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G-Protein-Coupled Receptors as Therapeutic Targets for Glioblastoma

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KEYNOTE

G-protein-coupled receptors as therapeutic targets for glioblastoma

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumour in adults. Treatments include surgical resection, radiotherapy, and chemotherapy. Despite this, the prognosis remains poor, with an impacted quality of life during treatment coupled with brain tumour recurrence; thus, new treatments are desperately needed. In this review, we focus on recent advances in G-protein-coupled receptor (GPCR) targets. To date, the most promising targets are the chemokine, cannabinoid, and dopamine receptors, but future work should further examine the melanocortin receptor-4 (MC4R), adhesion, lysophosphatidic acid (LPA) and smoothened (Smo) receptors to initiate new drug-screening strategies and targeted delivery of safe and effective GBM therapies.

Keywords: G-protein-coupled receptors (GPCRs); Glioblastoma multiforme (GBM)

Introduction

Tumours involving glial cells, or gliomas, are the most common tumours of the central nervous system (CNS) and comprise two subgroups; diffuse gliomas and nondiffuse gliomas.[1] Diffuse infiltrating gliomas (diffuse IGs) account for 80% of primary malignant gliomas and are histopathologically classified as astrocytoma isocitrate dehydrogenase (IDH)-mutant, astrocytoma IDH-wild-type, and oligodendroglioma.[2,3] IDH1 and IDH2 are key enzymes involved in cellular metabolism, epigenetic regulation, redox states. and DNA repair.[4] Genes that encode IDH1 and IDH2 are frequently mutated in multiple types of cancer, whereby ~ 90% of mutations are of the IDH1 type.[2,5] Examples of such cancers include acute myeloid leukemia (AML), myeloid malignancies, and gliomas.[6] The molecular classification of an oligodendroglioma is such that it has complete chromosome 1p and 19q co-deletion. Oligodendrogliomas account for < 10% of diffuse gliomas.[7]

The glioma tumour grading system of the WHO ranges from grade I to grade IV,[8] assigned based on pathological features, such as vascular proliferation, mitotic activity, necrosis, and proliferative potential.[9] GBM is an example of a grade IV tumour because it is a more advanced tumour with more malignant features, including vascular proliferation and necrosis.[10] According to the Central Brain Tumour Registry of the USA (CBTRUS), GBM is the most commonly occurring primary malignant brain and CNS tumour, accounting for 14.5% of all CNS tumours and 48.6% of all malignant tumours.[11] The median survival rate for patients with GBM is 8 months.[11]

GBMs are classified as either IDH wild-type or IDH mutant. IDH-wild-type GBMs develop quickly and are molecularly distinct from the IDH-mutant type, which usually results from a lower grade glioma. IDH-mutant GBMs are primarily observed in younger patients, with a median age of 45 years, and has a better prognosis compared with IDH wild-type, where the median

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age is 60 years.[1,3,12] The IDH-wild-type form accounts for 90% of GBMs and, consequently, has more related research performed.[12]

Current therapeutic strategies in glioblastoma

Patients with brain tumours can present a variety of general symptoms, the most common include increased intracranial pressure (ICP), seizures, headache, fatigue, nausea, and vomiting, as well as cognitive dysfunction.[13] Patients with GBM have a median survival rate of 3–4 months without treatment, whereas the median survival rate for patients who receive treatment increases to 15 months.[14]

Current therapeutic strategies to treat GBM include surgical resection followed by radiotherapy and chemotherapy, which are referred to as the 'Stupp Protocol'.[15] Initial surgery remains a hallmark in the treatment of malignant brain tumours.[16] However, because of the invasive nature of GBM, there are cells that remain after surgery that contribute to tumour recurrence.[16] The success of cancer chemotherapy depends on the development of drugs that selectively destroy tumour cells or limit their proliferation without causing severe adverse effects.[17] Drug treatment in neurological diseases, such as brain tumours, are particularly difficult because of the protective CNS barrier, which comprises the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB). Many approved anticancer drugs do not readily cross the BBB, thus limiting the options for GBM treatment.[18]

The most used chemotherapeutic agents for the treatment of GBM are bevacizumab, sunitinib, lomustine (CCNU), procarbazine, carmustine (BCNU) and the gold standard, temozolomide (TMZ).[16,19]

Temozolomide

TMZ is the most frequently used chemotherapeutic agent for GBM treatment and has increased the patient median survival rate from 12.1 months to 14.6 months.[19] The percentage of patients alive at 2 years also increased from 10.4% to 26.5%.[19] TMZ belongs to a class of second-generation imidazotetrazine prodrugs, which were developed during the 1980s.[20] Given its small size (194 Da), stability at acidic pH, and lipophilic properties, TMZ can cross the BBB (Fig. 1).[20,19]

However, prolonged TMZ treatment leads to resistance and a poor response to subsequent treatments.[20] It also requires continuous administration because of its low solubility in physiological media and shorter plasma half-life (1.8 h). The dosage of TMZ delivered is 150–200 mg/m^2 for 5 days every 28 days for six cycles.[21] Although higher doses of TMZ allow for positive outcomes, such as tumour death, there are negative effects associated, such as cardiomyopathy, haematological toxicity, and pneumonia, which can result in the cessation of treatment.[22] The limitations associated with TMZ therapy have resulted in TMZ being used in conjunction with other therapeutics in a bid to improve its efficacy.[23]

Other chemotherapeutic agents for GBM

Sunitinib (Fig. 1) is an oral multi-targeted tyrosine kinase inhibitor that is used in the treatment of metastatic renal cell carcinoma and that has shown potential in the treatment of GBM.[24] To date, sunitinib has been part of a Phase II trial (ClinicalTrials.gov ID: NCT03025893) in conjunction with lomustine in patients with recurrent GBM.[25] Lomustine and procarbazine are both orally administered alkylating agents and can be used as a stand-alone treatment for GBM or in conjunction with TMZ.[26]

Carmustine wafers (CWs) are a form of the medication carmustine (BCNU) and are used in the treatment of recurrent GBM as an adjunct to surgery.[27] CWs are recommended for patients for whom near-total surgical resection is feasible or in whom craniotomy is indicated.[28] The success of CWs has led to the development of TMZ delivery through a polymer wafer, which can be used as an alternate or complimentary treatment.[29] Preclinical studies were carried out using CWs, TMZ wafers, and wafers that were coloaded with both BCNU and TMZ against an

