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Glioblastoma Multiforme (GBM): An overview of current therapies and mechanisms of resistance

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Abstract

Glioblastoma multiforme (GBM) is a WHO grade IV glioma and the most common malignant, primary brain tumor with a 5-year survival of 7.2%. Its highly infiltrative nature, genetic heterogeneity, and protection by the blood brain barrier (BBB) have posed great treatment challenges. The standard treatment for GBMs is surgical resection followed by chemoradiotherapy. The robust DNA repair and self-renewing capabilities of glioblastoma cells and glioma initiating cells (GICs), respectively, promote resistance against all current treatment modalities. Thus, durable GBM management will require the invention of innovative treatment strategies. In this review, we will describe biological and molecular targets for GBM therapy, the current status of pharmacologic therapy, prominent mechanisms of resistance, and new treatment approaches. To date, medical imaging is primarily used to determine the location, size and macroscopic morphology of GBM before, during, and after therapy. In the future, molecular and cellular imaging approaches will more dynamically monitor the expression of molecular targets and/or immune responses in the tumor, thereby enabling more immediate adaptation of tumor-tailored, targeted therapies.

Graphical Abstract

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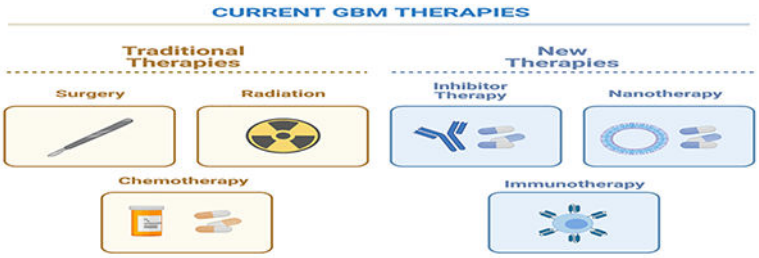

Keywords

glioblastoma; radiotherapy; chemotherapy; targeted therapy; nanotherapy; immunotherapy

2. Introduction

2.1 The epidemiology and etiology of GBM

Glioblastoma multiforme (GBM) is a WHO grade IV brain tumor which represents one of the most lethal human cancers. The incidence of GBM increases with age and shows the highest incidence in the 75 to 84-year old age group in the United States(1). The incidence is higher in men than women, as well as in Caucasians than in other ethnicities(2). According to gene profile analysis and genetic modeling of GBM in mice, there is evidence that GBM is derived from neural stem cells (NSCs), NSC-derived astrocytes, and oligodendrocyte precursor cells (OPCs)(3). GBM tumors derived from different cellular origins show different behaviors in animal models(3)(Fig.1). Exposure of the central nervous system (CNS) to ionizing radiation has been associated with an increased risk to develop malignant brain gliomas, excess relative risk (ERR) estimates for brain/CNS tumors ranged per Gy from 0.19 (95% confidence interval [CI]: 0.03, 0.85) to 5.6 (95% CI: 3.0, 9.4) (4).

2.2 The ongoing challenges of GBM treatment

Standard therapy for GBM encompasses surgical resection followed by chemoradiotherapy, using temozolomide (TMZ)(5). However, 5-year survival is only 7.2% in the United States according to CBTRUS Statistical Report 2020(6). Despite maximal surgical resection and aggressive adjuvant therapy, almost all GBM tumors locally recur after treatment(7). Ongoing challenges to GBM treatment include its incomplete resection, high degree of genetic heterogeneity, exclusive blood brain barrier (BBB), and immunosuppressive microenvironment.

2.2.1 High infiltration—The highly infiltrative nature of GBM makes complete resection at the cellular level nearly impossible(8). Also, abundant hypoxic regions provide perivascular niches for glioma initiating cells (GICs). These self-renewing cells can yield potentially more aggressive recurrent tumors that are radioresistant and chemoresistant (9, 10).

2.2.2 Intertumor and intratumor heterogeneity—Large intertumor and intratumor heterogeneity have complicated targeted therapy development(11). Based on their genetic

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and epigenetic markers, GBMs were previously classified by The Cancer Genome Atlas (TCGA) into four clusters: mesenchymal, classical, proneural, and neural(12, 13). Mesenchymal GBMs are characterized by the neurofibromin 1 (NF1) tumor suppressor gene mutation as well as by frequent mutations in the PTEN and TP53 tumor suppressor genes. The classical subtype is highly proliferative and characterized by EGFR amplification, but no TP53 mutation. Meanwhile proneural GBM is frequently associated with TP53 mutation, and uniquely with IDH1 and PDGFRA mutations. The neural subtype is characterized by many genes which also exist in the brain's normal noncancerous neurons(12, 14). Another GBM tumor transcriptome analysis described only three subtypes: proneural, classical, mesenchymal. The authors proposed that the neural subtype may be due to contamination of the original samples with non-tumor cells(13, 15). The mesenchymal and classical subtypes are typically more aggressive tumors, while the proneural subtype is less aggressive and more often seen in younger patients(13). The proneural-to-mesenchymal phenotype transition is associated with GBM resistance (16). Even though TCGA has categorized GBM into four subgroups, recent studies show that different GBM subgroups vary spatially and temporally within the same tumor(17). Patel et al. showed with single cell RNA sequencing that a single tumor can include a diversity of cells that comprise all the GBM subgroups(18)(Fig. 1).

2.2.3 Blood brain barrier (BBB)—Another challenge to GBM treatment is delivering chemotherapeutic drugs across the blood brain barrier (BBB). The BBB is a protective boundary between the circulatory system and the extracellular space of the central nervous system. The BBB is mainly composed of endothelial cells that form a tight barrier along the wall of blood vessels and selectively limit the compounds that can cross into the parenchyma(19). Tight junctions are less than 1 nm in size and prohibit penetration of >98% of small molecules. Unlike in healthy brain tissue, the BBB in GBM exhibits enhanced permeability due to poorly formed, leaky blood vessels, upregulated transporter proteins, and downregulated tight junction proteins(20, 21). However, the disruption of the BBB is not uniform throughout a given tumor, with some areas exhibiting blood vessels with higher permeability and other tumor areas with more intact vessels and/or vascular shunts. Even if a chemotherapeutic drug managed to extravasate into the tumor tissue, it can often not gain therapeutic levels in tumor cells due to upregulation of efflux pumps by glioblastoma cells (22). Ultimately there remains a substantial proportion of GBM with locoregional intact BBB and upregulated efflux pumps (23, 24).

