



# STAT3 in medulloblastoma: a key transcriptional regulator and potential therapeutic target

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Received: 4 April 2022 / Accepted: 8 June 2022 / Published online: 18 June 2022  
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## Abstract

Medulloblastoma is the most common malignant brain tumor of childhood accounting for about 60% of all pediatric embryonal tumors. Despite improvements in the overall survival rate, this tumor still lacks an efficient, reliable, and less toxic therapeutic approach. Characterization of the molecular mechanisms involved in medulloblastoma initiation and progression is a crucial step for the development of effective therapies. Signal transducer and activator of transcription 3 is a convergence point for several signaling cascades that are implicated in medulloblastoma tumorigenesis. Accumulated evidence has revealed the pivotal role of signal transducer and activator of transcription 3 in medulloblastoma pathogenesis such as proliferation, survival, angiogenesis, and immunosuppression as well as maintenance, drug resistance, and recurrence. In this review, we focus on the role of signal transducer and activator of transcription 3 in medulloblastoma tumorigenesis and discuss the recent advances of signal transducer and activator of transcription 3 inhibition as a promising developed strategy for medulloblastoma therapy.

**Keywords** Medulloblastoma · Signal transducer and activator of transcription 3 · Signaling pathway · Inhibitors

## Introduction

Medulloblastoma (MB) is the most common malignant brain tumor of childhood affecting mainly the cerebellum and accounting for about 20% of all pediatric central nervous system (CNS) neoplasms [1]. It mainly arises from the primitive neuroectoderm in the cerebellar vermis of the brain affecting mostly infants and children in their first years of life [2]. Males are more frequently diagnosed with MB than females with a male to female ratio of 1.7: 1 [3].

The incidence rate for children younger than 4 years is 0.55 per 100,000 population and 0.16 per 100,000 population for adolescents aged between 15–19 years [3].

MB can be either classified based on histological criteria or molecular features. Based on histological and cellular characteristics, MB is divided into four subtypes: classic MB, desmoplastic-nodular MB, MB with extensive nodularity, and large cell-anaplastic MB [4]. Recently, MB classification was updated according to different molecular phenotypes: wingless-activated MB (MB<sup>WNT-activated</sup>), sonic hedgehog-activated and TP53-wildtype MB (MB<sup>SHH-activated, TP53-wildtype</sup>), SHH-activated and TP53-mutant MB (MB<sup>SHH-activated, TP53-mutant</sup>), and non-WNT/non-SHH MB (MB<sup>non-WNT/non-SHH</sup>). The later includes two subtypes: MB<sup>non-WNT/non-SHH, Group 3</sup> and MB<sup>non-WNT/non-SHH, Group 4</sup> [5]. They exhibit highly distinct cytogenetic profiles, transcriptional signatures, mutational spectra, and most importantly, clinical phenotypes such as prognostic outcomes [6].

Currently, the standard regimens used for MB patients include surgical resection followed by adjuvant chemotherapy [7]. Multimodal treatments have improved overall survival (OS) to a 5 year of approximately 80% and 10 year

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of about 70% of MB patients [8]. However, recurrence, metastasis, and severe long-term side effects related to treatment toxicity remain the major clinical limitations [7]. In this regard, different treatment approaches have been used in MB such as immunotherapy, gene-targeted therapy, and drug repurposing [8, 9]. Nevertheless, a better understanding of the molecular mechanisms in the normal brain and also in MB that drive the tumor's phenotype will allow identifying a novel therapeutic target and development of safer and more effective treatment to interrupt such aberrant signaling. In this review, we focus our discussion on the role of Signal transducer and activator of transcription (STAT3) activation and signaling in MB pathogenesis highlighting the importance of STAT3 inhibitors as a promising therapeutic approach for targeting MB.

## Overview of STAT3 pathway

### STAT family

STAT family is a group of cytoplasmic transcription factors that were initially recognized as interferon mediators of several biological processes. In mammals, STAT family comprises seven members, STAT1, -2, -3, -4, -5a/b, and -6 [10]. STATs are activated by extracellular signaling proteins (cytokines, growth factors, and hormones) mainly via binding to specific cell surface receptor-associated Janus kinases (JAK) promoting a wide range of physiological events such as differentiation, proliferation, angiogenesis, apoptosis, and cellular immunity [11]. All STAT proteins share a common N-terminal domain (ND), a coiled-coil domain (CCD), DNA binding domain (DBD), linker domain (LD), Src-homology-2 domain (SH2), and the C-terminal transactivation domain (CTD) which contains two phosphorylation sites, Tyr705 and Ser727 involved in gene transcriptional activity [12].

### STAT3 activation and signaling

Among all the STATs, STAT3 is the most clinically relevant one due to its pivotal roles in a variety of biological events such as hematopoiesis, neurogenesis, embryogenesis, immune responses, and oncogenesis [12]. *STAT3* gene is almost expressed in all body cell types and can be proteolytically cleaved into two isoforms, STAT3 $\alpha$  and STAT3 $\beta$  [13]. STAT3 is induced by numerous cytokines and growth factors activating a transduction cascade which results in the amplification of target gene(s) [14]. Biological responses of STAT3 occur at two molecular levels: canonical STAT3 pathway and non-canonical STAT3 pathway.

The most prevalent canonical STAT3 pathway is the IL-6/STAT3 signaling pathway. Upon binding of IL-6 to

glycoprotein 130 (GP130) receptor [15], JAK (JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2)) is phosphorylated [16] inducing STAT3 phosphorylation at tyr705 residue in the STAT3 CTD. STAT3 can be also phosphorylated at another crucial residue Ser727 which is located at the CTD [17]. Phosphorylated STAT3 recruits another phosphorylated STAT3 to form a dimer, and translocate to the nucleus [11], where they bind to specific interferon-gamma (IFN- $\gamma$ )-activated sequence (GAS) sites [18] inducing the expression of target genes including pro-invasive factors such as metalloproteinases and heat shock proteins [19] as well as angiogenic factors such as vascular endothelial growth factor (VEGF) [20]. On the other hand, the non-canonical STAT3 signaling pathway has been implicated in STAT3 activity independently of tyr705 phosphorylation [21]. Evidence of this pathway indicated that an un-phosphorylated STAT3 monomer or dimer can translocate to the nucleus and regulate gene expression via binding to GAS [22].

### STAT3 regulation

Although phosphorylation plays a major role in STAT3 activation, post-translational modifications including acetylation, methylation, ubiquitination, and dephosphorylation are also essential for STAT3 signaling regulation [23, 24]. Acetylation is required for STAT3 dimerization and activation. This reversible mechanism is carried out by acetyltransferase p300 at Lys-685 residue of STAT3 [24]. STAT3 acetylation by CREB binding protein (CBP)-histone acetyltransferase enhances its translocation to the mitochondria where acetylated STAT3 participates in pyruvate metabolism by association with pyruvate dehydrogenase complex E1 (PDC-E1) resulting in ATP production. However, STAT3 role in pyruvate metabolism is terminated upon its deacetylation by sirtuin 5 (SIRT5) [25].