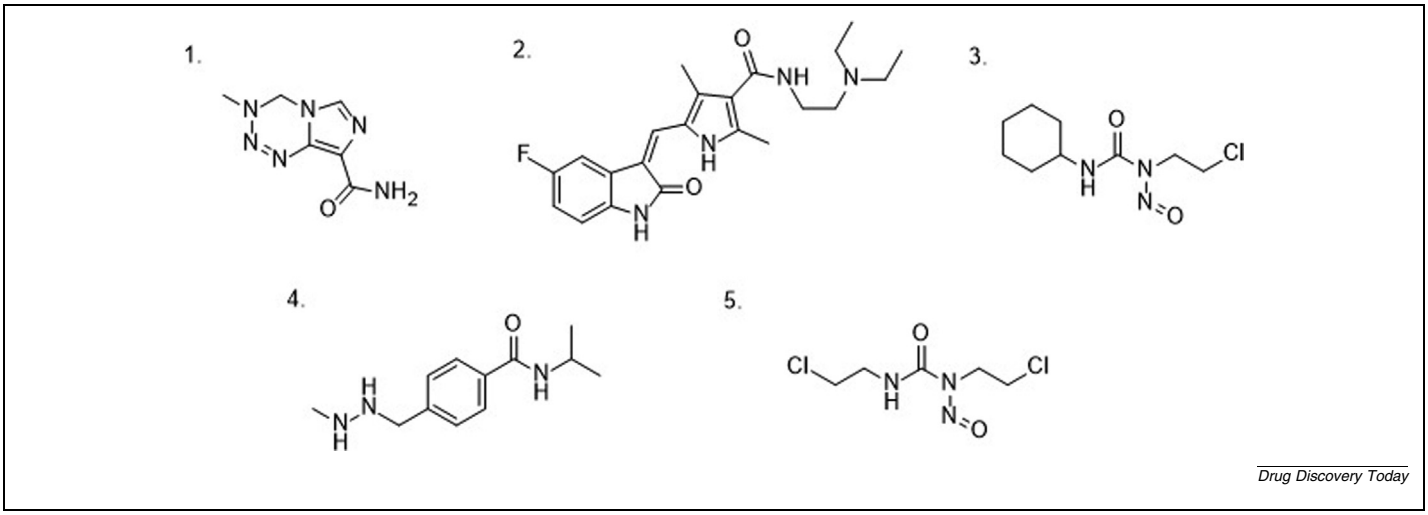

FIGURE 1

Structures of chemotherapeutics used in the treatment of glioblastoma multiforme (GBM). **1**. Temozolomide. **2**. Sunitinib. **3**. Lomustine. **4**. Procarbazine. **5**. Carmustine.

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intracranial 9L gliosarcoma model in F344 rats. Rats that received the TMZ wafer had a higher survival rate (18 days) over rats that received the CWs (15 days), whereas rats that received the BCNU-TMZ wafer had the highest overall survival rate (28 days), with 25% of them living long term. Despite this success of the BCNU-TMZ combination, it has not been delivered on a coloaded wafer successfully in a clinical setting. Nevertheless, this research can be used to improve local drug delivery and increase therapeutic options for patients with GBM.[29]

Bevacizumab is a humanised monoclonal antibody against vascular endothelial growth factor A (VEGF-A) and is used in the treatment of several different cancers, including cervical, colorectal and GBM.[30] It is used as a last in-line treatment for GBM following the failure of radiotherapy, lomustine, and TMZ.[31]

Search for novel protein targets for GBM

Most drug targets are in one of five protein families: GPCRs, ion channels, kinases, nuclear hormone receptors, and proteases.[32] GPCRs are considered one of the most important classes of pharmacological targets because they can act as both primary and secondary targets.[33] They also regulate numerous physiological processes, such as cell signalling and cell communication, as well as having druggable sites accessible at the cell surface.[34] It is estimated that 27% of all US Food and Drug Administration (FDA)-approved therapeutics that are sold target 108 GPCRs.[32] Recently, GPCRs have become an emerging oncogenic target class for GBM.

G-protein-coupled receptors

GPCRs comprise the largest family of membrane proteins, which include more than 800 members in humans.[32] Structurally, GPCRs share a core of seven transmembrane (TM) α-helices, an extracellular N terminus, and an intracellular C terminus, with various extracellular and intracellular loops.[35] Functionally, GPCRs regulate activities of intracellular signalling via G proteins and β-arrestin (Fig. 2).[35] Upon GPCR activation, G-proteins/β-arrestins translocate to the cell membrane, where they bind to the agonist-occupied receptors, triggering a variety of physiological responses, such as neurotransmission, metabolism, cell differentiation, inflammatory, and immune response, as well as vision, taste, and olfaction.[36]

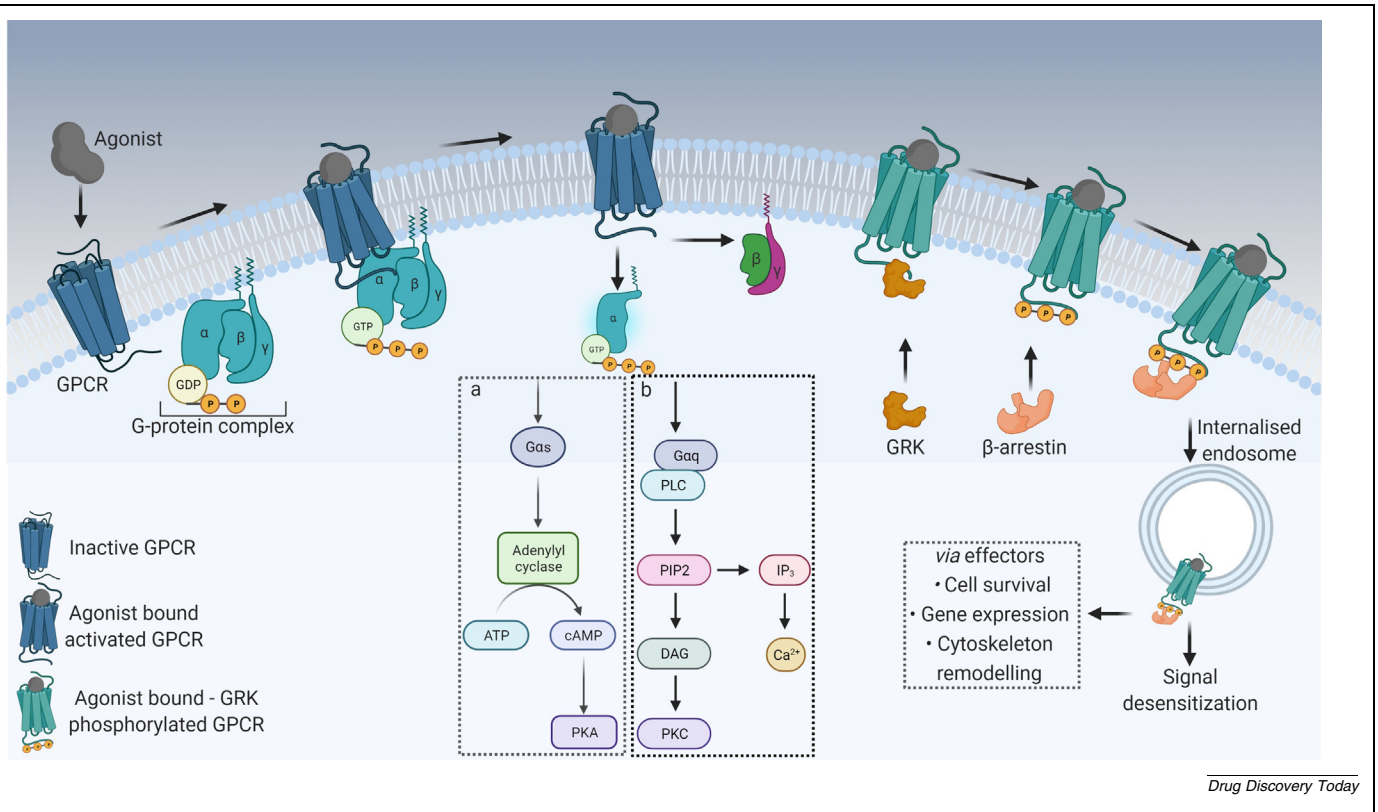

FIGURE 2

Schematic of diverse signalling pathways of G-protein-coupled receptors triggered upon activation of G-protein subunits (α, β and γ). Agonist-bound GPCR exchanges GDP for GTP on the Gα subunit, thus triggering Gα (s, i, q, 12) dissociation from the receptor and Gβγ. **(a)** Activated Gαs stimulates membrane-associated enzyme adenylyl cyclase (AC), which increases ATP–cAMP conversion. cAMP acts as a second messenger to activate protein kinase A (PKA), which can phosphorylate multiple downstream targets; whereas Gαi subunit inhibits AC. **(b)** Activated Gαq stimulates the membrane-bound phospholipase C (PLC) to cleave phosphatidylinositol biphosphate (PIP_2) into the second messengers inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 increases intracellular calcium concentrations (Ca^{2+}), whereas membrane-bound DAG activates PKC by translocating it from the cytosol to the plasma membrane. GPCR kinase (GRK) phosphorylates G-protein-independent ligand-bound GPCRs to initiate the recruitment of β-arrestin and blocks G-protein coupling. The GPCR-β–arrestin complex promotes endocytosis, trafficking ligand–GPCRs to sorting endosomes for either recycling to plasma membrane or signalling and regulation of various cellular processes. Figure prepared with BioRender (BioRender.com).