2.2.4 Immunosuppressive microenvironment—The microenvironment of GBM creates treatment challenges. Some authors refer to GBM with an immunosuppressive microenvironment as "cold tumors". These tumors lack pre-existing tumor T cell infiltration which results in tumor resistance to immune checkpoint inhibitors (25). These tumors are further characterized by their lack of tumor antigens, defects in antigen presentation, and high accumulation of immunosuppressive cells (26, 27). Treatment with immune checkpoint inhibitors shows limited efficacy (28). In contrast, "hot tumors" are infiltrated with swarms of T cells, and thus more immunogenic. Turning "cold" to "hot" may be achieved by combinatorial approaches that boost anti-tumor immunity (27).

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Given the genomic complexity and global alterations of multiple signaling pathways in GBM, consistent effort has been put forth to improve systemic therapies for glioblastoma. In the following sections, we will address how pharmacologic therapies have evolved, how mechanisms of resistance develop, and how new strategies have emerged.

3. GBM traditional therapy and resistance

3.1 Traditional therapy

3.1.1 Surgical resection—Surgical resection of brain tumors was revolutionized in the late ‘80s and early ‘90s with the development of frameless stereotaxy. It enabled the very precise placement of surgical instruments using image guidance and has been modernized with new imaging technologies(29). Additionally, cerebral cortical stimulation has enabled intraoperative localization of eloquent cortex regions of the brain that need be avoided during surgery. This technique, or “brain mapping” accounts for individual variations in anatomy or tissue reorganization to remove tumor in areas of great functional importance and that are responsible for quality of life(30, 31).

The extent of tumor resection has been positively correlated with survival time, with gross total resection (GTR) being desirable when possible(1, 32–36). There are, however, major challenges to achieving GTR, including successful identification of tumor margins as well as avoiding adjacent eloquent cortex. Even though the tumor margins can be roughly determined through imaging, GBM grows with microscopic, finger-like projections, imperceptible to presurgical or even intraoperative imaging techniques(37).

Imaging is an increasingly important tool for GBM resection(29). Images can guide biopsies(38–40), identify tumor margins(41–43), and localize critical brain structures that need to be spared(44). Computed tomography (CT) is valuable for emergent imaging, while MRI is the gold standard for brain tumor imaging due to its the higher anatomical resolution and higher soft tissue contrast. Depending on the MRI contrast and pulse sequence used, important features of the tumor and brain tissue can be elucidated including blood vessels, tumor necrosis, and hemorrhage (T1-weighted with contrast), cerebrospinal fluid (T2-weighted), blood perfusion (dynamic susceptibility contrast (DSC), dynamic contrast enhanced (DCE) and arterial spin labeling (ASL)(45, 46). Some studies have advocated supramaximal resection, thereby resecting beyond the T1 contrast enhancing portion(47).

Both intraoperative fluorescence imaging and MR imaging can help to address the challenging phenomenon of “brain shift” or movement of the brain during surgery, which can lead to discrepancies between the location of the tumor and critical brain structures on preoperative imaging studies and in the operating room. Some shifts can be up to 2 cm in distance and result from causes that are physical (e.g., patient position, gravity), surgical (types of equipment used, tissue/fluid loss during procedure), or biological (e.g., tumor type/location, drugs used to manage intracranial pressure). Unfortunately, this phenomenon worsens with increasing duration of the surgery and cannot be corrected for using neuronavigational devices that derive stereotaxic capabilities from pre-operative MRI images(48).

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Fluorescent imaging has been performed with fluorescein, indocyanine green (ICG), and 5-aminolevulinic acid (5-ALA)(49–52). Fluorescein is of historical relevance, as it is one of the first intraoperative imaging adjuncts. While fluorescein does not typically cross an intact the BBB, it does penetrate high grade gliomas (HGGs) in areas of enhanced permeability(53). Likewise, ICG has been of historical value, primarily for angiography and vessel delineation rather than parenchymal margins. Currently, 5-aminolevulinic acid (5-ALA) has become the standard of care among intraoperative fluorescent agents and has received regulatory approval in the US and Europe(49). 5-ALA is a precursor to heme that can cross the BBB and converts to the fluorescent compound protoporphyrin IX in the mitochondria. The mechanisms underlying its accumulation in brain tumors are still not well understood. It exhibits higher specificity over fluorescein (67% vs 33%)(54). Studies have also shown that GTR or near-GTR rate is higher in patients that underwent fluorescence-guided surgery with 5-ALA compared to patients that were operated under white light only (65% vs 36%)(50). Additional clinical trials are now being performed using IRDye800CW and ZW800–1 for targeted imaging(55, 56),

Intraoperative MRI and ultrasound (iMRI and iUS, respectively) help surgeons identify residual cancer in surgeries (57, 58). The surgeon resects a brain tumor, then performs a contrast-enhanced MRI in the operating room on an intraoperative MRI scanner. If residual enhancing tissue is found, then this residual tissue is removed in the same surgery.

3.1.2 Chemotherapy

3.1.2.1 Cytotoxic chemotherapy: The standard post-surgical treatment regimen includes 6 weeks of concomitant TMZ (75 mg/m^2) and radiation followed by adjuvant TMZ (150–200 mg/m^2) for 5 days every 28 days for six cycles(59). TMZ, the most widely used chemotherapeutic drug for GBM, is a small molecular alkylating agent that directly damages tumors by methylating the purine bases of DNA(60). The key cytotoxic action is through formation of O^6-methylguanine lesions which results in apoptosis, autophagy, and cellular senescence(61–64). In addition, it was discovered that TMZ has radiation-sensitizing properties. It increases the likelihood of radiation-induced DNA double strand breaks and cell death when the drug is administered at the same time as radiation therapy(65).