Methylation is another fundamental post-translational modification essential for STAT3 regulation. STAT3 can be di-methylated on Lys140 residue by histone methyltransferase (SET9) and demethylated by lysine-specific demethylase (LSD1). This process negatively regulates the expression of STAT3 target genes, knowing that methylation precedes STAT3 Tyr705 and Ser727 phosphorylation [26]. On the other hand, di-methylation of STAT3 on Lys49 by enhancer of zeste homolog 2 (EZH2) activates the transcription of IL-6-dependent genes [27].

STAT3 activity is also regulated by ubiquitin proteasomal degradation. In neonatal hippocampal neurons, calcineurin stimulation results in the degradation of STAT3 via ubiquitin proteasomal pathway through TATA element modulatory factor/androgen receptor coactivator 160 (TMF/ARA 160) [28]. Furthermore, tumor necrosis factor receptor-associated factor (TRAF6) is implicated in STAT3 negative regulation by mediating its ubiquitination and degradation [29].

An important mechanism for regulating the activity of STAT3 is achieved by a wide variety of phosphatases. Protein tyrosine phosphatase receptor T (PTPRT) dephosphorylates phosphorylated STAT3 at Tyr705 residue directly after STAT3 activation by JAK, while phosphorylated STAT3 is migrating to the nucleus, or after its translocation to the nucleus [23]. In addition, non-receptor tyrosine phosphatases Meg2 and SHP2 dephosphorylate STAT3 [30] in cytoplasm and nucleus [30, 31]. STAT3 activity is also influenced by serine/threonine phosphatases by which protein phosphatase 2A (PP2A) is involved in STAT3 dephosphorylation at Ser727 residue interrupting its normal function in transducing signals to the nucleus [32].

Furthermore, other molecules involved in STAT3 regulation are the suppressor of cytokine signaling-3 (SOCS3) and the protein inhibitor of activated STAT3 (PIAS3). SOCS3 acts as a negative feedback loop by binding to the GP130 receptor thus inhibiting JAK from activating STAT3 [33], whereas PIAS3 binds directly to STAT3 hindering its interaction with DNA and preventing the expression of target genes [34].

## Role of STAT3 in normal brain physiology

### STAT3 in neurodevelopment

STAT3 is expressed in the cerebral cortex, striatum, and hippocampus during early embryonic life, and continued to be detected till 3 months prenatally [35, 36]. Later, the activation of STAT3 in the brain is limited where it can be found in the subventricular zone of the forebrain and the subgranular zone in the hippocampus which comprises cells that constantly undergo division [37]. Takeda et al. have indicated that STAT3-deficient mice died between E6.5 and E7.5, preceding gastrulation [38]. Furthermore, the production of neural precursor cells (NPCs) and the neural stem cell markers decline significantly in STAT3 dominant-negative cells indicating the critical role of STAT3 in early neural development [39]. Newly formed neurons secrete gliogenic cytokines that activate the JAK/STAT pathway in the undifferentiated neuronal stem cells (NSCs) destined to become astrocytes where Notch signaling triggers the demethylation of astrocytic genes which are then activated when STAT3 and the co-activator p300/CBP bind to their promoters [40]. STAT3 downregulation enhanced hippocampal neuronal maturation through the crosstalk with MAPK signaling. However, STAT3 overexpression induced hippocampal neurospheres characterized by stem cell properties and further attenuated the terminal differentiation of hippocampal neurons [41] (Fig. 1A and B).

### STAT3 in synaptic plasticity

JAK/STAT3 signaling is critical in inducing N-methyl-D-aspartate receptor (NMDAR) dependent-long term depression (NMDAR-LTD) in the hippocampus [42]. NMDAR-LTD has been implicated in spatial reversal learning by eliminating previous memory traces to permit new traces to form when needed [43]. Interestingly, STAT3 anchored to the postsynaptic site was translocated to the nucleus in response to certain synaptic stimuli altering gene expression [44]. During the neonatal period, the activity of STAT3 was blocked by cytokine leptin resulting in the enhancement of glutamatergic synapses through the formation and maturation of dendritic spines in the hippocampal neurons [45]. Thus, STAT3 is implicated in synaptic plasticity and cognition [46] (Fig. 1C).