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KEYNOTE

TABLE 1

GPCR Glutamate, Rhodopsin, Adhesion, Frizzled, Secretin (GRAFS) classification with characteristics, biological roles, and representative examples for each group[a].

GRAFS; no. of known human sequences	Characteristics	Biological roles	Representative examples
Glutamate (Class C); 22	Presence of nine conserved cysteine residues known as cysteine-rich domain (CRD) or NCD3G in N-terminal region	Neurobiological roles, gustatory roles (sweet and sour tastes)	Metabotropic glutamate receptors, GABA receptors, taste-1 receptors
Rhodopsin (Class A); 719	Presence of D/ERY in TM3, NPxxY in TM7, and disulfide bond(s)	Olfactory and vision stimuli, neurotransmitter signalling, cardiovascular and immunological functions, etc.	Adrenergic receptors, opioid receptors, chemokine receptors
Adhesion (Class B2); 33	Long N termini containing multiple functional domains and numerous sites for glycosylation	Developmental biology of CNS, immunological functions, etc.	Latrophilin receptors, brain-specific angiogenesis inhibitor proteins
Frizzled (Class F); 11	Characterised by presence of ten conserved cysteine residues known as FZ_CRD domain or FZ domain between N-terminal and TM regions	Developmental biology, cancer development, perception of bitter taste; also known as receptors for Wnt proteins	Smo receptor, taste-2 receptors
Secretin (Class B1); 15	Evolved from Adhesion receptor family	Endocrine and metabolic disorders	Glucagon receptor, GLP-1 receptor, growth hormone-releasing hormone receptor

[a]Data from[36,37]

A widely used classification system for GPCRs, referred to as the A-F system (Table 1), separated the GPCRs into six classes based on their amino acid sequences and their functional similarities for both vertebrates and invertebrates.[36] Class A is the largest family, accounting for ~ 80% of GPCRs, and is referred to as the 'rhodopsin-like family'. Class B contains 70 receptors and is referred to as the 'secretin receptor family'. Class C examples include the glutamate family, GABA receptors, and calcium-sensing receptors. Class D includes fungal mating pheromone receptors; Class E contains cAMP receptors, and Class F contains frizzled/smoothened receptors.[36] A new classification system was subsequently devised referred to as the 'GRAFS' system (Table 1),[36,37] based on structural similarity and the phylogenetic tree of ~ 800 human genome sequences.[37] This system comprises five families: Glutamate (G), Rhodopsin (R), Adhesion (A), Frizzled/Taste2 (F), and Secretion (S).[36] A variety of GPCR family members are considered as potential GBM targets, and the remainder of this review focuses on GPCRs expressed on GBM cells.

Rhodopsin type GPCRs and GBM

Chemokine receptors

Chemokine receptors are classified as typical (GPCRs) and atypical chemokine receptors (ACKRs).[38] ACKRs are cell surface receptors with seven TM domains and are structurally homologous to chemokine GPCRs; however they fail to induce classical signalling and cellular responses, which are characteristic of GPCRs. Nevertheless, ACKRs are important in terms of health and disease.[39] CXCR4 is an example of a typical chemokine receptor, whereas CXCR7 is an atypical receptor.[40] Along with their CXCL12 ligand (stromal derived factor 1/SDF-1 ligand), these are the most widely studied chemokine systems involved in tumour growth, metastasis, and angiogenesis.[41] CXCR4 and CXCL12 are upregulated in brain cancers, such as meningiomas and GBM.[42] The significance of CXCL4/CXCR12 expression and function has been studied in both children and adults with GBM.[42] CXCR4 expression corresponds to tumour grade and it is overexpressed in GBM stem cells (GSCs), which drives GBM progression.[43] CXCR4 expression is observed in hypoxic areas of the tumour.[43] In addition, hypoxia enhances CXCL12 secretion, thus driving angiogenesis and feeding tumour development.[42] This is also observed when GBM is treated with radiation because this triggers upregulation of hypoxia-inducible factor 1α (HIF-1α) and CXCR4.[42,43] Therefore, a CXCR4 antagonist preventing this protein–protein interaction, between CXCR4 and CXCR12, could be a valuable therapeutic in the treatment of GBM. Both *in vitro* and *in vivo* studies using CXCR4 antagonists in combination with current GBM treatment have also been pursued.[43]

A study of the CXCR4 antagonist plerixafor (Fig. 3) showed that tumour recurrence was prevented in orthotopic xenografts of GBM in a mouse model. Plerixafor selectively inhibits CXCL12 binding to CXCR4 as well as inhibiting CXCL12-mediated chemotaxis.[44] In the 3-week *in vivo* study, the GBM tumours continued to shrink and did not recur even when exposure to plerixafor ceased.[44,45]

From this experimental model, the dose and schedule of administration of plerixafor was used in an adjuvant treatment study of a 66-year-old patient with GBM.[45] The patient was treated after chemoradiotherapy, receiving a total of 60 Gray (Gy) in 30 fractions with a concurrent dose of TMZ. The patient was then treated with plerixafor and a combination of a mammalian target of rapamycin (mTOR), a sirtuin 1 (SIRT1), and an epidermal growth factor receptor variant III (EGFRvIII) inhibitor. After 1 year of treatment, the use of TMZ and the EGFRvIII inhibitor was stopped, whereas the plerixafor and mTOR and SIRT1 inhibitors were continued. The patient was in remission at 30 months from the initiation of the adjuvant treatment, highlighting the potential of plerixafor as an adjuvant treatment for GBM.[45]