The most common side effect from TMZ is hematologic toxicity(66). Thrombocytopenia has been identified in 10–20% of patients. In a Phase II clinical trial, the addition of a thrombopoietin receptor agonist, Romiplostin to adjuvant CCRT (concurrent chemoradiation therapy) has increased the rate of completed regimens(67). Nonhematologic toxicities from TMZ are less common and include nausea, anorexia, fatigue, and hepatotoxicity(68).

Several nitrosourea reagents have also been explored for GBM treatment. Carmustine (also known as BCNU) is a small nitrogen mustard compound and alkylating agent. It induces interstrand crosslinks between the guanine and cytosine bases in DNA(69). Carmustine rose to prominence with the advent of Gliadel® wafers, which are FDA (U.S. Food and Drug Administration Agency)-approved biodegradable discs that are intraoperatively placed in the resection cavity to provide a slow release of drug over two weeks(70). The alkylating therapeutic effects of carmustine can be reversed by the enzyme alkyl guanine transferase (AGT). Therefore, carmustine is sometimes used in concert with AGT inhibitors to ensure

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therapeutic efficacy(71). Side effects include abnormal wound healing and intracranial infections since the wafers present as foreign bodies(72). Another alkylating anti-tumor reagent is lomustine. Due to its high lipophilicity and small size, lomustine can cross the BBB. Thus, lomustine can be given orally(73). Fotemustine, another molecule in this family of drugs, has been used for melanoma and is now tested for its efficacy with recurrent glioblastoma(74).

3.1.2.2 Anti-angiogenic chemotherapy: Bevacizumab is a humanized monoclonal antibody with anti-angiogenic properties and is administered intravenously. It binds to and inhibits vascular endothelial growth factor A (VEGF-A) which initiates growth of new blood vessels when bound to its receptor. The levels of VEGF-A in GBM are estimated to be approximately 30 times higher than in low grade astrocytomas making it an attractive therapeutic target. While bevacizumab appears to improve progression-free survival, it does not significantly improve overall survival for patients with newly diagnosed glioblastoma(75). Therefore, it is predominantly used to treat recurrent glioblastomas. The use of bevacizumab with irinotecan a small molecular prodrug that is converted a topoisomerase I inhibitor, is being explored(76, 77). Bevacizumab's side effects most commonly include hypertension and leukopenia(74, 78).

3.1.3 Radiation therapy (RT)—Radiation therapy can be utilized in several forms (e.g., x-ray photons, gamma photons, protons), but not all have become validated for standard of care (Table 1). Conceptually, it can be administered to help provide local control for the microscopic disease unaddressed by surgical resection. In CCRT, patients receive 3D conformal RT which uses x-ray photons that are directed at the tumor target from several different angles. CT and MRI images are used to plan the delivery of the therapeutic dose to the tumor site. Radiation therapy is usually distributed over 6 weeks in 2 gray (Gy) fractions for a total dose of 40–60 Gy(79). A typical device for RT possesses elaborate multi-leaf collimators which allow very specific shaped beams to target the tumor and provide a small (1–2 cm) margin around the periphery. Treatment with x-rays confers low linear energy transfer which results in both direct and indirect biological damage. Direct damage to DNA makes up approximately one-third of the treatment effect, and indirect effects (namely, ionization of water to produce free radicals which damage DNA), make up approximately two-thirds(80).

Conformal RT has been preferred in the clinic over whole brain radiation since recurrent gliomas tends to appear within 2 cm from the original tumor site in 80–90% of cases(81). Thus, conformal RT is designed to target the majority of residual GBM cells while sparing healthy brain tissue and thereby, minimize cognitive side effects. Intensity-modulated RT also uses multi-leafed collimators, but can redistribute ionizing radiation across the target depending on its location and sensitivity(82). This strategy has the added complexity of employing several collimated beams in order to avoid injury to critical structures including the cornea, optic nerves, and brainstem(83).

Another form of focal radiation includes stereotaxic radiosurgery (SRS) where even larger radiation doses can be applied by delivering multiple non-parallel, converging radiation beams. The dose is given in fewer fractions (1–5) and at higher doses per fraction (15+ Gy).

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The gamma radiation is derived from a cobalt-60 source and directed at the tumor through collimators(84). The heat transfer properties of gamma rays enable high doses of radiation to be delivered very specifically at the tumor target while sparing adjacent healthy tissues(85). SRS has been commonly used for brain metastases, particularly to avoid the cognitive side effects of whole brain RT, and may be appropriate in some cases of GBM(86).

Brachytherapy is being explored and involves local implantation of one or more radioactive vectors into the tumor bed at the time of surgery. The most common isotopes used for brachytherapy in brain tumors are iodine125 ($t_{1/2}$ = 59.5 days) and iridium-192 ($t_{1/2}$ = 73.8 days)(87). Unfortunately, two major trials by Laperriere et al. and Selker et al. did not show a statistically significant differences in survival between patients receiving brachytherapy and those that did not(88, 89). Another Phase I trial was halted early due to high toxicity(90). Adverse effects have included symptomatic radiation necrosis, vascular injury, and radiation exposure to close contacts(91).

Charged heavy proton radiotherapies such as carbon ion irradiation spares normal tissue and concentrates energy deposition on the tumor(92). This disrupts GIC viability(93) through the induction of pro-apoptotic pathways and thereby provides an antiangiogenic and immunopermissive tumor environment(94). Tumor regression and long-term local control were identified in xenograft mouse models and a clinical study of 50 patients with grade III and IV glioma measured an 18-month overall survival of 73% with dose equivalents of ≥ 60 gray appearing to be safe (93, 95). Indeed, combination therapies of carbon proton irradiation with TMZ treatment led to enhanced overall survival and progression-free survival compared to patients subjected to combination of TMZ with photon-induced irradiation(95).

An important impediment of effective RT is the hypoxic tumor environment. Oxygen generally enhances the response of cells to low linear energy transfer radiation. When tissue is irradiated and DNA radicals form, oxygen can react with the radicals to create permanent cell damage. In hypoxic tumor environments, the damaged DNA has added time to repair and reduce radiation injury(80). Hypoxic tissue is thought to require approximately three times the radiation dose to achieve the same therapeutic benefit(96).