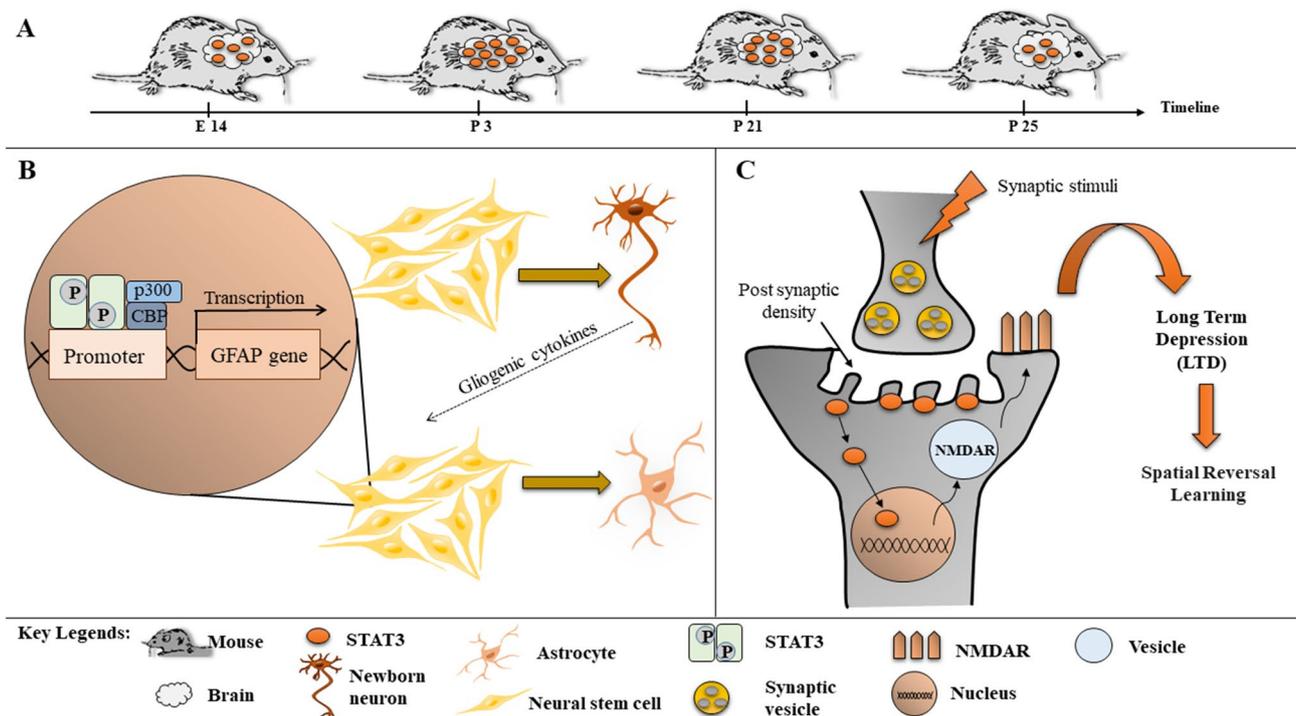
### STAT3 in neuroprotection

STAT3 is characterized by neuroprotection properties in response to various brain stress conditions including cerebral ischemia [47]. Several molecules such as estradiol, secretoneurin, GF-1, and IL-6 activated STAT3 which is, in turn, translocated to the nucleus and induced the transcription of neuroprotective genes such as *Bcl-2* [48]. Murase et al. demonstrated that activation of STAT3 regulated the transcription of *p53*, resulting in the activation of a survival signaling pathway in neurons upon the elevation of neuronal activity [49]. On the other hand, STAT3 pathway has been reported to be implicated in several brain pathophysiological conditions such as aging, Alzheimer's disease, Parkinson's disease, and brain tumors including glioma, neuroblastoma, and MB [50, 51].

## STAT3 and MB tumorigenesis

### STAT3 in MB growth and survival

STAT3 is a key cell survival protein involved in growth and anti-apoptotic mechanisms of several tumors including MB [52]. Phosphorylated STAT3 has been found in rapidly proliferated MB cells and implicated in MB cell growth and migration [53], and also attenuated apoptosis [54]. Notably, the constitutive activation of STAT3 via IL-6 and leukemia inhibitory factor (LIF) has induced the expression of a subset of genes such as *c-Myc*, *survivin*, *Bcl-2*, *Bcl-xl*, and *cyclin D1* mediating MB cell viability and proliferation [55, 56]. Furthermore, STAT3 besides other anti-apoptotic molecules such as epidermal growth factor receptor (EGFR) and matrix metalloproteinase-9 (MMP-9) was shown to protect MB cells against apoptosis [57] (Fig. 2). Interestingly, White et al. have indicated the sex-specific role of STAT3 in the initiation and development of SHH MB, by which targeting



**Fig. 1** Role of STAT3 in neurodevelopment. **A** STAT3 fluctuations during the perinatal period in the brain of rodents. Prenatally, STAT3 was detected at embryonic day (E14), whereas postnatally, it was identified at postnatal day (P3) and continued to increase till P21 where the amounts of STAT3 declined. **B** STAT3 role in astrogenesis during neurodevelopment. Newly formed neurons release gliogenic

cytokines driving the differentiation of neuronal stem cells into astrocytes by activating JAK/STAT pathway where STAT3 and p300/CBP bind to the promoters of astrocytic genes such as GFAP. **C** STAT3 role in synaptic plasticity and cognition through the induction of NMDAR-long term depression in the hippocampal synapse

STAT3 in SHH MB mouse model would protect the male mice from MB initiation with no effect on female mice [58].

### STAT3 in MB Angiogenesis

Angiogenesis—which is an essential process for MB in order to maintain its pathogenesis and survival—was shown to be dependent on STAT3 signaling [59]. STAT3 has been reported to induce neovascularization via binding to *VEGF* promoter inducing its expression and upregulation in many cell lines including MB [60]. Craveiro et al. revealed that constitutive activation of STAT3 enhanced the phosphorylation of VEGF2/3, the key receptors in angiogenesis [61] (Fig. 2).

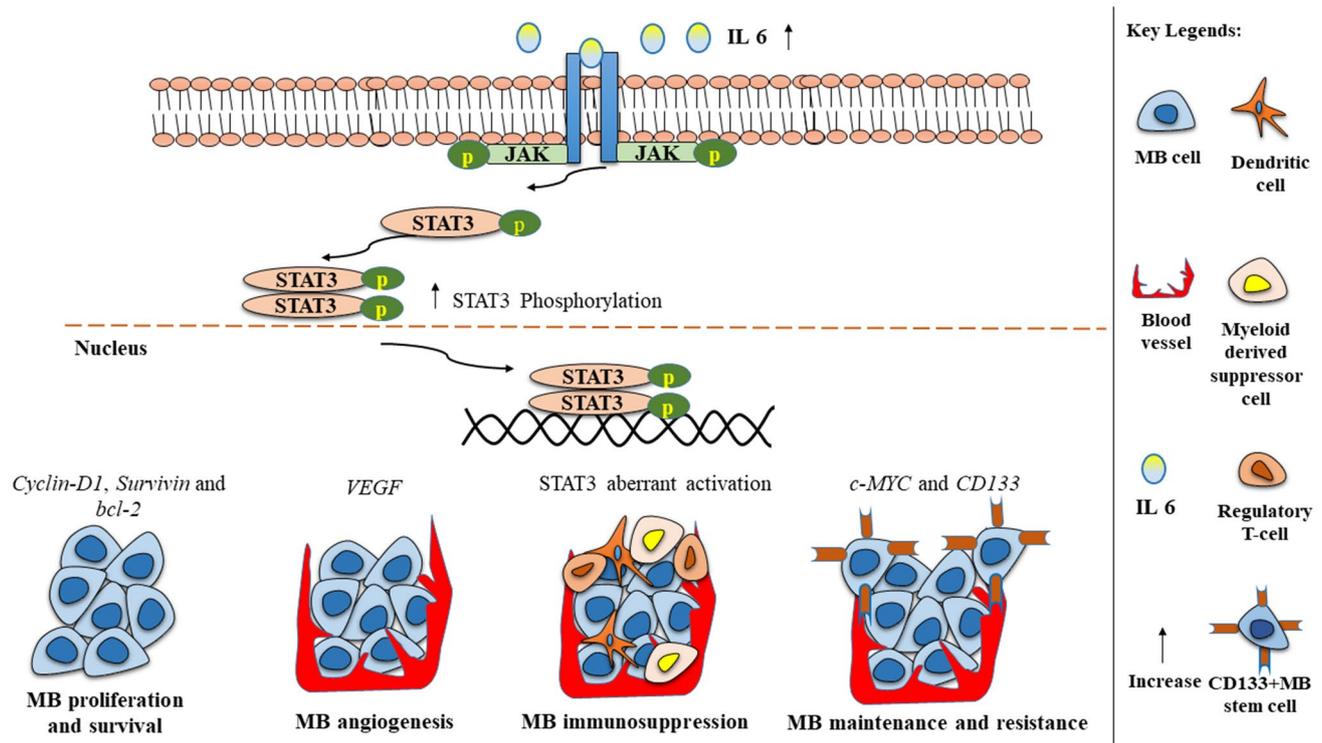
### STAT3 in MB immunosuppression

Although several studies have revealed the role of innate and adaptive immune components as extrinsic tumor suppressors, tumor cells including MB usually develop several mechanisms to evade the immune responses [2]. STAT3, one of the most common factors in tumors, acts as a negative regulator of inflammatory responses in immune cells

[62, 63]. Interestingly, activated STAT3 was shown to drive myeloid-derived suppressor cells (MDSCs) expansion that is involved in the immunosuppressive process [64]. Abad et al. have demonstrated the involvement of STAT3 in MDSCs to sustain this population in MB [63]. The constitutive activation of STAT3 in MDSCs promoted their survival and inhibited apoptosis in the MB mice model [63]. In addition, STAT3-intact MDSCs were able to regulate other cell types including dendritic cells (DCs) and regulatory T-cells (Tregs) within MB microenvironment to reinforce immunosuppression and promote MB growth [63]. Moreover, overexpression of B7-H3, an immunosuppressive immune checkpoint, was associated with increased activity of STAT3 which may indicate a more malignant and aggressive phenotype of MB [65] (Fig. 2).

