenicity of GBM cells have been found enhanced when *H19* is overexpressed (Jiang et al., 2016). The LncRNA *LOC441204* has been found highly expressed in glioma tumor specimens and cell lines. Tumor cell proliferation has been found suppressed by knockdown of *LOC441204* in glioma. On the other hand, *LOC441204*-induced tumor cell growth has been shown to be modulated by the stabilization of the β-catenin pathway (Lin et al., 2017). Regarding the role of other LncRNAs, evidence has also been reported about the fact that the high expression of other LncRNAs such as maternally expressed gene 3 (*MEG3*), metastasis associated lung adenocarcinoma (*MALAT1*), cancer susceptibility candidate 2 (*CASC2*), taurine-upregulated gene 1 (*TUG1*), DBH antisense RNA 1 (*DBH-AS1),* AC005035.1, AC010336.2, AC108134.2, AC116351.2, *Clorf132, C10orf91, LINC00475, MIR210HG* could be associated with poor outcome in GBM cases (Zeng et al., 2018).

## ROLE OF THE GBM TUMOR MICROENVIRONMENT

The brain is distinguished from the other organs by the presence of the blood-brain-barrier (BBB). The BBB provides a selective barrier between the systemic circulation and the brain, thus representing a limit for the delivery of many therapeutic agents

**FIGURE 1 |** Immune checkpoint inhibitors (ICIs) targets in lymphnodes and in glioblastoma (GBM) tumor microenvironment (TME). Cytotoxic T lymphocyte protein 4 (CTLA-4) blockade mainly acts by targeting Tregs expressing CTLA-4 in lymphnodes. In the context of the GBM TME, programmed cell death protein receptor 1 (PD-1)/programmed death ligand (PD-L1) blockade can overcome the T cell exhaustion and reverse immunosuppression.

(Chen et al., 2012; Miura et al., 2013). However, a loss of BBB integrity could be displayed in the presence of cancer, in particular during the cancer progression. This seems to be the reason why several agents, including ICIs, that are known to

be not capable of penetrating the BBB, have however shown in some extent a clinical efficacy (de Vries et al., 2006; van Tellingen et al., 2015). Specialized endothelial cells, pericytes, and astrocytic foot processes, dictating junctional integrity, are the elements that
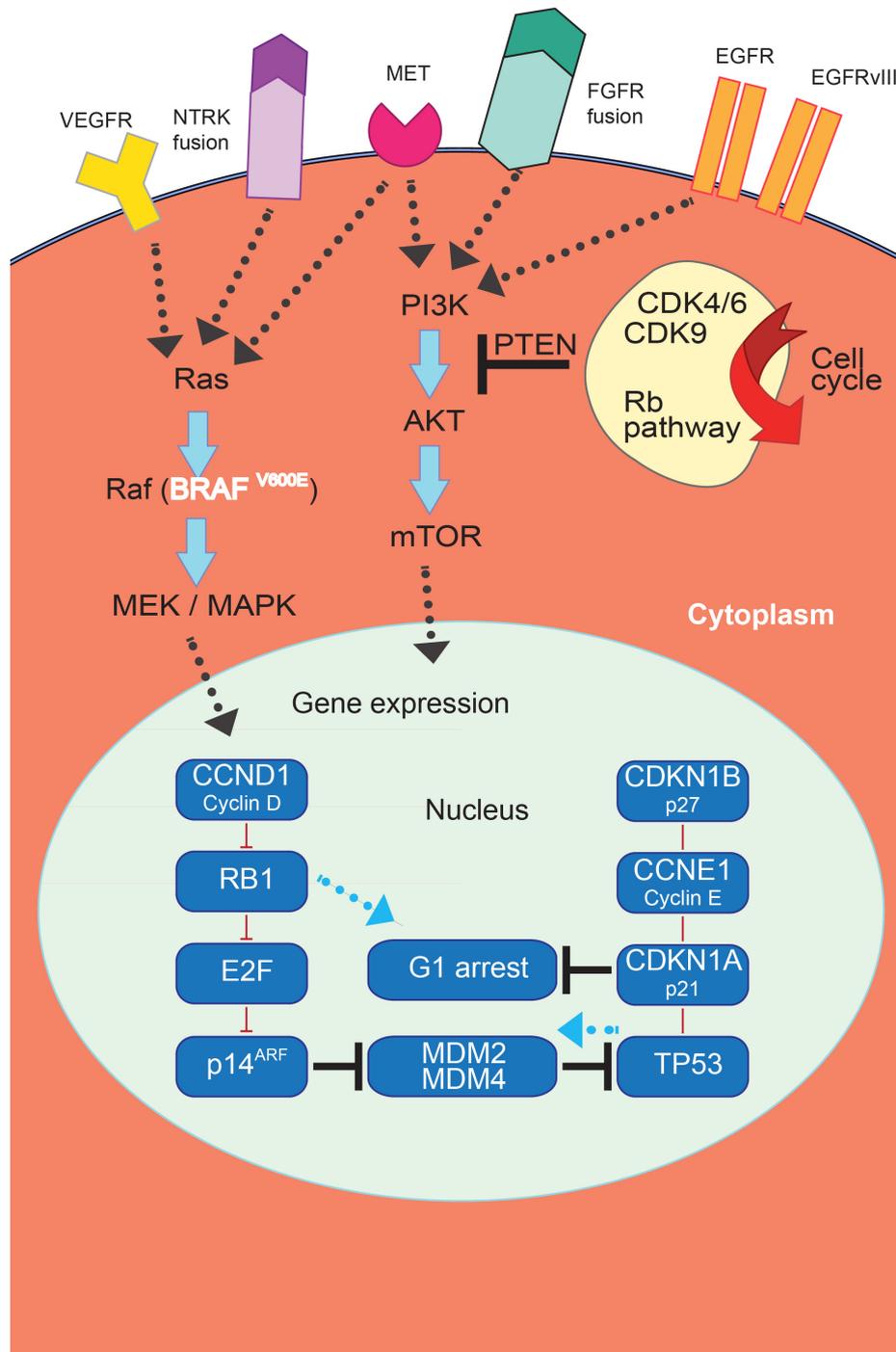
**FIGURE 2 |** Targeted therapies in glioblastoma (GBM). The introduction of novel targeted therapies has been allowed by the comprehensive characterization of the molecular landscape of somatic genomic alterations identifying a series of mutated genes and abnormal rearrangements potentially utilizable as therapeutic targets.

constitute the BBB. Moreover, BBB integrity can be also regulated by microglia, being these cells capable of repairing the BBB in a purinergic receptor P2RY12-dependent manner in case of injury (de Vries et al., 2006; van Tellingen et al., 2015).

The complex crosstalk of TME components is involved in the regulation of tumor progression (Quail and Joyce, 2013, 2017; Gritsenko et al., 2012; Nakasone et al., 2012; Quail and Joyce, 2013, 2017). The composition of ECM of normal brain is distinctive, with specific tissue-resident cell types such as neurons, astrocytes and microglia. Moreover, the BBB physically protects the ECM from inflammation (Novak and Kaye, 2000; Mahesparan et al., 2003). The most common component of the brain ECM is hyaluronic acid which is localized in the intraparenchymal region (Kim et al., 2018). The haptotactic cues from the vascular basement membrane, the enrichment of vascular derived chemotactic cues, as well as interconnected axon tracts can determine the therapeutic resistance of GBM cells in the perivascular space further providing haptotactic cues for cellular invasion (Giese and Westphal, 1996; Nimsky et al., 2005; Gritsenko et al., 2012).

A diffuse invasion pattern characterizes GBM (Young et al., 2015). Healthy tissue beyond the tumor margin is infiltrated by the tumor cells, generally enriched in the GSC stem cell fraction, that either migrate individually or collectively practically impeding complete surgical resection (Eyler and Rich, 2008; Sherriff et al., 2013). On the other hand, GBM tumors rarely intravasate and metastasize from the brain to distant organs (Quail and Joyce, 2013, 2017).

Glioblastoma frequently develop in a hypoxic microenvironment which can modify the metabolic pathways of GBM cells. The brain has a high metabolism level in which the glucose is the major energy substrate and lactate, ketone bodies, fatty acids and aminoacids can also be employed. The metabolic homeostasis of the brain is maintained by the interaction among its various constituent cells such as astrocytes, neurons and microglia (Gritsenko et al., 2012; Nakasone et al., 2012; Quail and Joyce, 2013, 2017). This equilibrium can be altered by genomic aberrations and biochemical variations in GBM cells that often metabolize glucose into lactate even when oxygen is present in a process called Warburg effect. GBM cells can also increase intracellular lipid, aminoacid and nucleotide levels. These metabolic adaptations can favor GBM tumor growth (Gritsenko et al., 2012; Nakasone et al., 2012; Quail and Joyce, 2013, 2017).