3.2 GBM therapy resistance

3.2.1 DNA repair mechanism

3.2.1.1 Methyl guanine methyl transferase (MGMT): TMZ causes tumor cytotoxicity by transferring methyl groups to DNA (70% at N^7-guanine sites, 10% at N^3-adenine sites and 5% at O^6-guanine sites)(97). The O^6 site alkylation on guanine leads to attachment of a thymine rather than a cytosine during the DNA replication process. The altered configuration is the primary cause of cell death(98). This alkylation damage can be reversed by the DNA repair enzyme MGMT (O^6-methylguanine-DNA methyltransferase) by removing O^6-methylguanine adducts. Thus, the methylation status of the MGMT gene promoter has great clinical significance(99, 100). MGMT promoter methylation is more prevalent in recurrent GBMs compared to primary GBMs (75% vs. 36%)(101). The level of MGMT protein expression has been associated with the efficacy of alkylating drugs to

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cancer cells in glioma tumor models (98, 102). GBM with MGMT promoter methylation (evaluated by methylation-specific PCR (MS-PCR)) and its subsequent loss of MGMT protein expression (measured by immunohistochemical staining) showed better response to TMZ therapy(103). MGMT inactivation or silencing is associated with significantly improved overall survival and progression-free survival (PFS)(104–106). The role of TMZ in CCRT has made MGMT one of the most pertinent prognostic markers.

Understanding the regulation of MGMT may improve the development of targeted therapies. An important gene related to MGMT expression is p53, a negative regulator of MGMT in GBM tumors (107–109). Clinically, GBM patients with lower MGMT expression have better prognoses with proneural phenotypes expressing p53(110). The proline-rich domain of p53 has been confirmed to be non-essential for MGMT-dependent DNA repair(111). However, p53 may down-regulate MGMT via interaction with the Sp1 transcription factor(112). This was supported when BACH overexpression competitively interfered with p53 and Sp1 binding, and antagonized MGMT expression(108). Sp1 is an imperative nuclear transcription factor, which suggests the role of transcriptional factors in regulating MGMT expression. In silico analysis by Transcription Elements Search System (TESS, http://www.cbil.upenn.edu/tess) identified putative consensus sequences for the binding of nuclear transcription factors in the promoter region of MGMT. These include Sp1, NF-κB, CEBP, AP-1, AP-2 and NF-IL6 at CpG dinucleotides. The literature has also supported Sp1(112–114), AP-1(102, 115), NF-κB(116, 117) in the activated transcription of MGMT. Thus, methylation of CpG dinucleotides may hinder the binding of these transcription and compress the transcriptional activation of the MGMT gene(102, 118, 119). Other transcription factors, such as hypoxia-inducible factor-1 (HIF-1), when activated in the hypoxic GICs niche, will enhance MGMT expression(120–122).

Besides transcriptional factors, epigenetic modifications of MGMT have also shown a relevant role in MGMT regulation(123, 124). Acetylation of lysine residues on histones H3 and H4 (H3Ac and H4Ac) is correlated with high MGMT expression(125) while di-methylation of lysine 9 of histone 3 (H3me2K9) leads to silencing of MGMT expression(102).

Thus, targeting transcriptional factors or epigenetic modifications related to MGMT activation may be options in GBM therapy.

3.2.1.2 Mismatch repair (MMR): O^6-methylguanine (O^6-MeG) generated by therapeutic alkylating agents is the dominant cytotoxic lesion. It can either be removed by MGMT or tolerated by MMR deficiency, to induce prominent therapy resistance(126). MMR addresses inappropriate nucleotide base pairing to maintain DNA replication fidelity. MMR is collectively achieved by several proteins including MLH1, MLH3, MSH2, MSH3, MSH6, PMS1, and PMS2(127). Outside the context of TMZ treatment, MMR deficiency or epigenetic silencing of MMR gene expression leads to cancer development(128, 129). MMR deficiency results in strong resistance to the alkylating agents, such as the alkylating agents N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), TMZ, or procarbazine. When MGMT is not present to remove the methyl group, thymine is erroneously inserted opposite O6-MeG by DNA polymerase. The O6-MeG:T mismatch is recognized by the MMR complex, and

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bound by MutSα (an MSH2•MSH6 heterodimer), subsequently, a gap is generated in a new stretch of single-stranded DNA, DNA polymerase once again inserts thymine to complete mismatch repair. It is highly likely that the futile repair generates double strand breaks leading to cell cycle arrest and cell death(130).

Perazzoli et al. noticed an interesting inverse correlation between MGMT and MMR (131). *In vitro* studies investigating an MGMT-methylated GBM cell line (U251), which survived TMZ treatment, ultimately developed MMR deficiency(132). Studies from TCGA suggest that at least one of the MMR genes - MLH1, MSH2, MSH6 or PMS2 - possess a methylated MGMT promoter(133). MSH6 inactivation exhibited a crucial role in conferring tolerant tumor cell growth. It has been shown that a modest decrease of MMR components MSH2 and MSH6 is associated with TMZ sensitivity(134). Sun et al reported that a large cohort of GBM clinical samples have enhanced expression of MMR genes especially MSH6 after long-term TMZ treatment(135). Whether the incidence of MSH6 mutation is induced by therapy is still unknown and remains controversial. The German Glioma network noticed significantly lower MSH2, MSH6, and PMS2 protein expression in recurrent GBM compared to primary GBM tumors(136), which is in line with some additional investigations(137). Meanwhile, recurrent GBM tumors frequently possess MMR6 mutations coinciding with microsatellite instability(138).