### STAT3 in MB maintenance and resistance

The cancer stem cell (CSC) hypothesis suggests that CSCs originate from normal stem cells after multiple mutations, and are engaged in tumor formation, growth, and relapse [66, 67]. In MB, it was reported that tumor-associated astrocytes secreted chemokine C–C ligand 2 (CCL2) on



**Fig. 2** STAT3 signaling pathway in MB. The constitutive hyper-activation of STAT3 due to elevated IL-6 level resulted in STAT3 dimerization and translocation to the nucleus inducing the expression of its target genes such as *cyclin D1*, *survivin*, *c-Myc*, *VEGF*, *CD133*, and *Bcl-2* that mediate MB proliferation and survival, angiogenesis, in

addition to maintenance and resistance. Moreover, STAT3 aberrant activation resulted in MB immunosuppression via driving myeloid derived suppressor cell (MDSC) which in turn regulated dendritic cells and regulatory T-cells (Treg)

MB stem cells (MBSCs) maintaining their stem-like properties by activating the Notch pathway via JAK2/STAT3 signaling [68]. Stemness and maintenance of MB cells showed dependency on STAT3 by which STAT3 was associated with the expression of stemness markers such as *c-Myc* and *CD133*, increased proliferation, and decreased cell death [69]. STAT3 was also activated in *CD133*<sup>+</sup> MBs promoting metastasis, tumorigenesis, and recurrence by the regulation of *c-MYC* expression [70]. Moreover, it was demonstrated that the self-renewal *CD133*<sup>+</sup> MBSCs are triggered by STAT3 pathway supporting tumor survival [71] (Fig. 2).

Furthermore, it was reported that increased STAT3 activity has been associated with MB radio- and chemotherapy drug resistance [71, 72]. Sreenivasan et al. showed an elevated level of activated STAT3 in MB cell lines. Chemoresistant group 3 MB cell lines displayed an increased IL-6/STAT3 activity which is in turn involved in developing drug resistance. However, drug sensitivity was restored in MB cells lacking STAT3 or IL-6 receptors [72]. Therefore, due to the aberrant role of STAT3 signaling in MB tumorigenesis, STAT3 has been shown to be a potential therapeutic target for developing anti-tumor drugs.

## STAT3 inhibitors

Recently, STAT3 has been suggested as a promising molecular target for tumor therapy including MB. Various STAT3 inhibitors have been developed and well-characterized for their efficacy in targeting in vitro and in vivo MB models. In the following section, targeting STAT3 for the prevention and treatment of MB will be discussed.

## Multi-kinase inhibitors

### Pazopanib

Multi-kinase inhibitors (MKi) target tumor angiogenesis by blocking VEGF receptor (VEGFR) signaling [73]. Pazopanib, one of the MKi members, has the ability to penetrate the blood–brain barrier (BBB) and inhibit oncogenic kinases in neoplastic cells [73]. In MB cell lines, Pazopanib inhibited proliferation, arrested cell growth, promoted apoptosis, and reduced MB cell migration through blocking the phosphorylation of STAT3 by targeting upstream kinases and suppressing STAT3 synthesis after long-term

exposure. Furthermore, Pazopanib hindered tumor growth and increased the survival of the MB mice model [73] (Table 1; Part 1).

### Sorafenib

Sorafenib, an oral MKi, has the ability to inhibit angiogenesis and promote apoptosis in tumors via targeting the Raf-MEK-MAPK pathway [74]. Similar to Pazopanib, Sorafenib reduced cell viability, cell growth, proliferation, and angiogenesis, and induced apoptosis in MB cell lines by reducing STAT3 phosphorylation. Moreover, Sorafenib reduced tumor growth and improve survival in vivo [73, 75] (Table 1; Part 1).

### Vandetanib

Vandetanib is characterized by its antiangiogenic capability by regulating angiogenesis genes which further impact the survival of tumor cells. It also reduced tumor growth by inhibiting target receptors VEGFR 1/2, EGFR, and c-RET [76]. Vandetanib showed antitumor activity against MB where it reduced cell viability and proliferation, enhanced apoptosis, and inhibited cell migration in MB cell lines by decreasing STAT3 phosphorylation and expression [61] (Table 1; Part 1).

### Axitinib

Axitinib, an FDA-approved drug for advanced renal cell carcinoma, is an angiogenic inhibitor that targets VEGFR 1/2, platelet-derived growth factor receptor (PDGFR  $\alpha/\beta$ ), and c-kit. In MB, Axitinib displays an antitumor activity by decreasing the phosphorylation of protein kinase B (AKT or PKB) and STAT3 [77]. When combined with GDC-0941 (PI3K inhibitor), Axitinib displays augmented cytotoxicity and anti-neoplastic efficacy in MB cell lines. Compared to other MKi, Axitinib possesses a higher affinity to STAT3 and AKT, thus it is suggested as a promising therapeutic drug to facilitate MB treatment [77] (Table 1; Part 1).

### Receptor tyrosine kinase inhibitor: sunitinib

Sunitinib, a receptor tyrosine kinase (RTK) inhibitor, suppresses tumor growth directly by targeting STAT3 and RTKs or indirectly by inhibiting angiogenesis, representing a promising treatment option for MB patients [52, 78]. Sunitinib exhibits anti-cancerous activity in MB via arresting cell cycle and inducing apoptosis which were associated with the inhibition of STAT3 phosphorylation [52]. Unfortunately, Sunitinib cannot cross the BBB, thereby it can be delivered locally to the cerebrospinal fluid by convection-enhanced

delivery in order to contribute to a more effective antitumor effect [52] (Table 1; Part 2).