Hypervascularity is a characteristic of GBM tumors with an increment in angiogenesis compared to healthy brain tissue. This tumor-associated vasculature is not completely formed, with leaky vessels, and associated with an increase in interstitial fluid pressure. A necrotic core softer than the surrounding tissue characterizes the TME of GBM (Brat and Van Meir, 2004; Brat et al., 2004; Persano et al., 2011; Hambardzumyan and Bergers, 2015; Chen and Hambardzumyan, 2018). High density regions called pseudopalisades are formed when cells migrate away from the hypoxic regions. Increased matrix production with respect to both necrotic regions and healthy tissues characterizes these regions (Brat and Van Meir, 2004; Brat et al., 2004; Persano et al., 2011; Hambardzumyan and Bergers, 2015; Chen

and Hambardzumyan, 2018). GBM cells are capable of rapidly invading vasculature (Akiyama et al., 2001; Ponta et al., 2003; Zimmermann and Dours-Zimmermann, 2008; Dicker et al., 2014; Schiffer et al., 2018).

Circulatory and immune systems are connected by the lymphatic system that is involved, together with blood vessels, in the exchange of various elements including fluid, waste, debris as well as immune cells (Engelhardt et al., 2017). Together with the absence of a classic lymphatic drainage system, the CNS exhibits several other peculiar features, such as the presence of tight junctions in the BBB, as well as the limited rejection of foreign tissues within the CNS (Louveau et al., 2015; Schiffer et al., 2018).

There are functional lymphatic vessels in the CNS with the presence of different types of antigen-presenting cells (APCs), including microglia, macrophages, astrocytes and canonical APC such as dendritic cells (DCs; **Figure 1**; Louveau et al., 2015; Schiffer et al., 2018). In the brain, microglia are the predominant APCs whereas DCs carry out a less relevant role (Lowe et al., 1989; Ulvestad et al., 1994; Weiss et al., 2009; Goldmann et al., 2016).

Activated T cells can invade the CNS. These activated T cells can cover these compartments in an unrestricted manner. On the other hand, antigens can be presented locally or in the draining cervical lymph nodes. Moreover, the BBB can be compromised, thus allowing a relevant infiltration of multiple immune cell types from the peripheral circulation (Weiss et al., 2009). However, GBM tumors present low numbers of tumor-infiltrating lymphocytes (TILs) and other immune effector cell types compared to other tumor types (Schiffer et al., 2018). The interaction of TILs with the TME can cause their re-education. In particular, the local TME can alter T cell effector function in the process related to anti-tumor immunity even in the CNS, where T cell-mediated inflammatory responses are considered poor under normal physiological contexts. The number of antigen-specific TILs can remain relatively low besides frequently displaying an exhausted phenotype. The peculiar immune environment of the brain can be responsible for this reduced quantity and limited activity of T cells in GBM. In particular, there is a specific need of avoiding unrestrained inflammation in the brain given its solid enclosure and the potential for damage from increased intracranial pressure (Quail and Joyce, 2013, 2017; Gajewski et al., 2017; Keskin et al., 2019). This need is not present with the same extent in peripheral organs. In fact, this environment in which both inflammatory and adaptive immune responses are tightly regulated is specific of the brain; besides there is a variety of immunosuppressive mechanisms at both the molecular and cellular levels (Perng and Lim, 2015). In particular, stromal cells of the brain produce high levels of the classic immunosuppressive cytokines transforming growth factor β (TGFβ), interleukin-10 (IL-10) in response to inflammatory stimuli, including those derived from GBM tumors, in order to maintain homeostasis (Vitkovic et al., 2001; Gong et al., 2012). Furthermore, the accumulation of regulatory T cells (Tregs) is stimulated by IDO which can suppress T cell activity by depleting tryptophan from the microenvironment. Microglia and tumor- infiltrating myeloid cells can also inhibit T cell proliferation and function through the production of high levels of arginase that causes the

TABLE 1 | Clinical trials in glioblastoma (GBM) using tyrosine kinase inhibitors (TKIs).

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| mTORC1/mTORC2 kinase inhibitor AZD2014 in previously treated glioblastoma multiforme | Glioblastoma multiforme | Drug: AZD2014 | NCT02619864 | Completed | Phase I |
| Gefitinib and radiation therapy in treating patients with glioblastoma multiforme | Adult giant cell Glioblastoma Adult glioblastoma Adult gliosarcoma | Drug: Gefitinib | NCT00052208 | Completed | Phase I, II |
| Study of AEE788 in patients with recurrent/relapse glioblastoma multiforme (GBM) | Glioblastoma multiforme | Drug: AEE788 | NCT00116376 | Completed | Phase I, II |
| Clinical trial on the combination of avelumab and axitinib for the treatment of patients with recurrent glioblastoma | Recurrent glioblastoma (WHO-grade IV glioma) | Drug: Axitinib Drug: Avelumab | NCT03291314 | Completed | Phase II |
| AZD8055 for adults with recurrent gliomas | Glioblastoma Multiforme Anaplastic astrocytoma Anaplastic oligodendroglioma Malignant glioma Brainstem glioma | Drug: AZD8055 | NCT01316809 | Completed | Phase I |
| Sunitinib in treating patients with recurrent malignant gliomas | Astrocytoma Adult diffuse astrocytoma Adult giant cell Glioblastoma Adult glioblastoma and other 5 | Drug: Sunitinib malate | NCT00499473 | Completed | Phase II |
| Study to assess safety, pharmacokinetics, and efficacy of oral CC-223 for patients with advanced solid tumors, non-hodgkin lymphoma or multiple myeloma | Multiple myeloma Diffuse large B cell Lymphoma Glioblastoma multiforme Hepatocellular carcinoma and other 4 | Drug: CC-223 | NCT01177397 | Completed | Phase I,II |
| Trial of ponatinib in patients with bevacizumab-refractory glioblastoma | Glioblastoma | Drug: Ponatinib | NCT02478164 | completed | Phase II |
| A phase II exploratory, multicentre, open-label, non-comparative study of ZD1839 (iressa) and radiotherapy in the treatment of patients with glioblastoma multiforme | Glioblastoma | Drug: Gefitinib | NCT00238797 | Completed | Phase II |
| A study of the safety and efficacy of tarceva in patients with first relapse of grade IV glioma (glioblastoma multiforme) | Glioblastoma | Drug: Erlotinib HCl (OSI-774) | NCT00337883 | Completed | Phase II |
| Study of tesevatinib monotherapy in patients with recurrent glioblastoma | Glioblastoma Recurrent glioblastoma Brain tumor | Drug: Tesevatinib | NCT02844439 | Completed | Phase II |
| Oral tarceva study for recurrent/residual glioblastoma multiforme and anaplastic astrocytoma | Glioblastoma multiforme Anaplastic astrocytoma | Drug: Erlotinib | NCT00301418 | Completed | Phase I,II |
| Gefitinib in treating patients with newly diagnosed glioblastoma multiforme | Adult giant cell glioblastoma Adult glioblastoma Adult gliosarcoma | Drug: Gefitinib | NCT00014170 | Completed | Phase II |
| Erlotinib and sorafenib in treating patients with progressive or recurrent glioblastoma multiforme | Adult giant cell glioblastoma Adult glioblastoma Adult gliosarcoma Recurrent adult brain tumor | Drug: erlotinib hydrochloride Drug: sorafenib tosylate | NCT00445588 | Completed | Phase II |
| AZD2171 in treating patients with recurrent glioblastoma multiforme | Adult giant cell Glioblastoma Adult glioblastoma Adult gliosarcoma Recurrent adult brain tumor | Drug: cediranib maleate | NCT00305656 | Completed | Phase II |
| Dasatinib in treating patients with recurrent glioblastoma multiforme or gliosarcoma | Adult giant cell glioblastoma Adult glioblastoma Adult gliosarcoma Recurrent adult brain neoplasm | Drug: Dasatinib | NCT00423735 | Completed | Phase II |