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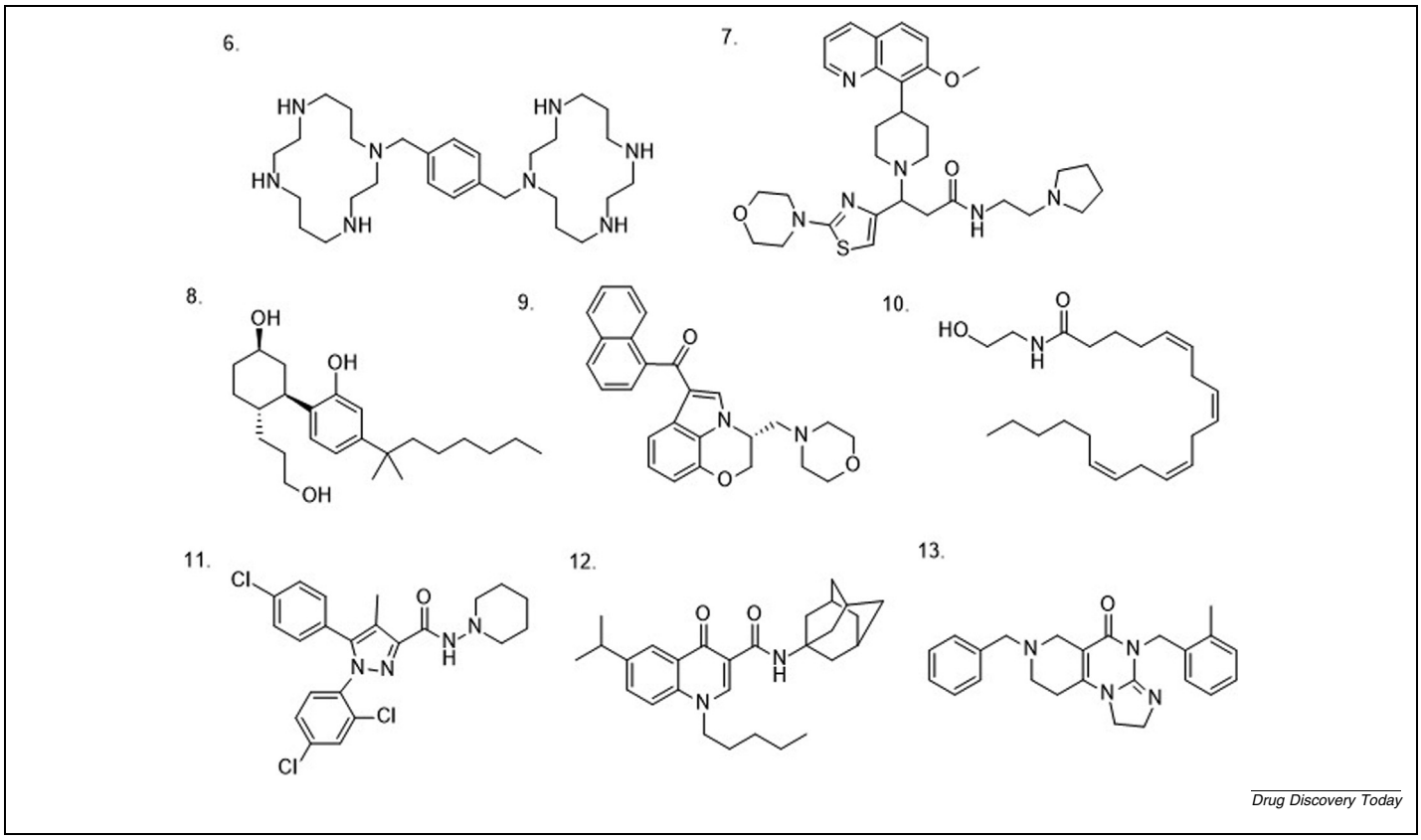

FIGURE 3

6. Structure of chemokine receptor ligand, Plerixafor. **7**. Structure of CXCR7 inhibitor CCX771. **8**. Structure of synthetic cannabinoid CB1 agonist CP55-940. **9**. Structure of selective synthetic cannabinoid CB1 agonist WIN 55,212–2. **10**. Structure of endogenous cannabinoid AEA. **11**. Structure of selective CB1 antagonist drug rimonabant. **12**. Structure of selective CB2 agonist COR167. **13**. Structure of DRD2 antagonist ONC201.

A Phase I/II clinical trial was performed in 2013 (ClinicalTrials.gov ID: NCT01977677) in which the use of plerixafor and TMZ after radiation therapy was studied in patients with newly diagnosed high-grade glioma to determine the optimum treatment dosage.[46] The hypothesis was that the recovery of the tumour was driven by CXCL12 secretion and, thus, blocking the CXCL12–CXCR4 axis would stop tumour recurrence. A total of 29 patients completed the study with a recommended dose of 16.6 μg/kg/hr of plerixafor with no deleterious effects observed; seven out of nine patients were alive at the end of the study, with the longest survival since diagnosis being 18 months.[46]

A novel antagonist of CXCR4, PRX177561 (structure not available because of commercial sensitivity) was used in conjunction with bevacizumab and sunitinib to determine its antitumour activity in a mouse GBM xenograft model.[47] A panel of 12 human GBM cells lines and five patient-derived GBM stem cell cultures were used. PRX177561 alone inhibited tumour growth and increased the efficacy of both bevacizumab and sunitinib, resulting in a significant reduction in tumour growth in animal models of GBM. PRX177561 also enhanced survival in combination with both bevacizumab and sunitinib in the animal model. After 35 days, all treatment was stopped, and the animals were checked for overall survival determination. The use of CXCL12–CXCR4 targeted, combined therapy allowed for a clinical trial (ClinicalTrials.gov ID: NCT02765165), which was terminated for business reasons rather than concerns related to safety.[47]

The therapeutic drug, fingolimod, has been proposed as a therapeutic option for the treatment of GBM because of its ability to cross the BBB, its lipophilicity, and its ability to accumulate in CSF. Fingolimod is a structural analogue of sphingosine 1-phosphate (S1P) and acts as an agonist of S1P receptors,[48] inhibiting the CXCR4 receptor and its ligand S1P. Cancers, such as primary brain tumours, produce and secrete more S1P, which has been shown to establish premetastatic niches in organs, such as the brain, through mechanisms involving S1PR1.[48] Fingolimod inhibited S1P signalling in multiple myeloma and it was revealed that metastasis to the bone marrow was due to involvement of the CXCR4–CXCL12 pathway.

Metastasis specific to the brain has not yet been studied with respect to S1P; however, the expression of CXCL12 is positively correlated with brain metastasis in solid tumours.[49] *In vitro*, fingolimod has shown the ability to induce apoptosis in multiple GBM cell lines as well as inhibiting migration and invasion through the modulation of matrix metalloproteinases. It is sensitive to two GBM cell lines, U87 and U-251 MG, and induces autophagy and apoptosis in U251-MG cells in response to TMZ.[48] According to the American Type Culture Collection (ATCC), the origins of the U87 and U-251 MG cell lines used in research laboratories are different from that of the original cell

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line. However, these cell lines are genuine human GBM of an unknown origin.[50] A further study treated glioma-bearing Wistar rats with fingolimod for a period of 14 days. Fingolimod inhibited the growth, migration, and invasion of glioma by inhibiting the CXCR4–CXCL12 pathway in these rats.[51]

CXCR7, or atypical chemokine receptor 3 (ACKR3), has a ten-fold higher affinity for CXCL12 than for CXCR4.[42] The expression of CXCR7 increases with the increasing malignancy of tumours. CXCR7 is expressed in GBM cells and facilitates the binding of CXCL12 to endothelial cells during hypoxia.[52]

An experimental model was carried out in which 24 6-week-old mice were injected intracranially with U251-MG human GBM cells. After a 3-week period of tumour establishment, the mice were given whole-brain irradiation and then injected subcutaneously with the CXCR7 inhibitor, CCX771. The results showed that inhibition of CXCR7 by CCX771 post irradiation blocked tumour recurrence and prolonged survival.[53] This suggests CXCR7 antagonists as a potential route to decrease the spread of tumour cells, their metastasis, and angiogenesis.

Therefore, by inhibiting the interaction between CXCR4 and/or CXCR7 and the ligand CXCL12, potential therapeutics to target GBM treatment and GBM recurrence could be developed.