### Peptide inhibitors: S3-NTDi

S3-NTDi is a penetrating-tagged cell-permeable peptide inhibitor that selectively inhibits the N-terminal domain of STAT3 [79]. Treating MB cell lines with S3-NTDi inhibited phosphorylated STAT3 as well as the expression of its target genes including *Bcl-2*, *c-Myc*, and *cyclin D1*. Notably, S3-NTDi reduced the efficiency of STAT3 to bind to its target gene promoters. Moreover, it was shown that S3-NTDi attenuated STAT3 signaling pathway via increasing the expression of protein inhibitor of activated STAT3 (PIAS3) mediated by the downregulation of miRNA-21 [79]. Furthermore, MB cells treated with S3-NTDi were subjected to apoptosis and cell cycle arrest, in addition to suppressed cell migration. Interestingly, S3-NTDi enhanced the apoptotic effect of the chemotherapeutic drug cisplatin in MB cells. Thus, S3-NTDi and cisplatin worked in a synergistic way to sensitize MB cells to chemotherapy [79] (Table 1; Part 3).

### Small molecule inhibitors/non-peptide inhibitors

#### LLL12 and LLL12B

Non-peptide inhibitors selectively inhibit the function of STAT3 SH2 domain regardless of the STAT3 phosphorylation state. They also inhibit STAT3 activation, dimerization, and nuclear translocation as well as the induction of apoptosis in STAT3-dependent cancer cells [80]. LLL12 is a non-peptide small molecule that inhibits STAT3 phosphorylation. It inhibited proliferation and induced apoptosis in MB cell lines [55]. LLL12 inhibits the phosphorylation of STAT3 but not ERK1/2, AKT, and STAT1, indicating that it is specific for STAT3. It also blocks the ability of STAT3 to bind to DNA and suppresses the transcription of STAT3 target genes such as *cyclin D1*, *survivin*, *Bcl-2*, and *Bcl-l*. Due to its efficacy and ability to cross the BBB, LLL12 should be further investigated as a treatment option in MB [55] (Table 1; Part 4).

LLL12B is a variant of LLL12 that displays greater bio-availability than its original compound. Chen et al. have demonstrated that LLL12B blocked IL-6-mediated STAT3 phosphorylation inducing growth arrest and apoptosis [81]. LLL12B possessed a better anti-neoplastic effect when combined with cisplatin compared to monotherapy in vitro and in vivo [81]. When combined with irradiation therapy, it decreased the expression of STAT3 downstream targets and exhibited enhanced tumorsphere suppression, cell migration, and invasion [54]. Thus, LLL12B offers a novel treatment option that can be used in combination with cisplatin chemotherapy or radiotherapy (Table 1; Part 4).

**Table 1** Summary table shows the different drug families used in targeting STAT3 signaling in vitro and in vivo MB models

Drug	In vitro studies: MB cell lines	In vivo studies: animal model used	Target	Mode of action	Combination	Biological effects	Reference
<b>Part 1: Multikinase inhibitors</b>							
Pazopanib	Daoy, MEB-Med-8A, D283 Med, D341 Med	MEB-Med-8A orthotopic xenograft mice (NOD/SCID IL2R $\gamma$ <sup>Null</sup> or NSG)	VEGFR	Inhibition of VEGFR pathway	N/A	Inhibited proliferation	[73]
			STAT3	Inhibition of STAT3 phosphorylation		Induced apoptosis Reduced cell migration Inhibited tumor growth Improved animal model survival	
Sorafenib	Daoy, MEB-Med-8A, D283 Med, D341 Med	MEB-Med-8A orthotopic xenograft mice (NOD/SCID IL2R $\gamma$ <sup>Null</sup> or NSG)	VEGFR	Inhibition of VEGFR pathway	N/A	Inhibited proliferation	[73]
			STAT3	Inhibition of STAT3 phosphorylation		Induced apoptosis Reduced cell migration Inhibited tumor growth Improved animal model survival	
Vandetanib	Daoy, MEB-Med-8A, D283 Med; D341	Daoy xenograft mice (Nude mice)	STAT3	Inhibition of STAT3 phosphorylation	N/A	Inhibited proliferation	[75]
			Cyclin D1/D2/D3, cyclin E and Mcl-1 Raf-MEK-MAPK pathway	Inhibition of the expression of cyclin D1/D2/D3, cyclin E and Mcl-1		Induced apoptosis Inhibited tumor growth Decreased survival Reduced cell viability and proliferation	
Axitinib	Daoy, D283 Med, MEB-Med-8As	N/A	VEGFR 2/3	Inhibition of VEGFR and EGFR	GDC-0941	Induced apoptosis Inhibited cell migration enhanced cytotoxicity Reduced cell viability	[61]
			EGFR STAT3	Reduction of STAT3 phosphorylation		Decreased cell growth Increased pro-apoptotic activity	
Sunitinib	Daoy, Primary cell culture of MB VC312	N/A	VEGFR1/2	Inhibition of VEGFR1/2 PDGFR $\alpha/\beta$ and c-kit	GDC-0941	Reduced cell viability	[77]
			c-kit AKT	Downregulation of STAT3 phosphorylation			
<b>Part 2: Receptor tyrosine kinase inhibitor</b>							
Sunitinib	Daoy, Primary cell culture of MB VC312	N/A	RTK	Inhibition of VEGFR, PDGFR, and c-kit	N/A	Inhibited cell growth and proliferation	[52]
			VEGFR	Inhibition of STAT3 and AKT phosphorylation		Inhibited cell migration Induced apoptosis	

Table 1 (continued)

Drug	In vitro studies: MB cell lines	In vivo studies: animal model used	Target	Mode of action	Combination	Biological effects	Reference
<b>Part 3: Peptide inhibitor</b>							
S3-NTDi	Daoy, D341	N/A	STAT3 NH2 terminal domain microRNA-21	Downregulation of phosphorylated STAT3 and its target genes Downregulation of microRNA-21	Cisplatin	Induced cell cycle arrest Induced apoptosis Suppressed metastasis Sensitized MB cells to chemotherapy	[79]
<b>Part 4: Small molecule inhibitors</b>							
LLL12	Daoy, UW426, UW2881, D341, D283	N/A	STAT3 Tyr705 residue	Inhibition of STAT3 phosphorylation	N/A	Inhibited proliferation and viability Induced apoptosis	[55]
LLL12B	D283, Daoy, D425; UW426, UW288	D283 and D425 xenograft nude mice	STAT3 SH2 domain	Downregulation of STAT3 target genes Inhibition of STAT3 phosphorylation	Cisplatin	Inhibited cell viability Induced apoptosis Inhibited tumor growth	[81]
LY5	D283, D425, UW288, UW426	N/A	STAT3	Downregulation of STAT3 target genes Inhibition of STAT3 phosphorylation	Irradiation	Inhibited cell viability Suppressed cell migration Suppressed cell invasion Increased apoptosis	[54]
LY5	UW426, UW2881, DAOY	N/A	STAT3	Inhibition of STAT3 phosphorylation	Cisplatin and X-ray irradiation	Increased apoptosis Reduced angiogenesis Prevented cell migration Reduced cell viability	[82]
LLY17	D283, D425UW288; UW426	N/A	STAT3	Downregulation of STAT3 target genes ( <i>cyclin D1</i> , <i>survivin</i> , and <i>Bcl-xl</i> ) Inhibition of STAT3 phosphorylation	Irradiation	Inhibited cell viability Suppressed cell migration and invasion Prevented tumorsphere formation Increased apoptosis	[54]
				Downregulation of STAT3 target genes			