3.2.2 Glioma initiating cells (GICs)—A subpopulation of cells in GBM described as “cancer stem cell (CSC)” or “glioma initiating cells (GICs)” is speculated to possess specific characteristics that support tumor development, recurrence, and therapeutic resistance(10, 139, 140). GICs are believed to be a distinct subgroup of cells, that can undergo self-renewal and initiate tumorigenesis. Singh et al. first described a population of CD133+ cells, but not CD133- cells, that initiated tumor growth in NOD-SCID (non-obese diabetic, severe combined immunodeficient) mouse brains(141). Although CD133 is a recognized marker of GICs, it is also expressed in normal neural stem cells(142). Therefore, multiple surface markers are needed to characterize GICs. Other recognized markers of GICs include CD44 and ATP binding cassette transporters (143–145). We further elaborate on the molecular mechanisms of resistance in GICs.

3.2.2.1 Enhanced DNA repair capacity: In addition to protective responses by MGMT and MMR expression following chemotherapy, GICs may exhibit sensitizing mechanisms to radiation(146). Checkpoint kinases (Chks), in particular Chk1 and Chk2, play primary roles in cell cycle control. In response to DNA damage, Chk1 signal will be activated and holds the cells in the G2 phase until DNA is repaired and ready for the mitotic phase, whereas Chk2 is activated under double stranded break (DSB) and prevents cells from dividing in an uncontrolled manner. Bao et al reported that GBM displayed aberrant DNA damage response to radiotherapy. They observed that the addition of specific inhibitors of Chk1 and Chk2 checkpoint kinases restored the radiosensitivity of GICs(147). More recently, Ahmed, et al. observed that GICs have a prolonged cell cycle arrest at the G2 phase and noted that the inhibition of the Chk1 pathway induced double stranded breaks under ionizing radiation, in an ATM- and ATRdependent manner(148).

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The polycomb group protein, BMI1, also shows enhanced expression in CD133+ GBM cells, BMI1 coprecipitated with DNA DSB response proteins, and preferentially associated with NHEJ repair proteins. This interaction enormously improved DNA damage response to radiation and cell viability(149).

Additionally, homologous recombination (HR) defects can contribute concurrently to the radioresistance of GICs. The inhibition of DNA repair protein RAD51 homolog 1 (RAD51) has been found to delay G2 cell cycle arrest, thereby sensitizing GICs to radiation(150). Due to the prominent cellular DNA damage response of GICs, targeting DNA repair pathways may provide a beneficial therapeutic approach for eliminating GICs.

3.2.2.2 ATP binding cassette (ABC) transporter: ABC transporters are ubiquitous and one of the largest transmembrane protein pump families. Normally, ABC transporters move endogenous bile acids, cholesterol, ions, and peptides across cell membranes. GICs express high levels of ABC transporters that are normally inactive in mature cells. In GICs, overexpression of ABC transporters further hinders drug delivery. ABC transporters promote therapy resistance by promoting efflux of exogenous compounds, such as TMZ, at the cellular and BBB level, in order to detoxify cells(151, 152).

ABC transporters include 49 members classified into seven gene subfamilies, designated ABCA–G. Thus far, ABCB1 (MDR1), ABCC1 (MRP1), and ABCG2 (BCRP1) are the most well-known pumps that have been identified in tumor stem-like cells. ABCG2 was first identified and associated with subpopulations of cells that are stem-like and multidrug resistant(153). ABCC1 is assumed to be another cause for GBM recurrence since the blockage of ABCC1 improved therapeutic response in GBMs(154, 155). GICs are enriched in hypoxic niches of the tumor(156). Low oxygen levels further promote the expression of MGMT, ABCC1 and ABCB1, and thereby lead to chemoresistance(157). Very recently, Lee, et al., proposed ABCB5 as a new marker for CD133+ GICs in chemoresistant GBM. The knockdown of ABCB5 inhibited GBM proliferation and sensitized the GBM cells to TMZ treatment(158).

Multiple pathways (SHH, Wnt-β-catenin pathway, Bcl-2, Akt, survivin, etc.) have been associated with the role of ABC transporters in therapy resistant GICs(151), which suggests that the ABC transporter is yet another target for which new therapies could be developed.

3.2.2.3 Hypoxia and autophagy: Tumor hypoxia is consistently associated with poor prognosis across multiple cancer types(159–161). GBM is characterized by extensive tissue hypoxia(162) and the hypoxic microenvironment has been regarded as an indispensable environmental cue for the preservation of GICs(120). In response to the reduction of oxygen tension, hypoxia-inducible factor 1 (HIF-1) is stabilized to maintain GICs and promote their tumorigenic ability(163, 164). In tumor tissue, microvascular thromboses congest vessels, further boosting intratumoral hypoxia. GICs can be found in the perivascular niche and the tumor core region, where are usually less oxygenated (1.25% O_2) than peritumoral areas (2.5% O_2)(165). The poorly oxygenated tumor tissue creates a perfect GIC niche and can stimulate downstream oncogenic pathways resulting in heterogeneity, invasiveness, and

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therapy resistance. Overall, the density and aggressiveness of GICs are negatively correlated with oxygen tension.

Several studies suggest that autophagy is induced by hypoxia as a cytoprotective mechanism, which is complementary to the ubiquitin system(166, 167). Autophagy is a survival-promoting process that contributes to the clearance of damaged proteins and organelles to maintain cellular homeostasis and genomic integrity. Moreover, it has been shown that autophagy supports cellular metabolism by generating metabolic precursors such as amino acids and lipids, which further supports the function of autophagy as an adaptive mechanism responding to metabolic stress(168, 169). In GBM, protective autophagy is triggered in GICs when challenged by cytotoxic therapies(168, 169).

It has been of great interest to investigate how autophagy sustains tumor growth and contributes to therapy resistance. Gene-9/Syntenin (MDA-9) is associated with advanced tumor grades in various cancer types. A recent study showed that MDA-9 is critical to maintaining GICs by regulating essential autophagy-related molecules, including BCL-2 and EGFR(170). Huang, et al., found that radiotherapy increases the level of MST4, which phosphorylates ATG4B and leads to autophagy. The inhibition of ATG4B significantly improved the survival benefit in radiation-treated mice(171). Inhibition of autophagy increased the massive accumulation of lipid peroxides and enhanced the sensitivity of GBM to TMZ treatment, which indicates initiation of ferroptosis in GICs(172). However, we observed an opposite role of autophagy. Aldehyde dehydrogenase 1A3 (ALDH1A3) has been regarded as a biomarker for various kinds of cancer stem cells, including GICs. We found that ALDH1A3 confers chemoresistance to GICs by deactivating toxic active aldehydes produced by lipid peroxidation under low concentrations of TMZ treatment. However, under high concentrations (500 μM) of TMZ, autophagy is induced, ALDH1A3 binds to p62 (an autophagic substrate) and is degraded along with ubiquitin cargo, ultimately enhancing the susceptibility of GICs to TMZ(173, 174).