Cannabinoid receptors

The endocannabinoid system (ECS) is an important neuromodulatory system comprising cannabinoid receptors, endogenous cannabinoids (endocannabinoids), and enzymes, which are responsible for the synthesis and degradation of endocannabinoids.[54] Cannabinoids and endocannabinoids have a variety of pharmacological effects. Cannabinoids are currently used in the palliative therapy of patients with cancer, in particular tetrahydrocannabinol (THC) or cannabidiol (CBD).[55] These substances inhibit nausea and vomiting, stimulate appetite, and reduce weight loss. Cannabinoids also affect cell proliferation, viability, and invasiveness of cancer cells in culture and *in vivo*.[55]

The two rhodopsin-type GPCRs primarily expressed in the human body are cannabinoid receptor type 1 (CB1) and 2 (CB2).[56] The identification of CB1 and CB2 led to the isolation and characterisation of endocannabinoids for these proteins, namely, anandamide (AEA) (Fig. 3) and 2-arachidonoylglycerol (2AG).[57]

CB1 receptors are expressed more abundantly in the body than other GPCRs and are found in high levels in the CNS, primarily on the preterminal axonal segment and the axons themselves.[54] They are also found in regions of the brain responsible for motor activity, memory, and cognition.[55] CB2 receptors are primarily expressed on immune cells, such as those derived from macrophages (e.g., microglia, osteoblasts, and osteoclasts).[54,56] CB2 receptors are also responsible for the anti-inflammatory effects of endocannabinoids.[56] Both CB1 and CB2 receptors signal through the inhibitory G-alpha proteins G_i and G_o.[56]

The expression of CB1 and CB2 receptors is altered in tumour types, such as brain, digestive tract, breast and prostate tumours, and has been related to cancer prognosis in GBM tumours expressing both CB1 and CB2 receptors.[58] There are high levels of CB2 receptors expressed in high-grade gliomas, such as GBM, and this expression positively correlates with the malignancy grade. CB1 receptor expression still requires characterisation because it has been reported to be increased, decreased, or unchanged in GBM compared with low-grade gliomas or nontumour control tissues.[58]

Cannabinoids and endocannabinoids can directly inhibit tumour growth *in vitro* in cancers such as GBM by inducing apoptosis, reducing tumour growth, and inhibiting neoangiogenesis.[59] In addition, hypoxia-induced inhibition of the endocannabinoid system aids the development of GBM. Hypoxia decreases the levels of CB1 and the astrocyte fibrillary acidic protein, and increases the levels of the enzyme responsible for the metabolism of endocannabinoids, VEGF, and cyclooxygenase-2 (COX-2). Cannabinoid receptor engagement induces cell death in GBM U-87 cells in normoxic conditions and cannabinoid receptor agonist cell death was associated with hypoxic conditions.[60]

The effects of the synthetic agonists CP55-940 and WIN 55,212–2 (Fig. 3) on cannabinoid receptors were first demonstrated during the 1990's when antinociceptive activity was displayed in mouse and rat tail flick tests.[61] A study by Ortega *et al.*, in which synthetic CB1 agonists CP55-940 and WIN 55,212–2, as well as the endogenous CB1 agonist, anandamide (AEA) (Fig. 3) were compared in terms of morphological changes, cell viability, and induction of apoptosis in primary astrocytes and two GBM cell lines, C6 and U251-MG. The study was performed to characterise the possible differential actions on brain tumour cells.[62] None of the CB1 agonists induced any changes in cell morphology or cell viability in primary astrocytes. By comparison, CP55-940 decreased cell viability in both GBM cell lines after 5 days of treatment, whereas WIN 55,212–2 and AEA only moderately decreased cell viability in both cell lines. Discrete morphological changes in U251 and C6 cells resulted from treatment with AEA and WIN 55–212- 2, whereas exposure to CP55-940 induced some degradation. CP55-940 also induced apoptosis in both C6 and U251-MG cell lines. This study proposed that apoptosis is the major mechanism contributing to cannabinoid-induced death of cancer cells.[62] The work also highlighted the need to compare effects of cannabinoids to design potential treatments against glial tumours.

To date, most studies have focussed on agonistic stimulation via CB receptors, stating that this is responsible for the antitumour effects of cannabinoids.[63] However, a study by Ciaglia *et al.* suggests that CB1 antagonists would also be useful in glioma therapy.[63,64] Pharmacological inactivation of CB1 by rimonabant (Fig. 3) inhibited glioma cell growth of the U251-MG cell line via cell cycle arrest and induction of caspase-dependent apoptosis. Rimonabant-induced MICA/B upregulation also directly correlated with the degree of CB1 expression and occurred in malignant glioma cells but not in normal human astrocytes.[64] As such, the use of CB1 antagonists could be explored further in animal studies as potential therapeutics for certain subsets of GBM with high CB1 expression levels.

An *in vivo* study was carried out on primary human GBM and anaplastic astrocytoma cells using a novel cannabinoid CB2 agonist, COR167. The CB2 agonist was found to inhibit the growth of the cells *in vitro* by a mechanism independent of apoptosis. These findings suggest that a role for the selective CB2 receptor as a therapeutic target for GBM therapy and further work

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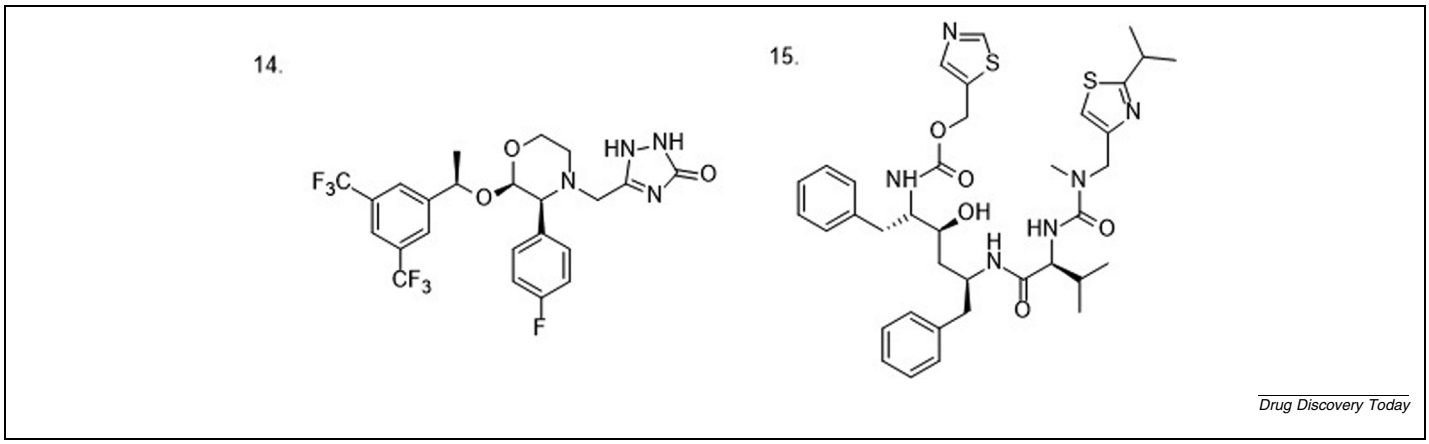

FIGURE 4

13. Structure of Neurokinin 1 receptor antagonist drug Aprepitant **14**. Structure of the antiviral drug ritonavir.

should be carried out to explore *in vivo* models of this CB2 agonist.[65]

Another challenge in treating GBM is the presence of GSCs, which express both cannabinoid receptors CB1 and CB2. The cannabinoids were shown to reduce the efficacy of GSCs to initiate glioma formation *in vivo* and was indicated by decreased cell proliferation.[58] Therefore, cannabinoid signalling has a role in inhibiting tumorigenesis and could have a role in the treatment of GBM.[43]

Dopamine receptors

There are five dopamine receptors (DR), D1–D5, which are members of the Rhodopsin subfamily and are responsible for the actions of the neurotransmitter, dopamine. The dopaminergic system controls brain functions, such as motor control, behaviour, and cognition, and is targeted by multiple therapeutically active drugs as well as drugs of abuse.[66] DRD2 has been the most extensively studied in cancer, and is overexpressed in high-grade gliomas, such as GBM. ONC201 (Fig. 3) was the first small-molecule DRD2 antagonist for oncology to demonstrate p53-independent antitumour activity in preclinical models of GBM and several other cancers. The compound crosses the BBB in rodents and exhibited antiglioma activity in an orthotopic model of GBM.[67] Currently, ONC201 is in an active Phase II trial (ClinicalTrial.gov ID: NCT02525692) to be used as treatment in recurrent GBM or recurrent Grade IV gliomas with a H3 K27M mutation.[67]