Table 1 (continued)

Drug	In vitro studies: MB cell lines	In vivo studies: animal model used	Target	Mode of action	Combination	Biological effects	Reference
Bazedoxifene	Daoy; UW288	N/A	IL-6/IL-6R/GP130 complex	Blockade of the formation of IL-6/IL-6R/GP130 complex	N/A	Inhibited proliferation Attenuated viability Decreased colony formation Impaired glycolysis	[56]
Part 5: Polyphenolic compounds							
Resveratrol	UW228-2, UW228-3	N/A	STAT3	Inhibition of STAT3 phosphorylation Attenuated the expression and phosphorylation of STAT3 Downregulation of STAT3 target genes ( <i>survivin</i> , <i>Cox-2</i> , <i>c-Myc</i> , and <i>cyclin-D1</i> ) Decreased STAT3 activity	N/A	Promoted neuronal differentiation of MB cells	[86]
Curcumin	Daoy, UW228-2, UW228-3	N/A	STAT3	Upregulation of PIAS3 Downregulation of SOCS3	N/A	Suppressed MB growth	[31]
Curcumin	DAOY; D283Med	N/A	STAT3 HHg IGF	Inhibition of STAT3, IGF, and SHH signaling pathways	N/A	Reduced MB growth Induced apoptosis Reduced the population of stem-like cells Arrested cell cycle	[90]
Part 6: Non-steroid anti-inflammatory drug							
Celecoxib	MB-derived CD133/ Nestin double-positive cells (MB-DPs, MB-derived CD133/Nestin double-negative cells (MB-DNs) isolated from Daoy cells	MB-DP orthotopic mice	STAT3	Downregulation of STAT3 signaling pathway	Ionizing radiotherapy	Induced apoptosis Suppressed MB tumorigenic potential Decreased the stemness Enhanced the sensitivity to radiotherapy	[95]

Table 1 (continued)

Drug	In vitro studies: MB cell lines	In vivo studies: animal model used	Target	Mode of action	Combination	Biological effects	Reference
<b>Part 7: Antipsychotic drug</b>							
Pimozide	Daoy	N/A	STAT3	Inhibition of STAT3 signaling pathway	N/A	Suppressed proliferation Inhibited colony formation Induced autophagy-mediated apoptosis	[98]
<b>Part 8: JAK/STAT3 inhibitors</b>							
AG490	UW228-3	N/A	STAT3	Inhibition of STAT3 signaling pathway Downregulation of <i>survivin</i> , <i>cyclin-D1</i> , <i>Cox-2</i> and <i>c-Myc</i>	N/A	Induced differentiation-like changes in MB Suppressed MB growth	[86]
Ruxolitinib	UW426, UW288, Daoy	N/A	JAK1/2	Reduced JAK/STAT3 phosphorylation	N/A	Reduced cell viability Suppressed colony formation and cell migration	[103]
Tofacitinib	UW426, UW288, Daoy	N/A	STAT3 JAK3	Reduced JAK/STAT3 phosphorylation	N/A	Induced apoptosis Reduced cell viability Suppressed colony formation and cell migration	[103]
<b>Cucurbitacin I</b>							
UW228;		N/A	STAT3 JAK2 STAT3	Inhibition of STAT3 phosphorylation Downregulation of <i>cyclin-D1</i> and <i>VEGF</i>	Temozolomide and Cisplatin	Induced apoptosis Inhibited cell growth Induced apoptosis Enhanced sensitivity to chemotherapy	[105]
Daoy, UW288	MB-CD133 <sup>+</sup> nude mice (BALB/c strain)		JAK2	Inhibition of JAK2/STAT3 phosphorylation	Cisplatin and radiation	Suppressed proliferation and CSCs properties Induced differentiation Inhibited tumorigenicity	[71]

Table 1 (continued)

Drug	In vitro studies: MB cell lines	In vivo studies: animal model used	Target	Mode of action	Combination	Biological effects	Reference
<b>Part 9: Src inhibitors</b>							
KX2-391	UW426, UW288, Daoy	N/A	STAT3	Inhibition of Src/STAT3 phosphorylation	N/A	Reduced cell viability Suppressed colony formation and cell migration Induced apoptosis Regulated EMT	[103]
Dasatinib	UW426, UW288, Daoy	N/A	Src STAT3	Inhibition of Src/STAT3 phosphorylation	Cisplatin	Reduced cell viability Suppressed colony formation and cell migration Induced apoptosis Regulated EMT	[103]
<b>Part 10: Other drugs</b>							
PG-S3-002	Med8A, D425, D458	STAT3 inhibitors-treated MB cells injected in NOD-SCID mice	STAT3	Inhibition of STAT3 dimerization Reduction in STAT3 phosphorylation	N/A	Reduced tumor size Treated NCSs showed a significant neurotoxicity	[70]
PG-S3-009	Med8A, D425, D458	D458 NOD-SCID mice	STAT3	Reduction in STAT3 phosphorylation	N/A	Reduced tumor size	[70]
PG-S3-010	Med8A, D425, D458	D458 NOD-SCID mice	STAT3	Reduction in STAT3 phosphorylation	N/A	Reduced tumor size	[70]
NSC 74859	Daoy, D283-Med, CHLA-01-Med	N/A	STAT3	Inhibition of STAT3 dimerization	Autophagy inhibitor CQ	Reduced cell death Reduced cell proliferation	[69]
WP1066	Daoy, D283-Med, CHLA-01-Med	D283-Med-Luc NSG (NOD SCID gamma) mice	STAT3	Inhibited STAT3 phosphorylation	Autophagy inhibitor CQ	Induced cell death Reduced proliferation Inhibited metastasis and tumor growth Increased survival	[69]

Table 1 (continued)

Drug	In vitro studies: MB cell lines	In vivo studies: animal model used	Target	Mode of action	Combination	Biological effects	Reference
LB100	Daoy, D283, D341	Daoy Immunocompromised (IcrTac: ICR- <i>P<sup>rhdc</sup></i> ) mice	STAT3 PP2A	Inhibition of PP2A Downregulation of phosphorylated STAT3 at Tyr-705 Inhibition of STAT3 target genes expression ( <i>cyclin D<sub>1</sub></i> , <i>c-Myc</i> , <i>survivin</i> , and <i>SNAIL</i> )	Cisplatin	Induced apoptosis suppressed tumor growth Decreased cell migration Inhibited proliferation	[112]