The autophagy inhibitor chloroquine could reduce GBM resistance to anti-angiogenic therapy(175). It also has been shown that chloroquine-treated patients have better median survival in adjuvant settings in a phase III clinical trial(176). Although the role of autophagy in GBM progression is controversial, nevertheless, it provides a new insight for targeted therapy and conquering therapy resistance of GICs.

3.2.2.4 Epigenetic regulation: There is growing evidence that non-genetic determinants, associated with epigenetic modifications, contribute to functional heterogeneity and maintenance of GIC hierarchies(177–180). Epigenetic regulation includes alterations in the chromatin structure as well as DNA methylation and post translational histone modification or even changes in noncoding RNAs including long noncoding RNAs and miRNAs (181, 182). Specifically, the subpopulation of CD133+ cells, which are generally regarded as GICs, were found to be H3K9me2 negative, while the majority of cancer cells expressed strong H3K9me2. This data indicates that H3K9me2 is an important switch for maintaining stemness of GICs by regulating CD133(183). In a study based on histone modification expression analysis of 230 tumor samples, patients were divided into 10 separate prognostic groups. The 10 groups showed significantly different progression-free ($P < 0.0001$) and

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overall survival ($P < 0.0001$), demonstrating that aberrant histone modifications are critical prognostic factors for GBMs(184).

Epigenetic deregulation of multiple GIC-related pathways enables cancer cells to gain self-renewal and drug resistance properties. Wnt/β-catenin signaling can be activated by DNA methylation and aberrant histone modifications (185). Wnt/β-catenin signaling stabilized the epigenetic regulator KDM4C, and enhanced tumorigeneses and survival of human GBM cells(186). Another pathway that regularly interacts with epigenetic regulators is Notch signaling. DNA methylation of NOTCH1 and NOTCH3 inhibits GBM cell proliferation(187). Another study used sodium butyrate (NaB), a DNA methylating agent, to induce GBM apoptosis by decreasing HEY1 expression, suggesting that promoter methylation may regulate Notch signaling(188). In parallel to Wnt/β-catenin and Notch signaling, GICs also have high Sonic Hedgehog (SHH) signaling which is associated with better epigenetic memory. Hedgehog signaling can be epigenetically triggered in CSCs by Shh promoter hypomethylation and HDAC1 expression induction(179). BRD4, a wellcharacterized “epigenetic reader”, is also a critical regulator of GLI1 and GLI2 transcription(189). Lysine acetyltransferase 2B (PCAF/KAT2B) is another epigenetic modulator which is important in regulating SHH signaling in GBM (190). Collectively, a sophisticated network of signaling pathways can be deregulated as a result of aberrant epigenetic modifications in GICs. These genetic alterations uphold the stemness of GICs and promote tumor progression (Fig. 2).

A better understanding of the mechanisms underlying GICs therapy resistance would improve the development of more effective therapies against GBM.

4. New therapies

Despite aggressive therapeutic measures, GBMs invariably continue to grow. Therefore, a broad search is underway for new and targeted therapies, such as inhibitors of specific molecular processes, nanoparticle carriers, and immunotherapy (Table.1 and Fig. 3).

4.1 Inhibitor therapy

Targeted inhibitors have been an additional therapeutic strategy for treating GBM(193). Inhibitors usually target a single biomarker or family of biomarkers that are markedly upregulated in malignant over healthy tissues. In general, inhibitors are developed either for extracellular targets like cell surface receptors or intracellular targets involved in signaling and activation of oncogenic pathways(193). These therapies have been tested alone and as a co-treatment with established therapeutics like TMZ or bevacizumab with varying but usually limited benefit.

4.1.1 PARP inhibitors—Poly (ADP ribose) polymerase (PARP) is a family of 17 nuclear enzymes that catalyze the cleavage of NAD+ molecules leading to the addition of ADP-ribose to acceptor proteins(194). The PARP1 enzyme is a prognostic marker and its elevated expression is associated with poor survival in some cancer patients(194). PARP is important for repair of DNA nicks, cell death, and genomic stability(195). Cancer therapies like radiation and alkylating agents rely on DNA breaks for their anti-tumor effect.

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Small molecule PARP inhibitors have been developed to improve the clinical efficacy of these therapies(196). PARP inhibitors can cause direct cytotoxic effects and potentiate the efficacy of alkylating agents. There is evidence to suggest that PARP inhibitors are most effective for GBM tumors with hypermethylated MGMT gene. In addition, PARP inhibitors can increase tumor sensitivity to TMZ chemotherapy, if the tumor has not previously been exposed to TMZ(196, 197). In addition to enhancing chemotherapy, PARP inhibitors synergize with radiation therapy because PARP1 activity increases 500-fold in the presence of DNA damage(198, 199). While several PARP inhibitors have been tested *in vitro*, only a few have been evaluated in GBM patients. A Veliparib-TMZ treatment regimen was evaluated in patients with recurrent GBM who had been previously treated with TMZ. Combination of TMZ and ABT-888 (veliparib) did not significantly improve PFS6 for either the bevacizumab-naïve or bevacizumab-failure patients (200). Similarly, co-treatment of children with diffuse intrinsic pontine glioma with veliparib, RT and TMZ did not show survival benefits(201). A phase I trial evaluating the safety and tolerability of cotreatment with olaparib and TMZ showed modest activity. Encouragingly, olaparib was found to have penetrated brain tumors both at the core and at the margins suggesting that the BBB was penetrated(202). Additional PARP inhibitors meriting further clinical study include niraparib and talazoparib (203–205). Many of these inhibitors show limited BBB penetration in preclinical models but evidence suggests the preclinical data might not faithfully predict clinical results(202, 204, 206). The true benefit, if any, of PARP inhibitor therapy for GBM patients remains to be determined.