Neurokinin 1 receptor

Neurokinin 1 receptor (NK-1) is the most studied tachykinin receptor and belongs to the Rhodopsin family of GPCRs.[43,68] The NK-1 receptor occurs in the nervous system and peripheral tissues.[68] It is also involved in cellular responses, such as pain transmission, vasodilation, and modulation of cell proliferation.[68] Substance P (SP) is a neuropeptide and the major endogenous ligand for the NK-1 receptor.[69,70] The number of NK-1 receptors expressed on tumour cells is greater than that expressed on normal cells and so is correlated directly with the malignancy.[69] Overexpression of the NK-1 receptor was found in 100% (10/10 cases) of GBM biopsy specimens studied.[70]

Aprepitant (Fig. 4) is the only NK-1 receptor antagonist drug with FDA approval and is used as a post-chemotherapy antiemetic drug.[71,72] Aprepitant and marketed nonchemotherapy drugs were tested on the glioma cell line, GAMG, to establish whether they had an ability to enhance the cytotoxicity of TMZ.[72] Four antiviral drugs were tested, acyclovir, cidofovir, maraviroc, and ritonavir, as well as Aprepitant and TMZ. No toxicity was found for acyclovir, cidofovir and maraviroc, whereas cytotoxicity for GAMG alone for both TMZ and ritonavir was 14% and aprepitant alone was 7%. However, the cytotoxicity value for all three drugs was 78%. This study concluded that both ritonavir and aprepitant should be added to the Stupp protocol because of the observed increased cytotoxicity.[72]

Melanocortin receptor-4

Melanocortins are peptides with anti-inflammatory and neuroprotective activity. There are five melanocortin receptors (MCRs; MC1–5R), with only MC4R being present in astrocytes and, hence, is referred to as a neural MCR.[73] Vaglini *et al.* evaluated the presence of MC4Rs in GBM cells and the selective inhibition of their activity through the MC4R antagonist, ML00253764, alone and in combination with TMZ *in vitro* and *in vivo*. *In vitro* studies were carried out on human GBM cells (U-87 and U-118) and *in vivo* studies were carried out on nude xenografted mice. The simultaneous combination of TMZ and ML00253764 determined a highly synergistic effect on GBM cells. The same combination *in vivo* showed a strong and significant decrease in GBM tumour volumes.[74] A Phase III clinical trial was also carried out (ClinicalTrial.gov ID: NCT02458508) to evaluate the possible predictive/prognostic role of MC4R in patients with GBM. There is currently no description of MC4R expression or activity in human cancer cells, including GBM, or their relationship with radiotherapy and chemotherapy, hence the aim of the study.[75] The study showed that the MC4R rs489693 genotype homozygous for allele A was associated with a significantly shorter progression-free survival and overall survival in patients treated with radiotherapy and TMZ.[75]

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KEYNOTE

Adhesion GPCRs

Adhesion GPCRs (aGPCRs) are the second largest group in the GPCR family and comprise 33 human receptors divided into eight groups.[76] The nomenclature for the receptors was recently updated by the International Union of Basic and Clinical Pharmacology and all aGCPRs now contain an ADGR prefix, followed by a letter indicating the receptor subfamily and a number for each receptor within that group.[76] They regulate various cellular functions in the body, such as migration, polarity, and adhesion.[77]

The adhesion receptors are distinguished by their large N-terminal regions, which can contain epidermal growth factor (EGF), cadherin, immunoglobulin domains, and novel lineage-specific structures. The complexity of these receptors, their size, and the fact that they are usually tethered to other cells have proven to be challenges in identifying and characterising them.[78]

The adhesion receptor GPR133 (or ADGRD1) has been reported to enhance tumorigenesis and is upregulated in GBM.[77] The presence of GPR133/ADGRD1 is observed in hypoxic GBM cells, and the levels at which it is expressed correlate directly with patient survival.[76] RNA sequencing was carried out on a primary human GBM culture to determine which genes were upregulated and downregulated in CD133+ cells. GPR133 was selectively expressed in the hypoxic areas of these cells and genetic inhibition of GPR133 with short hairpin RNA reduced the prevalence of CD133+ cell tumour proliferation and tumour formation *in vitro*.[79] Further studies using patient-derived cultures and xenografts of GBM showed that GPR133 expression is at its highest in GBM, suggesting that high GPR133 levels support tumour growth and are associated with a reduced patient survival rate. As such, GPR133 represents a novel therapeutic target in GBM to be explored further.[80]

EGF module-containing mucin-like hormone receptor 2 and 3, EMR2 and EMR3, are both upregulated in GBM and are also associated with poor survival.[81] Both receptors have unknown ligands and cellular function.[82] *In vitro* analysis showed that EMR3 has a key role in GBM migration and invasion, but no effect on cell proliferation.[83] However, EMR2 and EMR3 receptors have a complex structure with no mouse orthologs and there is limited information about their intracellular targets.[84]

Despite aGPCRs having been recognised as suitable targets for therapeutics, they remain largely unexplored with no FDA approved drugs as of late 2019.[85] A greater understanding of the mechanism of action of aGPCRs would facilitate new drug screening strategies to treat illnesses, such as GBM, which are linked to aGPCR dysfunction.[86]

Lysophospholipid receptors

Two agonistic lysophospholipids (LPLs) that have been most characterised are LPA and S1P.[87] They have a significant role as extracellular mediators by activating GPCRs and stimulating diverse cellular responses from their signalling pathways.[88] The effects of LPLs in a cancer microenvironment are widely studied because these lipids are secreted by various cell types, including cancerous cells.[89] S1P and LPA have a key role in particular in regulating the growth of tumour cells and manipulating the immune system.[89]

Lysophosphatidic acid

LPA (Fig. 5) is a small glycerophospholipid molecule (430–480 Da) that acts as an extracellular signalling molecule through at least six class A lysophosphatidic acid GPCRs [LPA(1–6)].[90]

LPA is involved in the tumorigenesis of cancers, including GBM.[91] LPA receptor (LPAR) agonists and LPA synthesis inhibitors have been proposed as promising drugs for cancer treatment.