*(Continued)*

**TABLE 1 |** Continued

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| Cediranib maleate and cilengitide in treating patients with progressive or recurrent glioblastoma | Adult giant cell Glioblastoma Adult glioblastoma Adult gliosarcoma Recurrent adult brain neoplasm | Drug: Cediranib maleate Drug: Cilengitide | NCT00979862 | Completed | Phase I |
| Sorafenib in newly diagnosed high grade glioma | Glioblastoma Gliosarcoma Anaplastic astrocytoma Anaplastic oligoastrocytoma Anaplastic oligodendroglioma | Drug: Sorafenib dose escalation | NCT00884416 | Completed | Phase I |
| E7050 in combination with E7080 in subjects with advanced solid tumors (dose escalation) and in subjects with recurrent glioblastoma or unresectable stage III or stage IV melanoma after prior systemic therapy (expansion cohort and phase 2) | Advanced solid tumors | Drug: E7050 Drug: Lenvatinib | NCT01433991 | Completed | Phase I,II |
| ZD 1839 in treating patients with glioblastoma multiforme in first relapse | Brain and central nervous system tumors | Drug: Gefitinib | NCT00016991 | Completed | Phase II |
| Ph I dasatinib + erlotinib in recurrent MG | Glioblastoma, Gliosarcoma | Drug: Erlotinib and dasatinib | NCT00609999 | Completed | Phase I |
| Bafetinib in treating patients with recurrent high-grade glioma or brain metastases | Adult anaplastic astrocytoma Adult anaplastic Ependymoma Adult anaplastic Oligodendroglioma Adult giant cell glioblastoma Adult glioblastoma and other 5 | Drug: Bafetinib | NCT01234740 | Completed | Phase I |
| A randomized phase II clinical trial on the efficacy of axitinib as a monotherapy or in combination with lomustine for the treatment of patients with recurrent glioblastoma | Glioblastoma multiforme | Drug: Axitinib Drug: Axitinib plus lomustine | NCT01562197 | Completed | Phase II |
| Cediranib in combination with lomustine chemotherapy in recurrent glioblastoma | Recurrent glioblastoma | Drug: Cediranib Drug: Lomustine chemotherapy Drug: Placebo Cediranib | NCT00777153 | Completed | Phase III |
| Radiation therapy and temozolomide followed by temozolomide plus sorafenib for glioblastoma multiforme | Glioblastoma multiforme | Drug: Temozolomide Drug: Sorafenib | NCT00544817 | Completed | Phase II |
| PTK787/ZK 222584 in combination with temozolomide and radiation in patients with glioblastoma taking enzyme-inducing anti-epileptic drugs | Glioblastoma | Drug: PTK787/ZK 222584 Drug: Temozolomide | NCT00385853 | Completed | Phase I |
| Study of imatinib mesylate in combination with hydroxyurea versus hydroxyurea alone as an oral therapy in patients with temozolomide resistant progressive glioblastoma | Glioblastoma multiforme Astrocytoma | Drug: Imatinib mesylate Drug: Hydroxyurea | NCT00154375 | Completed | Phase III |
| Open label trial to explore safety of combining afatinib (BIBW 2992) and radiotherapy with or without temozolomide in newly diagnosed glioblastoma multiform | Glioblastoma | Drug: Temozolomide Drug: BIBW2992 | NCT00977431 | Completed | Phase I |
| Ph. 2 sorafenib + protracted temozolomide in recurrent GBM | Recurrent glioblastoma Multiforme | Drug: Sorafenib and temozolomide | NCT00597493 | Completed | Phase II |
| Erlotinib and temozolomide with radiation therapy in treating patients with glioblastoma multiforme or other brain tumors | Adult giant cell glioblastoma Adult glioblastoma Adult gliosarcoma | Drug: Erlotinib hydrochloride Drug: Temozolomide | NCT00039494 | Completed | Phase II |
| Erlotinib and radiation therapy in treating young patients with newly diagnosed glioma | Brain and central nervous system tumors | Drug: Erlotinib hydrochloride | NCT00124657 | Completed | Phase I,II |
| Safety and efficacy study of tarceva, temodar, and radiation therapy in patients with newly diagnosed brain tumors | Glioblastoma multiforme Gliosarcoma | Drug: Tarceva Drug: Temodar | NCT00187486 | Completed | Phase II |

*(Continued)*

**TABLE 1** | Continued

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| Study of sunitinib before and during radiotherapy in newly diagnosed biopsy-only glioblastoma patients | Glioblastoma | Drug: Sunitinib | NCT01100177 | Completed | Phase II |
| Dasatinib and bevacizumab in treating patients with recurrent or progressive high-grade glioma or glioblastoma multiforme | Glioblastoma multiforme | Biological: Bevacizumab Drug: Basatinib | NCT00892177 | Completed | Phase II |
| Lapatinib in treating patients with recurrent glioblastoma multiforme | Brain and central nervous system tumors | Drug: Lapatinib ditosylate | NCT00099060 | Completed | Phase I,II |
| Gefitinib in treating patients with recurrent or progressive CNS tumors | Brain and central nervous system tumors | Drug: Gefitinib | NCT00025675 | Completed | Phase II |
| Radiation therapy, temozolomide, and erlotinib in treating patients with newly diagnosed glioblastoma multiforme | CNS tumor Adult | Drug: Erlotinib hydrochloride Drug: Temozolomide | NCT00274833 | Completed | Phase II |
| Sunitinib tumor levels in patients not on enzyme-inducing anti-epileptic drugs undergoing debulking surgery for recurrent glioblastoma | Glioblastoma Brain tumor | Drug: Sunitinib | NCT00864864 | Completed | Early Phase I |
| Study of bevacizumab plus temodar and tarceva in patients with glioblastoma or gliosarcoma | Glioblastoma Gliosarcoma | Drug: Bevacizumab Drug: Tarceva Drug: Temozolomide | NCT00525525 | Completed | Phase II |
| A Phase II trial of sutent (sunitinib; SU011248) for recurrent anaplastic astrocytoma and glioblastoma | Anaplastic astrocytoma Glioblastoma | Drug: Sunitinib malate | NCT00606008 | Completed | Phase II |
| Cediranib, temozolomide, and radiation therapy in treating patients with newly diagnosed glioblastoma | Adult giant cell Glioblastoma Adult glioblastoma Adult gliosarcoma | Drug: Cediranib maleate Drug: Temozolomide | NCT00662506 | Completed | Phase I,II |
| Dasatinib or placebo, radiation therapy, and temozolomide in treating patients with newly diagnosed glioblastoma multiforme | Brain and central nervous system tumors | Drug: Dasatinib Drug: Temozolomide | NCT00869401 | Completed | Phase I,II |
| Erlotinib compared with temozolomide or carmustine in treating patients with recurrent glioblastoma multiforme | Brain and central nervous system tumors | Drug: Carmustine Drug: Erlotinib hydrochloride Drug: Temozolomide | NCT00086879 | Completed | Phase II |
| Sorafenib combined with erlotinib, tipifarnib, or temsirolimus in treating patients with recurrent glioblastoma multiforme or gliosarcoma | Adult giant cell glioblastoma Adult glioblastoma Adult gliosarcoma Recurrent adult brain tumor | Drug: Sorafenib tosylate Drug: Erlotinib hydrochloride Drug: Tipifarnib Drug: Temsirolimus | NCT00335764 | Completed | Phase I,II |
| Phase II Imatinib + hydroxyurea in treatment of patients with recurrent/progressive grade II low-grade glioma (LGG) | Glioblastoma Gliosarcoma | Drug: Imatinib mesylate and hydroxyurea | NCT00615927 | Completed | Phase II |
| Sorafenib tosylate and temsirolimus in treating patients with recurrent glioblastoma | Adult glioblastoma Adult gliosarcoma Recurrent adult brain neoplasm | Drug: Sorafenib tosylate Drug: Temsirolimus | NCT00329719 | Completed | Phase I,II |
| Phase I : cediranib in combination with lomustine chemotherapy in recurrent malignant brain tumor | Recurrent glioblastoma Brain tumor | Drug: Cediranib Drug: Lomustine | NCT00503204 | Completed | Phase I |
| Ph I SU011248 + irinotecan in treatment of Pts w MG | Glioblastoma | Drug: SU011248 and irinotecan | NCT00611728 | Completed | Phase I |
| Ph I zactima + imatinib mesylate and hydroxyurea for pts w recurrent malignant glioma | Glioblastoma Gliosarcoma | Drug: Zactima, gleevec, hydroxyurea | NCT00613054 | Completed | Phase I |
| Imatinib mesylate and hydroxyurea in treating patients with recurrent or progressive meningioma | Glioblastoma Gliosarcoma | Drug: Hydroxyurea Drug: Imatinib mesylate | NCT00354913 | Completed | Phase II |
| Bevacizumab and sorafenib in treating patients with recurrent glioblastoma multiforme | Brain and central nervous system tumors | Biological: Bevacizumab Drug: Sorafenib tosylate | NCT00621686 | Completed | Phase II |
| BIBW 2992 (afatinib) with or without daily temozolomide in the treatment of patients with recurrent malignant glioma | Glioma | Drug: BIBW 2992 Drug: TMZ Drug: BIBW 2992 plus TMZ | NCT00727506 | Completed | Phase II |
| Bevacizumab and erlotinib after radiation therapy and temozolomide in treating patients with newly diagnosed glioblastoma multiforme or gliosarcoma | Brain and central nervous system tumors | Drug: Bevacizumab Drug: Erlotinib hydrochloride | NCT00720356 | Completed | Phase II |
| Ph I gleevec in combo w RAD001 + hydroxyurea for Pts w recurrent MG | Glioblastoma Gliosarcoma | Drug: Gleevec, RAD001, and hydroxyurea | NCT00613132 | Completed | Phase I |