4.1.2 Protein kinase inhibitors—

Protein kinases are a involved in biochemical phosphate transfer reactions. Receptor tyrosine kinases are a family of high affinity cell surface receptors that have an extracellular domain with a ligand-binding site for many polypeptides, including growth factors and cytokines(207). Receptor tyrosine kinase pathways are known oncogenic drivers for malignant cancers(208). Several tyrosine kinase receptors, including vascular endothelial growth factor receptor (VEGFR), platelet derived growth factor (PDGF), fibroblast growth factor receptor (FGFR), and epidermal growth factor receptor (EGFR), are mutated and/or upregulated in GBM and have become attractive targets for novel therapy development(209–213). GBM is known to have high levels of VEGF expression resulting in highly angiogenic tumors and abnormal vessel formation(214). Inhibition of VEGFR or its substrates aims to stymie the growth of new blood vessels and starve the tumor of needed nutrients. Cediranib, an inhibitor of VEGF receptor tyrosine kinases has been evaluated in recurrent GBM as a monotherapy(215) or in combination with cilengitide(216), lomustine(217) or gefitinib(218), but has yet to receive FDA approval. Cediranib has the advantage of oral administration, targeting of multiple tyrosine kinases, and the ability to target intracellular VEGF receptors(215). Cediranib has been shown to help normalize tumor vascularization in a subset of patients which improved perfusion, oxygenation, and response to therapy. These patients had higher overall survival (OS) than the non-responding cohort(219). It was also recently discovered that cediranib sensitizes tumors to PARP inhibitors by downregulating homology-directed DNA repair(220). This discovery may encourage further investigations of combined anti-angiogenic and PARP inhibitor chemotherapies. Unfortunately, to date, the use of anti-angiogenic therapies for GBM has shown limited efficacy in clinical trials. One reason may

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include inadequate patient selection (221). Lu-Emerson, et al., summarized results from recent anti-angiogenic trials for GBM and how to use biomarkers to select for patients in future trials(222).

Erlotinib is an inhibitor of EGFR and has been approved to treat non-small cell lung and pancreatic cancers. It garnered excitement as a possible therapy for GBM in the early 2000s with encouraging *in vitro* results. However, several clinical trials have failed to reproduce the positive results obtained in initial experimental studies or improve survival of patients with GBM(223–227). Gefitinib, another EGFR inhibitor, has similarly been tested with and without other therapies. A phase II trial showed some evidence of activity of gefitinib for recurrent GBM in a subset of patients. However, survival was worse for the majority of patients compared to treatment with TMZ at the first sign of relapse(228). Other clinical trials using gefitinib alone or with cotreatments have shown no significant improvements over currently approved therapies(229–231). While most clinicians agree that combination therapies will be needed to treat recurrent GBM, there has been little further investigation of these two EGFR inhibitors in recent years.

There are also a number of small molecular multi-targeted protein kinase inhibitors that have been evaluated in new and recurrent GBM. Imatinib was evaluated in phase II clinical trials in newly diagnosed GBM (inoperable or not fully resected) and recurrent GBM, with and without concurrent radiation, but showed no clinical activity(232–234). Imatinib was well-tolerated but suffered from poor BBB penetration, even after radiation therapy. Again, it is possible that lack of proper patient selection for tumors overexpressing the targeted protein kinases may have contributed to the trial's unfavorable results. Phase II trials of imatinib with the addition of hydroxyurea, which inhibits ribonucleotide reductase, again showed little to no anti-tumor activity(235–237). Dasatinib is another small molecular multi-targeted tyrosine kinase inhibitor. An early phase I/II clinical trial in recurrent GBM demonstrated toxicity issues and was underpowered such that clinical efficacy could not be determined(238). A trial evaluating the efficacy of adding dasatinib to bevacizumab did not have any added treatment benefit in patients with recurrent GBM compared to bevacizumab alone(239). Dasatinib was also tested in pediatric patients with progressive/recurrent GBM or diffuse intrinsic pontine gliomas with crizotinib, an oral c-Met inhibitor, but was poorly tolerated such that further investigation was discouraged in this patient population(240). Co-administration of dasatinib with lomustine (CCNU) unfortunately led to significant hematological toxicity(241). Sorafenib is another multi-targeted protein kinase inhibitor that has been tested in GBM patients(242). Several studies have evaluated the use of sorafenib with TMZ. Only one of these studies met its primary endpoint with 26% of patients achieving PFS at 6 months(243). The remaining trials with TMZ, bevacizumab, or erlotinib demonstrated safety of the combined therapy but no significant therapeutic efficacy(225, 244–246). Possible reasons leading to unfavorable study results included extensive prior therapy of some of the study participants and lack of selection of patients with tumors that expressed molecular targets for sorafenib (i.e., VEGFR, Raf-1 and wild-type B-Raf, PDGFR, c-KIT, and Flt-3A). Additional studies combining sorafenib with temsirolimus, an inhibitor of mammalian target of rapamycin (mTOR) or tipifarnib, a farnesyltransferase inhibitor, led to significant toxicities(247, 248). Recent evidence suggests that sorafenib may sensitize GBM cells to tumor-treating fields (249). Sunitinib is a similar

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multi-targeted protein kinase inhibitor that has been FDA-approved for the treatment of renal cell carcinoma but has failed to show efficacy for recurrent GBM in several clinical trials(250–252). Cabozantinib, another multi-targeted protein kinase inhibitor approved for the treatment of kidney cancer was evaluated in phase II trials for recurrent GBM and was welltolerated but did not meet predetermined statistical measures for success. Trials for pediatric patients are underway(253, 254).