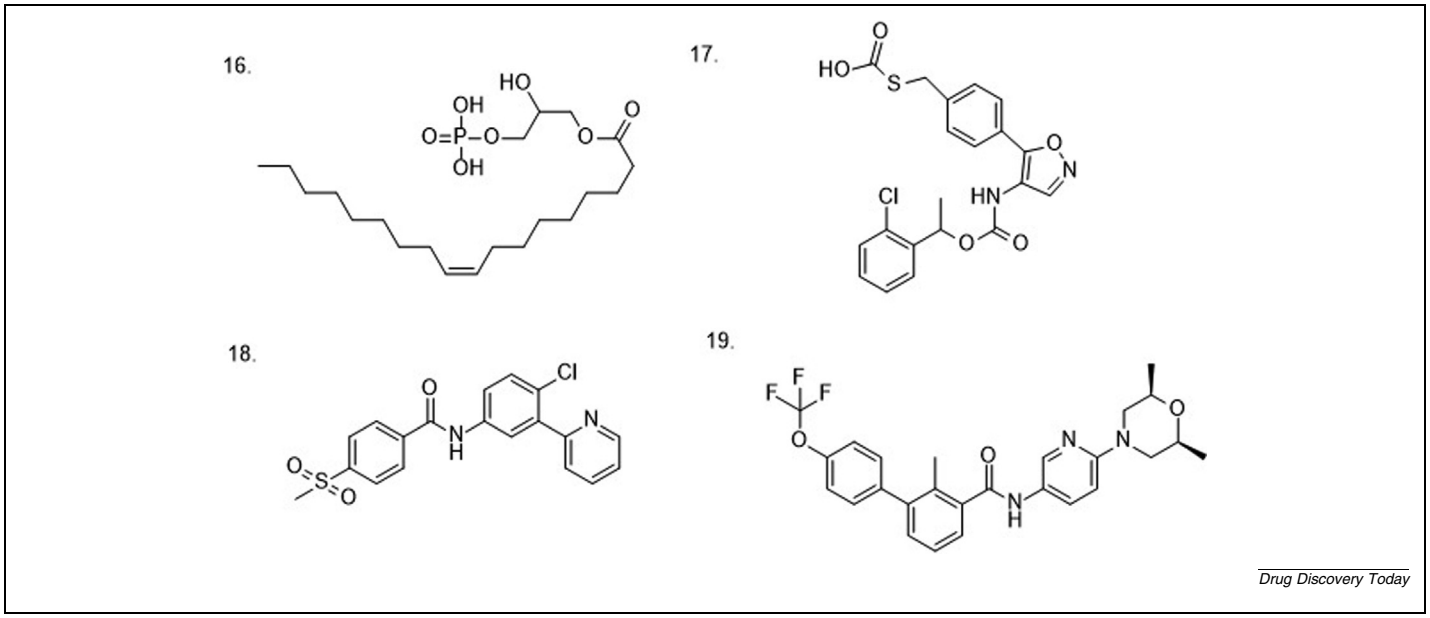

FIGURE 5

Structure of lysophospholipid receptor ligands. **15**. Lysophosphatidic acid (LPA). **16**. Structure of LPA receptor inhibitor Ki6425. Structures of Smoothened (Smo) receptor ligands **17** vismodegib and **18** Sonidegib.

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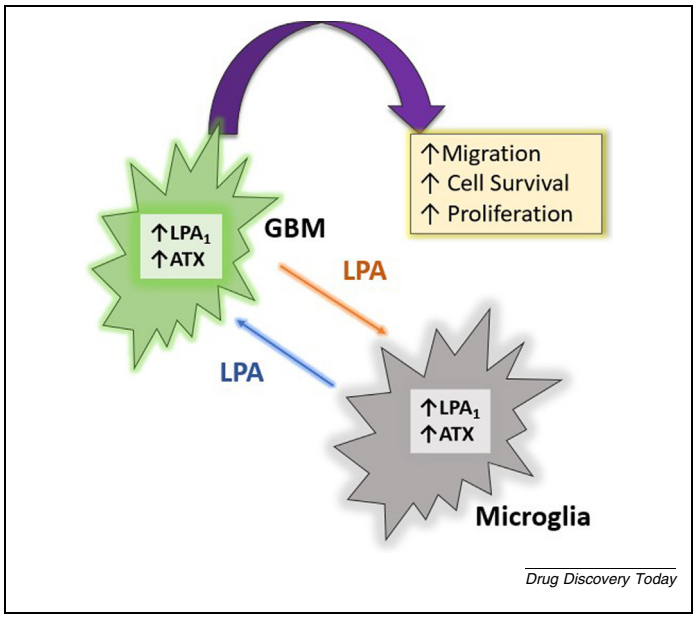

FIGURE 6

Interaction with lysophosphatidic acid (LPA) and GBM in a microglia co-culture system, in which elevated levels of LPA1 were associated with a worse prognosis.

LPA1 is the dominant LPAR in the CNS and is highly expressed in GBM.[92] LPA(1–3) are expressed at low levels in the normal human brain, although their expression is upregulated following brain injury.[92]

A study using a microglia co-culture system demonstrated that GBM secreted factors that increased LPA1 and ATX levels in microglia, which could be further increased by hypoxia. Microglial-induced GBM proliferation and migration could be inhibited through the pharmacological inhibition of LPA1 receptor. Increased levels of LPA1 were also observed in GBM in a microglia co-culture system compared with other gliomas and were associated with a poorer survival rate (Fig. 6).[93]

A preliminary study investigated the effects of the LPAR inhibitor, Ki6425, on the U87 GBM cell line. Given that GPCRs have a role in hypoxia, the study investigated which LPARs and downstream signalling pathways were involved under hypoxic conditions, finding that LPA1 was selective to the U87 cell line. From these results, the genetic profile of LPARs in the U87 cell line was investigated using qPCR. The study showed that EGFR was the main receptor tyrosine kinase (RTK), which was activated under hypoxic conditions in the U87 cell line. Inhibition of hypoxia-induced EGFR phosphorylation by the LPAR inhibitor Ki6425 was also observed in the U87 cell line.[94]

Furthering our understanding of LPA1 antagonists *in vitro* and *in vivo* could lead to the development of new therapeutics, given that LPARs are potential targets for GBM treatment.

Smoothened receptors

The Smo GPCR is the signal transducer of the Hedgehog (Hh) pathway and belongs to the F subfamily, frizzled/smoothened receptors.[95] The Hh signalling pathway provides instructional cues during development that contribute to postdevelopmental homeostasis and wound healing. Corruption of the Hh pathway can lead to developmental disorders and cancer.[95] Hh proteins are secreted ligands synthesised in discrete regions. The receptor of Hh is the multitransmembrane protein Patched (PTCH), which is expressed in cells close to the source of Hh. Binding of Hh to PTCH activates Smo, which signals through the transcription factor glioma-associated oncogene-1 (Gli-1) and regulates gene expression.[95] Therefore, targeting the Hh pathway and Smo has become an attractive therapeutic target for treating GBM.

Currently, there is much interest in Hh signalling pathway inhibitors as anticancer agents, particularly Smo receptor antagonists, resulting in the approval of two drugs: sonidegib and vismodegib (Fig. 5).[96] Although sonidegib has shown efficacy in Phase II clinical trials for medulloblastomas, it has not yet been used in the treatment of GBM.[97] Vismodegib is approved for the treatment of basal cell carcinoma and is an inhibitor of the Hh pathway that binds to Smo. It underwent a Phase II clinical trial (ClinicalTrial.gov ID: NCT00980343) for glioma because it demonstrated antitumour activity.[98] No mortality or serious adverse effects were reported. However, its underlying mechanisms have not yet been investigated in glioma. In 2018, there were 11 Hh signalling pathway inhibitor compounds in clinical trials for different cancers, ten of which were Smo antagonists.[96] Research has shown that Hh signalling is activated in glioma grades II and III but it has not been determined whether it is activated in GBM.[43,99]

The determination of Smo crystal structures now offers the possibility to perform computational structure-based screens for new antagonists.[100] By focusing screening on readily available molecules, hits can be tested quickly, and it also reduces the rate of false positives. This technique is particularly successful with GPCR structures for which hit rates are 17–58%.[100] For example, a study identified 21 novel smoothened ligands by using structure-based docking. A screen for analogues then revealed a further six compounds, one which was effective against the D473H mutant of Smo, which contributes to clinical resistance to vismodegib in cancer treatment.[100]

Concluding remarks

New therapeutic approaches are desperately needed for the treatment of GBM; thus, here we reviewed recent advances in GPCRs as potential emerging therapeutic targets for GBM. Although there is scope to develop new therapeutics by targeting GPCRs, additional research is needed.