*(Continued)*

**TABLE 1 |** Continued

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| GW572016 to treat recurrent malignant brain tumors | Glioma<br>Brain tumor<br>Glioblastoma multiforme<br>GBM<br>Gliosarcoma<br>GS | Drug: Lapatinib ditosylate | NCT00107003 | Completed | Phase II |
| Temozolomide and radiation therapy with or without vatalanib in treating patients with newly diagnosed glioblastoma multiforme | Brain and central nervous system tumors | Drug: Temozolomide<br>Drug: Vatalanib | NCT00128700 | Completed | Phase I,II |
| Ph II erlotinib + sirolimus for pts w recurrent malignant glioma multiforme | Glioblastoma<br>Gliosarcoma | Drug: Erlotinib + Sirolimus | NCT00672243 | Completed | Phase II |
| Afatinib (BIBW 2992) QTcF trial in patients with relapsed or refractory solid tumors | Neoplasms | Drug: BIBW 2992 | NCT00875433 | Completed | Phase II |
| Phase (Ph) II bevacizumab + erlotinib for patients (Pts) with recurrent malignant glioma (MG) | Glioblastoma<br>Gliosarcoma | Drug: Bevacizumab and erlotinib | NCT00671970 | Completed | Phase II |
| Everolimus and gefitinib in treating patients with progressive glioblastoma multiforme or progressive metastatic prostate cancer | Brain and central nervous system tumors<br>Prostate cancer | Drug: Everolimus<br>Drug: Gfitinib | NCT00085566 | Completed | Phase I,II |
| Sorafenib in treating patients with recurrent or progressive malignant glioma | Adult anaplastic astrocytoma<br>Adult anaplastic Oligodendroglioma<br>Adult giant cell Glioblastoma and other 2 | Drug: Sorafenib tosylate | NCT00093613 | Completed | Phase I |
| AZD7451 for recurrent gliomas | Glioblastoma multiforme | Drug: AZD7451 | NCT01468324 | Completed | Phase I |
| Gefitinib and radiation therapy in treating children with newly diagnosed gliomas | Untreated childhood anaplastic astrocytoma<br>Untreated childhood anaplastic oligodendroglioma<br>Untreated childhood brain stem glioma<br>Untreated childhood giant cell glioblastoma and other 4 | Drug: Gefitinib | NCT00042991 | Completed | Phase I,II |
| Erlotinib in treating patients with recurrent malignant glioma or recurrent or progressive meningioma | Adult anaplastic astrocytoma<br>Adult anaplastic oligodendroglioma<br>Adult giant cell glioblastoma<br>Adult glioblastoma and other 5 | Drug: Erlotinib hydrochloride | NCT00045110 | Completed | Phase I,II |
| Gamma-secretase inhibitor RO4929097 and cediranib maleate in treating patients with advanced solid tumors | Adult anaplastic astrocytoma<br>Adult anaplastic ependymoma<br>Adult anaplastic oligodendroglioma<br>Adult brain stem glioma<br>Adult giant cell glioblastoma<br>Adult glioblastoma and other 41 | Secretase inhibitor RO4929097<br>Drug: Cediranib maleate | NCT01131234 | Completed | Phase I |
| EGFR inhibition using weekly erlotinib for recurrent malignant gliomas | Brain cancer | Drug: Erlotinib | NCT01257594 | Completed | Phase I |
| Lapatinib in treating young patients with recurrent or refractory central nervous system tumors | Recurrent childhood anaplastic Astrocytoma<br>Recurrent childhood brain stem gliom<br>Recurrent childhood ependymoma<br>Recurrent childhood giant cell glioblastoma<br>Recurrent childhood glioblastoma and other 3 | Drug: Lapatinib ditosylate | NCT00095940 | Completed | Phase I,II |
| Erlotinib in treating patients with solid tumors and liver or kidney dysfunction | Astrocytoma<br>Adult anaplastic ependymoma<br>Adult anaplastic oligodendroglioma | | | | |

*(Continued)*

**TABLE 1 |** Continued

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| Adult brain stem glioma Adult diffuse astrocytoma Adult ependymoblastoma Adult giant cell glioblastoma and 79 more | | Drug: Erlotinib hydrochloride | NCT00030498 | Completed | Phase I |
| ZD1839 and oral irinotecan in treating young patients with refractory solid tumors | Glioblastoma Rhabdomyosarcomas Neuroblastoma Osteosarcoma | Drug: Irinotecan, Gefitinib | NCT00132158 | Completed | Phase I |
| Apatinib in recurrent or refractory intracranial central nervous system malignant tumors | Efficacy and safety | Drug: Apatinib Drug: Temodar | NCT03660761 | Completed | Phase II |
| Bevacizumab and cediranib maleate in treating patients with metastatic or unresectable solid tumor, lymphoma, intracranial glioblastoma, gliosarcoma, or anaplastic astrocytoma | Adult grade III lymphomatoid granulomatosis Adult nasal type extranodal NK/T-cell lymphoma Anaplastic large cell lymphoma Angioimmunoblastic T-cell lymphoma Childhood burkitt lymphoma and other 56 | Biological: Bevacizumab Drug: Cediranib maleate | NCT00458731 | Completed | Phase I |
| Erlotinib and Temsirolimus in Treating Patients With Recurrent Malignant Glioma | Adult anaplastic astrocytoma Adult anaplastic oligodendroglioma Adult diffuse astrocytoma Adult giant cell glioblastoma Adult glioblastoma and other 6 | Drug: Erlotinib Drug: Temsirolimus | NCT00112736 | Completed | Phase I,II |
| Pazopanib in combination with lapatinib in adult patients with relapsed malignant glioma (VEG102857) | Glioma | Drug: Pazopanib Drug: Lapatinib | NCT00350727 | Completed | Phase I |
| BIBF 1120 for recurrent high-grade gliomas | Glioblastoma Gliosarcoma Anaplastic astrocytoma Anaplastic oligodendroglioma Anaplastic oligoastrocytoma | Drug: BIBF 1120 | NCT01380782 | Completed | Phase II |
| Imetelstat sodium in treating young patients with refractory or recurrent solid tumors or lymphoma | Brain and central nervous system tumors Lymphoma Lymphoproliferative disorder Small intestine cancer Unspecified childhood solid tumor, protocol specific | Drug: Imetelstat sodium | NCT01273090 | Completed | Phase I |
| Imatinib mesylate in treating patients with gliomas | Brain and central nervous system tumors | Drug: Imatinib mesylate | NCT00039364 | Completed | Phase II |
| Imatinib mesylate in treating patients with recurrent malignant glioma or meningioma | Brain and central nervous system tumors | Drug: Imatinib mesylate | NCT00010049 | Completed | Phase I,II |
| Tumor tissue analysis in patients receiving imatinib mesylate for malignant glioma | Brain and central nervous system tumors | Drug: Imatinib mesylate | NCT00401024 | Completed | Phase I |
| Imatinib mesylate, vatalanib, and hydroxyurea in treating patients with recurrent or relapsed malignant glioma | Brain and central nervous system tumors | Drug: Hydroxyurea Drug: Imatinib mesylate Drug: Vatalanib | NCT00387933 | Completed | Phase I |
| Gefitinib plus temozolomide in treating patients with malignant primary glioma | Brain and central nervous system tumors | Drug: Gefitinib Drug: Temozolomide | NCT00027625 | Completed | Phase I |
| Imatinib mesylate and temozolomide in treating patients with malignant glioma | Brain and central nervous system tumors | Drug: Imatinib mesylate Drug: Temozolomide | NCT00354068 | Completed | Phase I |
| Erlotinib and sirolimus in treating patients with Recurrent malignant glioma | Brain and central nervous system tumors | Drug: Erlotinib + Sirolimus | NCT00509431 | Completed | Phase I |
| SU5416 in treating patients with recurrent astrocytoma or mixed glioma that has not responded to radiation therapy | Brain and central nervous system tumors | Drug: Semaxanib | NCT00004868 | Completed | Phase I,II |

*(Continued)*

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| Lenalidomide in combination with bevacizumab, sorafenib, temsirolimus, or 5-fluorouracil, leucovorin, oxaliplatin (FOLFOX) | Advanced cancers | Drug: Lenalidomide Drug: Bevacizumab Drug: Sorafenib Drug: Temsirolimus Drug: Oxaliplatin Drug: Leucovorin Drug: 5-fluorouracil | NCT01183663 | Completed | Phase I |

*Summarized in the Table 1 are the ongoing clinical trials present on ClinicalTrials.gov searching the keywords "glioblastoma multiforme" and "kinase inhibitor" The research has been done adding the following filters: "Completed"; "The research has been performed on October 21st, 2020."*

depletion of tissue arginine levels (Uyttenhove et al., 2003; Fecci et al., 2006a,b; Wainwright et al., 2012).