AKT Protein kinase B; Bcl-2 B-cell lymphoma 2; Bcl-xL B-cell lymphoma-extra-large; CSCs Cancer stem cells COX-2 Cyclooxygenase-2; EGFR Epidermal growth factor receptor; EMT Epithelial-mesenchymal transition; HHg Hedgehog molecule, IGF insulin like growth factor; JAK Janus kinase; MB Medulloblastoma; Mel-1 Myeloid cell leukemia-1; N/A Not applicable; NSCs Neuronal stem cells; NFκB Nuclear factor Kappa-B; PDGFR Platelet-derived growth factor receptor; PIAS3 Protein inhibitor of activated STAT3; PP2A Protein phosphatase 2A; Raf/MEK/MAPK Rapidly accelerated fibrosarcoma/MAPK-ERK kinase; RTK Receptor tyrosine kinase; SOCS3 Suppressor of cytokine signaling 3; Src Proto-oncogene tyrosine-protein kinase; STAT3 Signal transducer and activator of transcription 3; Tyr Tyrosine; VEGFR Vascular endothelial growth factor receptor

## LY5 and LLY17

LY5, another small molecule inhibitor, suppressed the constitutive activation of STAT3, inhibited its nuclear translocation, and downregulated the expression of downstream targets such as *survivin*, *cyclin D1*, *Bcl-x1*, and microRNA-21. LY5 treatment in combination with cisplatin and radiotherapy showed a potent effect in the reduction of cell viability [82]. Similarly, LLY17, another small-molecule inhibitor of STAT3, blocked IL-6-induced phosphorylation of STAT3 and enhanced the anti-tumor effect of irradiation [54]. Therefore, the inhibition of STAT3 by LY5 and LLY17 represents a possible strategy for MB treatment (Table 1; Part 4).

## Bazedoxifene

Bazedoxifene is an FDA-approved drug for postmenopausal osteoporosis prevention that is re-purposed for anti-cancer treatment. It suppresses tumor growth and promotes apoptosis via targeting IL-6/IL-6R/GP130 complex [56]. Furthermore, a recent study has shown that bazedoxifene inhibited GP130 signaling in order to overcome the chemoresistance in Group3 MB [83]. Bazedoxifene was also shown to inhibit STAT3 phosphorylation and decrease IL-6-mediated glycolysis and colony formation in MB in vitro models. It also resulted in reduced cell viability and proliferation due to the inhibition of the IL-6/GP130/STAT3 axis [56], thus representing a possible therapeutic drug (Table 1; Part 4).

## Polyphenolic compounds

### Resveratrol

Resveratrol is a natural polyphenolic compound used for targeting tumors due to its anti-neoplastic properties [84] and biochemical characteristics such as high permeability across BBB and low toxic effect [85]. In MB cell lines, resveratrol has inhibited the activation of STAT3 signaling, suppressed the expression of STAT3-related genes, and also induced neuron-oriented differentiation [31, 86] through Fas-independent pathway [87]. This may further drive MB to growth arrest and apoptosis as well as enhance radiosensitivity in resveratrol-treated MB [88] (Table 1; Part 5).

### Curcumin

Curcumin, a natural compound product, is served as an effective agent for pediatric brain tumors due to high BBB permeability [89]. It attenuates many signaling pathways in MB such as Wnt/β-catenin, NF-κB, SHH, and STAT3 which are crucial for MB pathogenesis [90–92]. Curcumin reduced the level of phosphorylated STAT3, induced a

dose-dependent decrease in the growth of MB cells, reduced the population of stem-like cells, arrested cell cycle, and induced apoptosis [90] (Table 1; Part 5).

### Non-steroid anti-inflammatory drug (NSAID): celecoxib

Celecoxib is a cyclooxygenase-2 (COX-2) inhibitor and non-steroid anti-inflammatory drug that shows an anti-tumor effect in various human cancers [93, 94]. Celecoxib mediates its anti-tumor effects either directly through suppressing COX-2 activity or in a COX-2 independent manner [94]. In MB, celecoxib inhibited STAT3 activation and suppressed MB stem cell-like properties. It also enhanced the sensitivity to ionizing radiotherapy, thus improving the survival of the animal model [95] (Table 1; Part 6).

### Antipsychotic drug: pimozide

Pimozide is an FDA-approved antipsychotic drug used clinically in treating psychological disorders and targeting human cancers through the inhibition of cell proliferation, angiogenesis, and metastatic activity [96]. Pimozide is considered a potential STAT3 inhibitor in targeting the tumor [97]. It was shown that pimozide targeted MB cells by inhibiting STAT3 pathway and its downstream pro-apoptotic markers such *c-Myc*, *Mcl-1*, and *Bcl-2* thus inducing autophagy-mediated apoptosis in the MB cell line [98] (Table 1; part 7).

### JAK inhibitors

#### AG490

STAT3 can be inhibited indirectly by selective inhibition of the upstream JAK, consequently preventing STAT3 phosphorylation which has been implicated in tumor growth [99]. It was revealed that selective inhibition of STAT3 phosphorylation by AG490 was accompanied by suppression of MB growth, downregulation of *survivin*, *cyclin-D1*, *Cox-2*, and *c-Myc*, and enhancement of differentiation-like changes in MB cells [86] suggesting the importance of STAT3 signaling in MB survival and maintenance (Table 1; Part 8).

#### Ruxolitinib and tofacitinib

Ruxolitinib and Tofacitinib are selective inhibitors targeting JAK1/2 [100] and JAK3 exerting a potent anti-tumor effect in several human malignancies [101]. Noteworthy, ruxolitinib and tofacitinib have a safety profile and can be effectively delivered across the BBB [102]. In MB, they reduced JAK/STAT3 phosphorylation a well reduced MB tumorigenesis by decreasing cell viability, colony formation, and

migration, and also enhanced apoptosis [103]. They could be an attractive drug for targeting MB (Table 1; Part 8).

### Cucurbitacin I

Cucurbitacin I (JSI-124) is also a JAK2/STAT3 inhibitor with an effective anti-neoplastic activity [104]. Suppression of STAT3 phosphorylation by cucurbitacin I has downregulated STAT3 target genes leading to inhibition of cell growth and significant cell death [105]. Moreover, the inhibition of STAT3 signaling pathway notably suppressed CSC-like characteristics and stemness gene expression and induced cell differentiation in MB-derived CD133<sup>(±)</sup> cells [71]. Interestingly, the inhibition of JAK2/STAT3 sensitized MB cells to chemotherapy and irradiation resulting in synergistic cell death [71, 105] (Table 1; Part 8).

### Src inhibitors

#### KX2-391

STAT3 signaling can be also activated by non-receptor tyrosine kinases (Src kinases) consequently mediating cancer tumorigenesis and drug resistance [106]. Activated Src were significantly detected in SHH-MB cell lines compared to normal brain cells making them a potential target for SHH-MB [103]. KX2-391 is a non-ATP-competitive inhibitor of Src kinase and tubulin polymerization. It blocked MB cell colony formation and migration through the inhibition of Src/STAT3 [103] (Table 1; Part 9).