The use of chemokines has been pursued in both *in vitro*, *in vivo*, and in Phase I/II clinical trials (Table 2). Given the complicatedness of the chemokine network, it is unlikely that any single chemokine or receptor could be a tumour marker effective enough for a GBM diagnosis. Therefore, a proteomic approach could be effective in the selection of potential tumour markers for gliomas. A study using sandwich ELISA and protein expression in GBM tumoral tissue by means of western blot confirmed that the proteomic approach was effective and identified the chemokine receptor CXCR4.[101] There is scope to use chemokine ligands in the treatment of GBM either by looking at biomarkers or by using them in conjunction with current therapeutics, as shown in a Plerixafor/TMZ study.[101]

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TABLE 2

Established and novel GPCR therapeutic targets to be potentially targeted in the treatment of GBM.

Receptor target	GBM tumour imaging studies	Protein Data Bank entries	Compound(s) and functional effect	Effect on glioma	GBM study phase	Refs
Chemokine receptor	CXCR4 can be targeted by diagnostic PET agent [68 Ga]Ga-PentixaFor and its therapeutic counterpart [177Lu]Lu-pentixather	CXCR4: 3ODU, 3OE0, 4RWS, 3OE8, 3OE9	Plerixafor: antagonist; inhibits CXCR4 binding to CXCL12	Inhibited tumour growth and recurrence	Phase II clinical trial	45,104,105
	CXCR4 expression increases intensity of T2-weighted MRI		PRX177561: antagonist; inhibits CXCR4 binding to CXCL12	Inhibited tumour growth; increased efficacy of combination therapy	*In vivo* study	47
			Fingolimod: agonist; inhibits CXCR4 binding to CXCL12	Inhibits growth, migration and invasion of glioma	*In vitro* study	51
			CCX77: agonist; inhibits CXCR7 binding to CXCL12	Inhibited tumour growth and recurrence	Post-irradiation *in vivo* studies	53
Cannabinoid receptor	N/A	CB1R: 5TGZ, 5U09, 5XR8, 5XRA, 6KPG	CP55-940: CB1R agonist	Decreased cell viability and induced apoptosis	*In vivo*	62
			Rimonabant: CB1R antagonist	Inhibited cell growth and induced apoptosis	*In vitro* study	64
			COR167: CB2R agonist	Inhibited cell growth	*In vitro* study	65
Dopamine receptor	PET imaging studies used in conjunction with MRI	DRD2: 6CM5, 6VMS, 6LUQ, 7DFP, 7JVR	ONC201: DRD2 antagonist	Inhibited cell growth	Phase II clinical trial	67,106
NK-1 receptor	Targeted alpha-radionuclide therapy	6E59, 6HLL, 6HLO, 6 J20, 6HLP	Aprepitant: NK-1 receptor antagonist	Increased cytotoxicity of TMZ and Ritonavir	*In vitro* co-treatment study	72,103
MC4R	Radiolabelled peptides for PET imaging	6 W25, 7AUE	ML00253764: antagonist	Inhibited cell growth and induced apoptosis; enhanced cytotoxicity of TMZ	*In vitro* co-treatment study	74,102
Lysophospholipid receptor	MRI and NIR fluorescence imaging	4Z35, 4Z36, 4Z34	Ki6425: EDG family LPAR antagonist	Inhibition of hypoxia	*In vitro* study	94,107
Smo receptor	N/A	4JKV, 4QIM, 4N4W, 6O3C, 5 V56	Vismodegib: antagonist	Antitumour activity	Phase II clinical trial	98

Although magnetic resonance imaging (MRI) is regularly used for GBM imaging, recent studies have explored the detection of GPCR expression in GBM tissue using labelled peptides/ligands and positron emission tomography (PET) imaging.[102] Targeted alpha-radionuclide therapy has been used to identify NK-1 receptors.[103] The CXCR4 chemokine receptor was targeted by the diagnostic PET agent [^{68}Ga]Ga-PentixaFor,[104] with some result variability observed compared with immunohistochemical analysis. It was concluded that, when high CXCR4 expression can be identified with [^{68}Ga]Ga-PentixaFor, these patients might be good candidates for targeted radionuclide therapy with [^{177}Lu]Lu-pentixather.[104] CXCR4 expression has also been shown to increase the intensity of T2-weighted MRI.[105] Research has combined PET imaging with MRI to identify dopamine receptors,[106] whereas MRI and NIR fluorescence imaging have been used to identify LPL receptors.[107] The ongoing work in this field is highlighted in Table 2.

The DRD2 antagonist ONC201 is currently undergoing Phase II clinical trials and has shown antitumour activity in GBM.[67] Pending the successful outcome of the clinical trial, there is huge potential for this and similar drugs.

The use of cannabinoids as a treatment for GBM has been explored, either by exploiting the use of CB1 receptor agonists or antagonists or of CB2 receptor agonists (Table 2). Cannabinoids are promising in the treatment of GBM because they target cancer hallmarks, such as programmed cell death, neoangiogenesis, and tissue invasion. A recent receptor crystal structure could further aid the rational design and development of new compounds in this space.[108] Furthermore, the effects of cannabinoids could be enhanced when used with other chemotherapeutic agents, although there is a need for further studies using cannabinoids given that there have only been a few *in vitro* and *in vivo* studies carried out to date.[63]

Both *in vitro* and *in vivo* studies showed a highly synergistic effect on GBM cells with TMZ and the MC4R antagonist, ML00253764. A significant decrease in tumour volumes was observed *in vivo*.[74] It has also been shown that there is a shorter progression-free survival and overall survival rate in patients who

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express the MC4R rs489693 AA genotype.[75] As such, targeting *MC4R* is a potential attractive treatment for GBM.

The overexpression of NK-1 is present in a variety of tumours, including glioma, in which the number of NK-1 receptors expressed on cells is greater than that observed on normal human cells and is correlated with the degree of malignancy.[109] Use of drug repurposing and/ or combination therapy to target NK-1 has become an attractive treatment, with work being carried out continuously in this area.

In terms of lesser studied GPCR targets, there are several with potential for GBM, but requiring further studies. Adhesion receptors are the second largest family of GPCRs. The challenges with EMR2 and EMR3 receptors are their complex structure with no mouse orthologues and limited information about their intracellular targets.[84] High GPR133 levels are associated with a reduced patient survival rate and, thus, there is scope for this receptor to be explored further. Currently, there are no approved drugs on the market that use aGPCRs as a therapeutic target and, therefore, more studies are required to better understand their mechanism of action.

LPA antagonists have only been studied in a microglia co-culture system, but an LPA1 antagonist has shown promise in the treatment of multiple fibrotic diseases. A further understanding of these receptors and their GBM role is needed to develop new targeted therapeutics. The final target of interest is the Smo receptor, because there is great interest in Hh signalling pathway inhibitors as anticancer agents. Vismodegib was used as part of a Phase II clinical trial for the treatment of glioma, but its mechanism has not been investigated fully. Although Hh signalling is activated in grade II and III gliomas, it is not clear whether it is activated in GBM. Thus, more studies are needed on these interesting receptor types.

Treating GBM is challenging because most approved anticancer drugs do not readily cross the BBB and there is the issue of tumour recurrence, thus limiting the options for treatment. Given the aggressive and complex nature of GBM, effective treatments that target the CNS are a serious unmet medical need. Therefore, the identification and validation of drug targets associated with GBM disease progression present an exciting opportunity to improve treatment of this devastating disease.

The introduction of new therapeutic targets for the treatment of GBM could improve the outlook for patients; however, a significant amount of research is required, such as computational work, which could allow novel ligands to be designed. Currently, there are no GPCR drugs approved for the treatment of GBM, with the most established targets being the chemokine, cannabinoid and dopamine receptors (Table 2). Future work should also focus on the adhesion GPCRs, LPAR and Smo receptors to initiate new drug screening strategies and targeted delivery of therapies to treat GBM in a safe and efficient manner.

KEYNOTE

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