Immune checkpoints exert a key role in central and peripheral tolerance by counteracting activating signaling (Xu et al., 2018). Under physiological conditions, immune checkpoint molecules represent a negative feedback to regulate inflammatory responses following T cell activation (Krummel and Allison, 1996; Chambers et al., 2001; Collins et al., 2002; Stone et al., 2009; Inarrairaegui et al., 2018). A mechanism used by tumors, including GBM, to inhibit and escape the anti-tumor immune response is represented by the expression of checkpoint molecules, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4) and PD1 (Stone et al., 2009; Francisco et al., 2010; Cheng et al., 2013; Bhandaru and Rotte, 2017; Hui et al., 2017; Wei et al., 2017, 2018, 2019a,b; Rotte et al., 2018; Kalbasi and Ribas, 2020; Sharma and Allison, 2020).

## SMALL MOLECULES FOR TARGETED THERAPIES IN GBM

The progresses in the molecular classification of GBM have allowed the identification of dysregulated pathways that could represent potential targets for new treatment strategies (**Figure 2**).

Glioblastoma is a vascularized tumor which is histologically characterized by the expression of VEGF and other proangiogenic cytokines involved in the stimulation of endothelial cell proliferation, migration and survival (Schiffer et al., 2018). In patients with a relapsed GBM, the TKI regorafenib has received approval in the GBM treatment (Lombardi et al., 2019).

Other TKIs targeting VEGF family components have been proposed for the treatment of GBM besides regorafenib. Of note, vascular normalization has been proposed as an alternative strategy for the employment of antiangiogenic therapies in which the objective is to modulate the tumor vasculature in order to reduce hypoxia, and to support physiological angiogenesis. This process could ultimately improve perfusion and drug delivery. In this context, promising results in reducing angiogenesis and normalizing vascularization have been shown by cediranib and sutinib (Batchelor et al., 2013; Grisanti et al., 2019).

The PI3K/mammalian target of rapamycin (mTOR) pathway is a targetable pathway in GBM. In this context, the mTOR inhibitor temsirolimus did not show a treatment efficacy as single agent in recurrent GBM (Chang et al., 2005). Similarly, the pan-PI3K inhibitor buparlisib did not demonstrate a treatment efficacy (Wen et al., 2019). Also treatment combinations of mTOR pathway inhibitors with radiotherapy and TMZ or in combination with radiotherapy only did not show efficacy (Ma et al., 2015; Wick et al., 2016).

Targeting MDM2 and mouse double minute 4 homolog (MDM4) activity has been suggested for GBM cases carrying *MDM2* or *MDM4* gene amplification (Wick et al., 2019).

Moreover, the *CDK4/6* inhibitor palbociclib failed to demonstrate the efficacy of this treatment in GBM (Taylor et al., 2018). CDK9 is an alternative targetable CDK (Taylor et al., 2018).

The use of TKIs targeting EGFR as single agents did not demonstrate significant activity for GBM treatment (Lassman et al., 2005; Hegi et al., 2011). It has not yet been agreed on the potential use of MET as target for GBM treatment. The use of the TKIs crizotinib and cabozantinib in recurrent GBM has achieved modest efficacy after several attempts (International Cancer Genome Consortium PedBrain Tumor Project, 2016; Wen et al., 2018). Tests have been carried out for larotrectinib and entrectinib in neutrophic tyrosine receptor kinase (NTRK) fusion-positive GBM without any confirmation on their efficacy (Ferguson et al., 2018). Notwithstanding the frequent expression in GBM of fibroblast growth factor receptors (FGFRs), a relevance as potential therapy target seems to be restricted to GBM exhibiting FGFR-transforming acidic coiled-coil containing protein TACC fusions (Singh et al., 2012), as shown by using the pan-FGFR kinase inhibitor erdafitinib (Di Stefano et al., 2015). A modest treatment efficacy has been obtained for the possible targeting of *BRAF*V600E mutation in GBM (Kaley et al., 2018). Finally, eribulin has been proposed to inhibit TERT activity in GBM (Takahashi et al., 2019). A list of the current clinical trials employing TKIs for GBM treatment is reported in **Table 1**.

## USE OF ICIs FOR GBM TREATMENT

Following the results of ICIs use in other cancers, the use of PD-1/PD-L1 inhibitors has been proposed for GBM (**Table 2**). Clinical trial results have shown that GBM patients with unresectable tumors do not benefit from monotherapy with nivolumab in terms of survival improvement when compared

**TABLE 2 |** Clinical trials in glioblastoma (GBM) using immune checkpoint inhibitors (ICIs).

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| Neoantigen-based personalized vaccine combined with immune checkpoint blockade therapy in patients with newly diagnosed, unmethylated glioblastoma | Glioblastoma | Biological: NeoVax Biological: Nivolumab Biological: Ipilimumab | NCT03422094 | Suspended | Phase I |
| Autologous dendritic cells, metronomic cyclophosphamide and checkpoint blockade in children with relapsed HGG | Childhood glioblastoma | Drug: depletion of regulatory T cells Biological: cancer vaccine Biological: checkpoint blockade | NCT03879512 | Recluting | Phase I,II |
| Cytokine microdialysis for real-time immune monitoring in glioblastoma patients undergoing checkpoint blockade | Glioblastoma | Drug: Nivolumab Drug: BMS-986016 | NCT03493932 | Recluting | Phase I |
| Laser interstitial thermotherapy (LITT) combined with checkpoint inhibitor for recurrent GBM (RGBM) | Glioblastoma Adult | Drug: Pembrolizumab at 7 days prior Drug: Pembrolizumab at 14 days post Drug: Pembrolizumab at 35 days post | NCT03277638 | Recluting | Phase I,II |
| Pilot surgical trial to evaluate early immunologic pharmacodynamic parameters for the PD-1 checkpoint inhibitor, pembrolizumab (MK-3475), In patients with surgically accessible recurrent/progressive glioblastoma | Brain cancer | Drug: MK-3475 | NCT02852655 | Active, not recruiting | Phase I |
| A study testing the effect of immunotherapy (ipilimumab and nivolumab) in patients with recurrent glioblastoma with elevated mutational burden | Recurrent glioblastoma Secondary glioblastoma | Biological: Ipilimumab Biological: Nivolumab | NCT04145115 | Not yet recruiting | Phase II |
| First-in-human, phase 1b/2a trial of a multipeptide therapeutic vaccine in patients with progressive glioblastoma | Glioblastoma Adult | Biological: Multiple dose of EO2401 | NCT04116658 | Not yet recruiting | Phase I,II |
| A phase 1 study of PVSRIPO and pembrolizumab in patients with recurrent glioblastoma | Glioblastoma Recurrent glioblastoma Supratentorial glioblastoma Brain tumor | Biological: PVSRIPO Biological: Pembrolizumab | NCT04479241 | Not yet recruiting | Phase I |
| Nivolumab, BMS-986205, and radiation therapy with or without temozolomide in treating patients with newly diagnosed glioblastoma | Glioblastoma | Biological: IDO1 Inhibitor BMS-986205 Biological: Nivolumab Drug: Temozolomide | NCT04047706 | Recluting | Phase I |
| Immunogene-modified T (IgT) cells against glioblastoma multiforme | Glioblastoma multiforme of brain Glioblastoma multiforme | Biological: Antigenspecific IgT cells | NCT03170141 | Enrolling by invitation | Phase I |
| An investigational immunotherapy study of nivolumab compared to temozolomide, each given with radiation therapy, for newly diagnosed patients with glioblastoma (GBM, a malignant brain cancer) | Brain Cancer | Drug: Nivolumab Drug: Temozolomide | NCT02617589 | Active, not recruiting | Phase III |
| Translational study of nivolumab in combination with bevacizumab for recurrent glioblastoma | Recurrent adult brain tumor | Drug: Nivolumab Drug: Bevacizumab | NCT03890952 | Recluting | Phase II |
| Immunological and functional characterization of cellular population CD45+ infiltrating human glioblastoma | Glioblastoma | | NCT03687099 | Recluting | Observational |