#### Dasatinib

Dasatinib, another FDA-approved Src inhibitor, is used clinically in myeloid leukemia patients and some solid malignancies including MB [103]. It is worthy to note that this drug can be administered orally and easily crosses BBB, hence confirming its potent efficacy in treating pediatric brain tumors [103]. Dasatinib showed a synergistic effect when combined with cisplatin which could significantly inhibit STAT3 phosphorylation and reduce colony formation and migration ability [103]. These results suggest that Src inhibitors could be a novel candidate for MB treatment (Table 1; Part 9).

### Other drugs

#### PG-S3-002, PG-S3-009 and PG-S3-010

PG-S3-002 is a STAT3 inhibitor targeting the SH2 domain preventing its dimerization. It has a selective activity toward CD133<sup>+</sup> MBSCs. Garg et al. have shown a decreased level

of phosphorylated STAT3 protein and reduced tumor size in group 3 MB xenograft model after treatment with PG-S3-002. However, treating normal neuronal stem cells with PG-S3-002 led to significant neurotoxicity [70]. Other inhibitors of STAT3, PG-S3-009, and PG-S3-010, are highly selective for recurrent MB. They have been shown to decrease tumor burden in vivo demonstrating effectiveness in targeting recurrent MB. Nevertheless, PG-S3-009-treated mice showed no survival benefits with a neurotoxicity side effect [70] (Table 1; Part 10).

#### NSC74859 and WP1066

NSC 74,859, also known as S3I-201, is a chemical probe STAT3 inhibitor. It prevents STAT3 dimerization and STAT3-DNA interaction thus suppressing its transcriptional activities [107]. WP1066, an AG490 analog, inhibits JAK2 and STAT3 phosphorylation and participates in JAK2 degradation, thereby, affecting STAT3 signaling pathway [108]. Nazio et al. have revealed through that NSC 74,859 and WP1066 decreased STAT3 phosphorylated level and the expression of its target genes including *c-Myc* and *cyclin-D2*. Interestingly, the combination of these two drugs with an autophagy inhibitor induced MB cell death and reduced cellular proliferation. WP1066 in combination with an autophagy inhibitor showed an antitumor activity via attenuating MB cell growth and inhibiting metastasis [69] (Table 1; Part 10). Furthermore, a phase I clinical trial is currently evaluating the safety, feasibility and tolerability of WP1066 in brain tumors including MB (ClinicalTrials.gov; NCT4334863). It is worth mentioning that a phase I clinical trial has also investigated the therapeutic effect of WP1066 in patients with recurrent malignant glioma (ClinicalTrials.gov; NCT01904123) where WP1066 showed systemic immune suppression of p-STAT3 with the absence of significant toxicity [109].

#### LB100

Inhibition of STAT3 activity by increasing Ser727 phosphorylation and decreasing Tyr705 phosphorylation was previously observed upon deactivation of serine/threonine phosphatase 2A (PP2A) [110]. LB100 is a PP2A inhibitor that was tested in preclinical experiments and showed safety and efficacy in targeting solid tumors. Moreover, it exhibited anti-neoplastic properties and was evaluated in a phase I clinical trial (ClinicalTrials.gov; NCT01837667) [111]. Ho et al. reported that LB100 inhibited STAT3 phosphorylation and its downstream targets as well as reduced MB tumorigenicity such as attenuating cell growth and migration and overcoming drug resistance in MB cells [112] (Table 1; Part 10). These results provide strong evidence for evaluating

LB100 as an adjunct to chemotherapeutic drugs in MB clinical trials.

## Other ways for targeting STAT3 in MB

### RNA Interference

RNA interference (RNAi) is a biological process of targeting the silencing of gene expression [113]. It is one of the used approaches for cancer treatment, brain disorders, and viral infection [114]. Kotipatruni et al. showed that downregulation of tumor invasion molecules including urokinase plasminogen activator receptor (uPAR) and MMP-9 via small hairpin RNAs (shRNA) disrupted EGFR-mediated activation of STAT3, thus preventing the recruitment of STAT3 at the promoter of *Bcl-2* and *survivin* genes consequently inhibiting gene expression and further inducing apoptosis in MB cell lines [57].

### Fusion Protein GMME1

Fusion protein technology represents one of the used therapeutic approaches in protein-drug development in order to increase drugs' stability, activity, and bioavailability for targeting diseases [115]. It comprises different domains encoded by distinct genes [116]. GMME1 is a fusion protein that consists of granulocyte-macrophage colony-stimulating factor (GM-CSF) and truncated C-C motif chemokine ligand 2 (CCL2). It specifically blocked C-C motif chemokine receptor 2 (CCR2)-associated STAT3 phosphorylation and upregulated BCL2-associated X protein (BAX) leading to cell death indicating its potential therapeutic utility [117].

### SPARC overexpression

Secreted Protein Acidic and Rich in Cysteine (SPARC), a calcium-binding glycoprotein, is a multifunctional component of extracellular matrix (ECM) that modulates various processes such as angiogenesis, counter adhesion, ECM remodeling, cellular migration, and proliferation [53, 118]. The infection of MB cells with SPARC full-length cDNA adenovirus (Ad-DsRed-SP) resulted in a decline in Notch1 expression as well as a reduction in STAT3 phosphorylation in MB tumor specimens. This enhanced neuron-like morphology and expression of neuronal markers in MB cell lines [53]. Another study demonstrated that SPARC expression suppressed MB cell proliferation and arrested cell cycle at G2/M phase and also decreased STAT3 phosphorylation at Tyr705 residue [119]. These findings indicate that targeting STAT3 signaling via SPARC could be valuable for rational cancer therapy.

## Conclusion and future perspectives

The aggressive heterogeneous nature of MB requires more understanding of the underlying abnormal signaling pathways in order to prevent further development of MB, avoid long-term toxicity of the current treatment regimens and prolong patients' OS. Considering that STAT3 is a well-known oncogene factor in different cancers, and its involvement in the tumorigenesis MB, several studies have attempted to decipher the molecular mechanisms for constitutive activation of STAT3 that are implicated in the malignancy and growth of MB, thereby facilitating the development of more comprehensive and efficient therapeutic strategies. Targeting STAT3 is a promising approach in MB treatment that has shown to have anti-neoplastic effects in preclinical studies. Indeed, several drugs directed toward STAT3 pathway are considered a major part of MB therapies making STAT3 a focal topic in cancer research. Future research and clinical interventions are indeed needed for better delineating the detailed aberrant molecular mechanisms of STAT3 and support further exploration of STAT3 inhibitors in MB therapies.

**Author contributions** AZ and SN conceived the concept and idea of the present review. AZ and SN worked on the study design strategy and selected the topics to be discussed. AZ, ZA, FS, ZS, RN and HB did literature searches and screened titles and abstracts for relevance. AZ, ZA, FS and ZS abstracted the data from the eligible full text articles, analyzed and interpreted the data and drafted the manuscript. HH, RN and HB revised the final draft of the manuscript. SN critically revised the manuscript with input from the entire team. All authors have read and approved the final draft.

**Funding** Not funded.

**Data availability** Not applicable.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** None.

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

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