*(Continued)*

**TABLE 2 |** Continued

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| Avelumab in patients with newly diagnosed glioblastoma multiforme | Glioblastoma Multiforme of brain | Biological: Avelumab | NCT03047473 | Active, not recruiting | Phase II |
| Capecitabine + bevacizumab in patients with recurrent glioblastoma | Glioblastoma | Drug: Capecitabine Drug: Bevacizumab | NCT02669173 | Recluting | Phase I |
| VXM01 plus avelumab combination study in progressive glioblastoma | Recurrent glioblastoma | Biological: VXM01 Biological: Avelumab | NCT03750071 | Recluting | Phase I,II |
| Immunotherapy (nivolumab and ipilimumab) before and after surgery for the treatment of recurrent or progressive high grade glioma in children and young adults | Glioblastoma Malignant glioma Recurrent glioblastoma Recurrent malignant glioma Recurrent grade III Glioma Grade III GLioma | Biological: Ipilimumab Biological: Nivolumab Drug: Placebo Administratio | NCT04323046 | Not yet recruiting | Phase I |
| CART-EGFRvIII + Pembrolizumab in GBM | Glioblastoma | Biological: CARTEGFRvIII T cells Biological: Pembrolizumab | NCT03726515 | Active, not recruiting | Phase I |
| INO-5401 and INO-9012 delivered by electroporation (EP) in combination with cemiplimab (REGN2810) in newly diagnosed glioblastoma (GBM) | Glioblastoma | Biological: INO-5401 Biological: INO-9012 Biological: Cemiplimab Drug: Temozolomide | NCT03491683 | Active, not recruiting | Phase I,II |
| Combination adenovirus + pembrolizumab to trigger immune virus effects | Brain cancer Brain neoplasm Glioma Glioblastoma Gliosarcoma and other 3 | Biological: DNX-2401 Biological: Pembrolizumab | NCT02798406 | Active, not recruiting | Phase II |
| GMCI, nivolumab, and radiation therapy in treating patients with newly diagnosed high-grade gliomas | Glioma Malignant | Biological: AdV-tk Drug: Valacyclovir Drug: Temozolomide Biological: Nivolumab | NCT03576612 | Recluting | Phase I |
| Nivolumab, BMS-986205, and radiation therapy with or without temozolomide in treating patients with newly diagnosed glioblastoma | Glioblastoma | Biological: IDO1 Inhibitor BMS-986205 Biological: Nivolumab Drug: Temozolomide | NCT04047706 | Recluting | Phase I |
| Study of the IDO pathway inhibitor, indoximod, and temozolomide for pediatric patients with progressive primary malignant brain tumors | Glioblastoma Multiforme Glioma Gliosarcoma Malignant brain tumor Ependymoma and other 3 | Drug: Indoximod Drug: Temozolomide Drug: Cyclophosphamide Drug: Etoposide | NCT02502708 | Active, not recruiting | Phase I |
| A phase 0 study of AZD1775 in recurrent GBM patients | Glioblastoma | Biological: AZD1775 | NCT02207010 | | Early phase I |
| Nivolumab in people with IDH-mutant gliomas with and without hypermutator phenotype | Glioma Glioblastoma High grage glioma Low grade glioma Malignant glioma | Drug: Nivolumab | NCT03718767 | Recluting | Phase II |
| A pilot study to evaluate PBR PET in brain tumor patients treated with chemoradiation or immunotherapy | Intracranial tumors Glioblastoma Melanoma | Biological: Cancer immunotherapy Radiation: Radiation and chemotherapy | NCT02431572 | Completed | |

*(Continued)*

**TABLE 2 |** Continued

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| HSV G207 with a single radiation dose in children with recurrent high-grade glioma | Neoplasms High grade glioma Glioblastoma multiforme Malignant glioma of brain Anaplastic astrocytoma of brain and other 3 | Drug: Biological G207 | NCT04482933 | Not yet recruiting | Phase II |

*Summarized in the Table are the ongoing clinical trials present on ClinicalTrials.gov searching the keywords "glioblastoma multiforme" and "Checkpoint." The research has been done adding the following filters: "Not yet recruiting"; "Recruiting"; "Enrolling by invitation"; "Active, not recruiting"; "Suspended"; "Terminate"; "Completed"; "Withdrawn"; "Unknown status"; "The research has been performed on October 21st, 2020."*

to bevacizumab (Reiss et al., 2017). Moreover, pembrolizumab showed limited activity for GBM (Reardon et al., 2014, 2016; Schwartz et al., 2016; Reardon et al., 2017; Reiss et al., 2017; Wen et al., 2018).

Recent tests have been carried out involving patients with newly diagnosed or relapsed GBMs for the use of ICIs (e.g., nivolumab or pembrolizumab) in neoadjuvant and/or adjuvant administration, although no straightforward results have been obtained (Cloughesy et al., 2019; Schalper et al., 2019).

Glioblastoma tumors of cases non-responsive to ICIs have shown an enrichment in mutations of the *PTEN* gene (Zhao et al., 2019) that has been associated with an immunosuppressive TME characterized by the presence of GBM cells expressing CD44. *PTEN* mutant tumors were characterized by highly clustering tumor cells with a lack of T cell infiltration (Peng et al., 2016; George et al., 2017). Furthermore, the poor responsiveness to ICIs of GBM cases carrying *PTEN* mutations has been related to a low PD-L1 expression for the involvement of the PI3K-mTOR pathway that is downstream to PTEN (Lastwika et al., 2016).

Responsiveness to ICI was associated with the presence of mutations of *BRAF*/protein tyrosine phosphatase non-receptor type 11 (*PTPN11*). In this subset of *BRAF*/*PTPN11* GBM patients, treatment combinations of ICIs and MAPK inhibitors could be introduced (Toso et al., 2014; Toso et al., 2014; Ebert et al., 2016; Wang et al., 2016).

The heterogeneous response rate to ICIs highlights the need of identifying the subgroups of patients who could benefit the most from the use of this immunotherapy treatment. PD-L1 expression was the first marker evaluated as predictor of a clinical response to ICIs (Ansell et al., 2015). PD-L1 expression in gliomas was associated with *IDH* status (Berghoff and Preusser, 2016; Garber et al., 2016; Berghoff et al., 2017). Importantly, mesenchymal GBM has been found having high levels of PD-L1 expression that may suggest that the expression of immune checkpoint proteins and aggressiveness of GBM tumors may be correlated (Garber et al., 2016). More recently, the tumor mutational burden has been proposed as a predictive marker of responsiveness to ICIs. However, it has not generally been demonstrated that the tumor mutational burden is capable of sufficiently predicting long term clinical benefits (Champiat et al., 2014; Rizvi et al., 2015; Schumacher et al., 2015; Le et al., 2017). Moreover, recent studies have shown that higher somatic mutation and neoepitope loads have not been found in GBM cases responsive to ICIs (Zhao et al., 2019). The infiltration of mutation-reactive class I and class II T cells into the tumor seems not to be precluded by a low mutational load in GBM (Cloughesy et al., 2019; Schalper et al., 2019; Zhao et al., 2019). The presence of alterations in the *MMR* genes is another proposed biomarker (Cloughesy et al., 2019; Schalper et al., 2019). The expression of MHC class I molecules has been associated to responsiveness to ICIs since it is involved in the presentation of antigens and characterized by highly heterogeneous expression levels in GBM (Indraccolo et al., 2019).

## DISCUSSION

Surgery followed by radiotherapy and chemotherapy with alkylating agents constitutes the standard first-line treatment of GBM (Stupp et al., 2005; Canoll and Goldman, 2008; Levine et al., 2015). Complete resection of the GBM tumors is generally not possible given its high invasive features. Although this combination therapy can prolong survival, the prognosis is still poor due to several factors including chemoresistance. Multiple mechanisms appear to be involved in the development of drug resistance in GBM including overexpression of drug efflux transporter pumps such as p-glycoprotein, the presence of a GSC population, a relevant activity of DNA repair mechanisms and dysregulated apoptosis processes such as MGMT, the MMR pathway, the base excision repair (BER) pathway and the TP53 pathway (Walker et al., 1992; Bobola et al., 1996; Qian and Brent, 1997; Jaeckle et al., 1998; Chen et al., 1999; Esteller et al., 2000; Middlemas et al., 2000; Paz et al., 2004; Hegi et al., 2005; Helleday et al., 2005; Bryant and Helleday, 2006; Zawlik et al., 2009; van Nifterik et al., 2010; Malmstrom et al., 2012; Reifenberger et al., 2012; Armstrong et al., 2013; Brennan et al., 2013; Wiestler et al., 2013; Wick et al., 2014, 2018; Erasimus et al., 2016; Peng et al., 2016; Sun et al., 2018; Gupta et al., 2018; Zhang et al., 2018; Christmann and Kaina, 2019; Hafner et al., 2019; Mantovani et al., 2019). Tumor/TME interactions also contribute to the development of drug resistance in GBM tumor cells (Hanahan and Weinberg, 2011; Ab and Jn, 2012; Rodriguez-Hernandez et al., 2014; Munoz et al., 2015).

Systemic delivery uses existing vessels to deliver anti-tumor drugs to the tumor. To overcome the impediment of the

**TABLE 3 |** Clinical trials in glioblastoma (GBM) using chimeric antigen receptor-T (CAR-T).
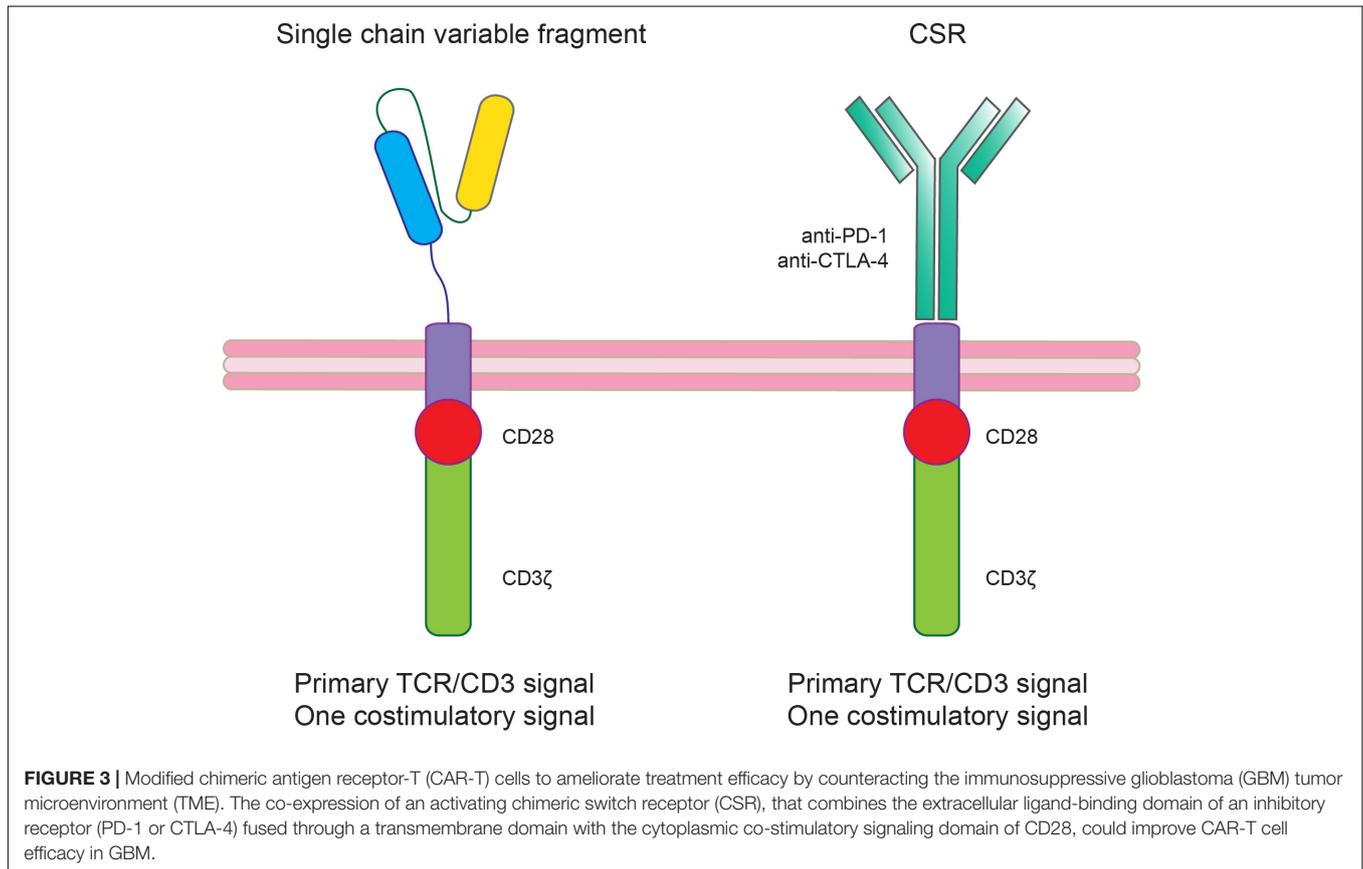
| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| Pilot study of autologous anti-EGFRvIII CAR-T cells in recurrent glioblastoma multiforme | Glioblastoma multiforme | Biological: anti-EGFRvIII CAR-T cells drug: cyclophosphamide Drug: Fludarabine | NCT02844062 | | Phase I |
| Pilot study of B7-H3 CAR-T in treating patients with recurrent and refractory glioblastoma | Recurrent glioblastoma Refractory glioblastoma | Drug: B7-H3 CAR-T Drug: Temozolomide | NCT04385173 | Recruiting | Phase I |
| B7-H3 CAR-T for recurrent or refractory glioblastoma | Recurrent glioblastoma Refractory glioblastoma | Drug: Temozolomide Biological: B7-H3 CAR-T | NCT04077866 | Not yet recruiting | Phase I, II |
| CD147-CAR-T cells in patients with recurrent malignant glioma | Recurrent glioblastoma CD147 positive | Biological: CD147-CAR-T | NCT04045847 | Active, not recruiting | Early phase I |
| CART-EGFRvIII + pembrolizumab in GBM | Glioblastoma | Biological: CART-EGFRvIII T cells Biological: Pembrolizumab | NCT03726515 | Active, not recruiting | Phase I |
| EGFRvIII CAR-T cells for newly diagnosed WHO grade IV malignant glioma | Glioblastoma Gliosarcoma | Biological: EGFRvIII CAR-T cells | NCT02664363 | Terminated | Phase I |
| Chimeric antigen receptor (CAR- T) cells with a chlorotoxin tumor-targeting domain for the treatment of recurrent or progressive glioblastoma | Recurrent glioblastoma Recurrent malignant glioma recurrent WHO grade II glioma recurrent WHO grade III glioma | Biological: Chlorotoxin (EQ)-CD28-CD3zeta-CD19t-expressing CAR-TTlymphocytes NCI SYs | NCT04214392 | Recluting | Phase I |
| IL13Ralpha2-targeted chimeric antigen receptor (CAR-T) T cells with or without nivolumab and ipilimumab in treating patients with recurrent or refractory glioblastoma | Recurrent glioblastoma Refractory glioblastoma | Biological: IL13Ralpha2-specific Hinge-optimized 4-1BB-co-stimulatory CAR/Truncated CD19-expressing autologous TN/MEM Cells Biological: Ipilimumab Biological: Nivolumab | NCT04003649 | Recluting | Phase I |
| Autologous T cells redirected to EGFRVIII-with a chimeric antigen receptor in patients with EGFRVIII+ glioblastoma | Patients with residual or reccurent EGFRvIII+ glioma | Biological: CART-EGFRvIII T cells | NCT02209376 | Terminated Result | Phase I |
| NKG2D-based CAR-T-cells Immunotherapy for patient with r/r NKG2DL+ solid tumors | Hepatocellular carcinoma Glioblastoma Medulloblastoma Colon cancer | Biological: NKG2D-based CAR-T-cells | NCT04270461 | Not yet recruiting | Phase I |
| Pilot study of autologous chimeric switch receptor modified T Cells in recurrent glioblastoma multiforme | Glioblastoma multiforme | Biological: Anti-PD-L1 CSR T cells Drug: Cyclophosphamide Drug: Fludarabine | NCT02937844 | | Phase I |
| Intracerebral EGFR-vIII CAR-T cells for recurrent GBM | Recurrent glioblastoma Recurrent gliosarcoma | Biological: EGFRvIII-CARs | NCT03283631 | Recluting | Phase I |
| Combination of immunization and radiotherapy for malignant gliomas (*In situ* Vac1) | High grade glioma Glioblastoma Glioma of brainstem Glioma Malignant | Combination Product: Combined immune adjuvants and radiation | NCT03392545 | Recluting | Phase I |
| CAR-T cell receptor immunotherapy targeting EGFRvIII for patients with malignant gliomas expressing EGFRvIII | Malignant glioma Glioblastoma Brain cancer Gliosarcoma | Biological: Epidermal growth factor receptor (EGFRv)III Chimeric antigen receptor (CAR) transduced PBL Drug: Aldesleukin Drug: Fludarabine Drug: Cyclophosphamide | NCT01454596 | Completed | Phase I, II |
| Immunogene-modified T (IgT) cells against glioblastoma multiforme | Glioblastoma multiforme of brain glioblastoma multiforme | Biological: Antigen-specific IgT cells | NCT03170141 | Enrolling by invitation | Phase I |
| CMV-specific cytotoxic T lymphocytes expressing CAR-T targeting HER2 in patients with GBM (HERT-GBM) | Glioblastoma multiforme | Biological: HER.CAR-TCMV-specific CTLs | NCT01109095 | Completed | Phase I |
| Genetically modified T-cells in treating patients with recurrent or refractory malignant glioma | Malignant glioma Refractory brain neoplasm Recurrent brain neoplasm Glioblastoma | Biological: IL13Rα2-specific, hinge-optimized, 41BB-costimulatory CAR/truncated CD19-expressing Autologous T lymphocytes Biological: Vaccine Therapy | NCT02208362 | Recluting | Phase I |

*(Continued)*

**TABLE 3 |** Continued

| Title | Condition or Disease | Intervention/Treatment | NCT Number | Status | Status Phase |
|---|---|---|---|---|---|
| Memory-enriched T cells in treating patients with recurrent or refractory grade III-IV glioma | Glioblastoma HER2/Neu positive Malignant glioma Recurrent glioma Refractory glioma WHO grade III glioma | Biological: CD19CAR-CD28-CD3zeta-EGFRt-expressing Tcm-enriched T-lymphocytes Biological: CD19CAR-CD28-CD3zeta-EGFRt-expressing Tn/mem-enriched T-lymphocytes | NCT03389230 | Recluting | Phase I |

*Summarized in the Table are the ongoing clinical trials present on ClinicalTrials.gov searching the keywords "glioblastoma multiforme" and "CAR-T." The research has been done adding the following filters: "Not yet recruiting"; "Recruiting"; "Enrolling by invitation"; "Active, not recruiting"; "Suspended"; "Terminate"; "Completed"; "Withdrawn"; "Unknown status"; "The research has been performed on October 21st, 2020."*



**FIGURE 3 |** Modified chimeric antigen receptor-T (CAR-T) cells to ameliorate treatment efficacy by counteracting the immunosuppressive glioblastoma (GBM) tumor microenvironment (TME). The co-expression of an activating chimeric switch receptor (CSR), that combines the extracellular ligand-binding domain of an inhibitory receptor (PD-1 or CTLA-4) fused through a transmembrane domain with the cytoplasmic co-stimulatory signaling domain of CD28, could improve CAR-T cell efficacy in GBM.

BBB several strategies have been proposed including chemical modification of the drugs, high dose chemotherapy capable of inducing a transient BBB disruption, nanoparticle-based drug delivery and peptide-based drug delivery. Nevertheless, no straightforward results have still been reached (Siegal, 2013).

Glioblastoma stem cell cell population has been shown to induce a certain degree of radio- chemoresistance given their high expression of anti-apoptotic proteins, ATP-binding cassette pumps, their increased capability of DNA damage repair, as well as their high capacity of migration and invasion (Bao et al., 2006; Calabrese et al., 2007; Eyler and Rich, 2008; Diehn et al., 2009; Pietras et al., 2014). GSCs have been found capable of secreting angiogenic factors which in turn are responsible for an enhancement in the formation of tumor blood vessels, this has been frequently associated with high tumor aggressiveness. Moreover, the TME cell components can promote GSC survival by VEGF secretion (Wada et al., 2006). The interaction of TME with GSCs can facilitate tumor progression and consequently therapeutic resistance (Chen and Liu, 2012; Miura et al., 2013).

Over the past 10 years, the knowledge regarding genomic features of GBM has been greatly increased by comprehensive multiplatform genome-wide analyses. As a result of these analyses, it has emerged that GBM comprises a group of highly heterogeneous tumor types, each with peculiar molecular/genetic features (Tcga, 2008; Brennan et al., 2013; Wang et al., 2016).

In GBM the phase I/II and III trials investigating the use of therapies molecularly targeting oncogenic alterations did not generally show straightforward results and, consequently, their

clinical utilization is still limited. However, although limited activity or no therapeutic efficacy has so far been produced by the use of TKIs, improvement in understanding the mechanisms of action of these compounds could help to determine how to better incorporate their use in the existing treatment modalities. Redundancies are frequently present in the molecular pathways that can be targeted which makes the inhibition of any pathway largely ineffective (Tcga, 2008; Brennan et al., 2013; Wang et al., 2016). The failure of targeted therapies can also be ascribed to another possible reason such as the fact that several genomic alterations are important only for the initial stages of tumor progression whereas other molecular mechanisms outweigh their role in the later stages. On the other hand, several genomic alterations in GBM can interfere with GBM cell metabolism. In particular, alterations in the growth factor signaling pathways that can control metabolic flux have been found in high frequency as well as recurrent mutations in *IDH1* and *IDH2* genes, whose encoded proteins are part of the tricarboxylic acid (TCA) cycle. Alterations of the cellular metabolism, which is controlled also by the biochemical microenvironment, could contribute to the failure of the proposed targeted therapies (Tcga, 2008; Brennan et al., 2013; Wang et al., 2016). A better understanding of the interactions constituting this interplay between altered genome and biochemical microenvironment could contribute to finding more effective treatment strategies in the reverting of altered cellular metabolism of GBM cells.

The TME of GBM is largely immunosuppressive, therefore efficiency of ICI treatments can be strongly affected by this condition (Akiyama et al., 2001; Brat and Van Meir, 2004; Brat et al., 2004; Nimsky et al., 2005; Zimmermann and Dours-Zimmermann, 2008; Persano et al., 2011; Sherriff et al., 2013; Dicker et al., 2014; Hambardzumyan and Bergers, 2015; Young et al., 2015; Chen and Hambardzumyan, 2018). GBM patients frequently present reduced levels of circulating CD4^{+} and CD8^{+} lymphocytes as a consequence of chemotherapy treatments (Gustafson et al., 2010; Mirzaei et al., 2017). A clear molecular/immunological signature that can be predictive of response to ICI treatments has not yet been identified (Motzer et al., 2015; Goldberg et al., 2016; Reck et al., 2016; Schwartz et al., 2016; Reiss et al., 2017; Reardon et al., 2018; Cloughesy et al., 2019; Schalper et al., 2019).

The treatment of different cancers has markedly been revolutionized by immunotherapy. Nevertheless, the data obtained so far concerning the use of ICIs for the treatment of GBM patients seem to be not sufficient to propose this type of immunotherapy as a standard treatment for GBM (Reardon et al., 2014, 2016; Motzer et al., 2015; Goldberg et al., 2016; Reck et al., 2016; Schwartz et al., 2016; Reardon et al., 2017; Reiss et al., 2017; Wen et al., 2018; Cloughesy et al., 2019; Schalper et al., 2019).

Another immunotherapy approach that can be used also in combinations with ICIs is the chimeric antigen receptor-T (CAR-T) cell therapy targeting specific tumor associated antigens. The introduction of CAR-T cell therapy approaches also in solid tumors including GBM has been favored by the success of this therapy in hematological malignancies (Neelapu et al., 2017; Maude et al., 2018). Concerning GBM treatment, several clinical trials have been proposed showing that there are still substantial obstacles including TME immune suppression (**Table 3**; Morgan et al., 2010; Brown et al., 2015, 2016; Zah et al., 2016; Ahmed et al., 2017; Walseng et al., 2017; Richman et al., 2018). To increase CAR-T treatment efficacy several CAR-T modifications have been proposed such as the knocking out of genes encoding T cell inhibitory receptors or signaling molecules (e.g., PD-1 or CTLA-4) or the co-expression of activating chimeric switch receptor (CSR; **Figure 3**; Prosser et al., 2012; Shin et al., 2012; Ankri et al., 2013; Kobold et al., 2015; Liu et al., 2016).

Understanding the molecular and immunological complexity of GBM more and more could provide the grounds for the introduction of other immunotherapeutic approaches such as the use of CAR-T cell therapy, in combination with ICIs or TKIs, in the treatment paradigm of GBM.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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