4.1.3 Miscellaneous inhibitors—

Additional molecular targets for inhibitors include myeloid cell leukemia-1 (MCL-1), and topoisomerase I inhibitors. MCL-1 is associated with PTEN deletion/mutation which occurs in 30–60% of GBM patients(255). Loss of PTEN in GBM cells led to upregulation of MCL-1 which is associated with resistance to apoptosis. There is preclinical evidence that the use of MCL-1 inhibitors in GBM may be an effective therapeutic strategy(255–257). A few MCL-1 inhibitors have gone to clinical trial (e.g., AZD5991, S64315, AMG 176, and AMG 397) mostly for the treatment of blood cancers but some have been halted due to concerns with cardiac toxicity(256, 257). Gossypol (AT-101), a polyphenolic compound that permeates cells and inhibits several dehydrogenase enzymes, has shown modest benefit in treating recurrent GBM, though few subsequent clinical trials have been initiated(258). Another biomarker under investigation is mTOR. Temsirolimus and everolimus are small molecular inhibitors that have been developed for mTOR which plays a role in glioma induction, growth, and progression(259). With these inhibitors, little to no radiographic improvements or benefit to progression-free survival have been observed when used as monotherapy and no added benefit was observed when used with bevacizumab or when compared against TMZ for patients with unmethylated MGMT promoters(260–265). The addition of everolimus and bevacizumab to radiation and TMZ as first-line treatment for GBM gave similar results compared to other phase II trials where bevacaizumab was added to first-line treatment(266). Additional mTOR inhibitors are currently being evaluated as potential cancer therapies (e.g., AZD-8055, OSI-027, and CC-115)(267). Irinotecan is a small molecular prodrug that, upon hydrolysis, is converted to SN-38, a topoisomerase I inhibitor(76). As a monotherapy, irinotecan has shown little to no benefit(268). It has been shown to have some activity in recurrent GBM although with some toxicity concerns(269–271). Use of irinotecan with TMZ for newly diagnosed GBM following concurrent chemoradiation therapy did not improve OS compared to TMZ alone and resulted in significant toxicities(272).

Targeted therapies for GBM have largely failed in clinical trials. A greater emphasis is now being placed on selecting patients whose tumors express the specific biomarkers targeted by the therapy(273, 274).

4.2 Immunotherapy

Immunotherapy has seen great success in the treatment of numerous cancers, but GBM has not been among the immunotherapy success stories. Several candidate therapies spanning antibodies and vaccines have reached phase III trials but yielded only modest gains in terms of clinical endpoints and survival. These experiences have underscored that additional interventions may be needed to achieve robust immune activation, potent effector cell activity, and durable treatment response. Here, we describe some of the most advanced

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immunotherapies studied to date for GBM and the specific mechanisms of resistance that accompany each strategy.

4.2.1 Checkpoint inhibitors—Checkpoint inhibitors are among the most successful innovations in immunotherapy. They are monoclonal antibodies designed to interrupt binding of regulatory receptors on T cells. In the normal cell, downstream signaling by these activated checkpoint receptors exist to prevent excessive inflammation(275). However, tumor cells can also produce the corresponding ligand, thereby abrogating and eluding an immune response that would lead to tumor clearance. Hence, such receptors are sometimes considered markers of T cell exhaustion, as they are no longer able to mount an immune response despite their successful tumor infiltration(276). A number of these receptors and inhibitors have since been identified, with anti-CTLA4 (cytotoxic T-lymphocyte-associated protein 4), anti-PD-1(programmed cell death protein 1), and anti-PDL1 already being used in the clinic (Fig. 4).

For GBM, the greatest translational success has been seen with anti-PD-1 agents, nivolumab and pembrolizumab. When PD-1 binds to its ligand, there is downregulation of T cell activation(275). A trial from Dana-Farber Cancer Institute revealed patients who received pembrolizumab prior to surgery showed significant improvement in overall survival(281). However, in a Phase 3 clinical trial, CheckMate 143, nivolumab monotherapy failed to demonstrate an increase in overall survival versus bevacizumab (9.8 vs 10 months) for recurrent GBM(282). Additionally, monotherapy was found to be better tolerated than dual therapy with anti-CTLA4. Further findings in phase III trials, CheckMate 498 and CheckMate 548, also did not identify improvement in overall survival for standard therapy with and without anti-PD-1 in patients with MGMTmethylated GBM(283, 284).

Several checkpoint inhibitor-specific mechanisms of resistance have been described across cancers and may generalize for GBM. One transcriptome analysis of melanoma biopsies found an “innate PD-1 resistance gene signature” that could predict response to anti-PD-1(285). In this study, resistant tumors exhibited increased expression of genes regulating mesenchymal transition, cell adhesion, extracellular matrix remodeling, angiogenesis, and wound healing. Other work in a lung tumor model has shown that anti-inflammatory immune cells in the tumor microenvironment (TME) can decrease the efficacy of checkpoint inhibitors. For example, tumor-associated macrophages (TAMs) have been observed to remove anti-PD1 antibodies bound to CD8+ T cells (286). Additional preclinical studies on several cell lines have suggested that Treg apoptosis due to TME-related oxidative stress can generate adenosine release and anti-PD-L1 resistance(287).

Various mechanisms also prevent checkpoint inhibitors from generating a robust or durable T cell response. This is, in part, due to impaired epigenetics and memory cell formation, resulting in T cell exhaustion and immune evasion of the tumor(288, 289). Furthermore, several other checkpoint molecules may compensate for blocked PD-1 function(290). LAG3 is one example found on CD4, CD8, and Tregs. Like PD1, LAG3 normally inhibits T cell receptor activation and cytokine production(291). TIM3 is another promising checkpoint target, seen during the late stages of disease and T cell exhaustion. Binding to its ligand can lead to effector T cell apoptosis(292). Both LAG3 and TIM3 inhibitors are being assessed in

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combination with anti-PD1 in GBM Phase I clinical trials. (NCT02658981, NCT03961971). Given the complex interplay of checkpoint regulators, clinical trials have also begun to explore combination therapies.

4.2.2 Vaccines—Vaccines can be largely divided into peptide-based vaccines and cell-based vaccines. While the vehicles may differ, both ultimately require antigen presentation and T cell activation. Much of the early enthusiasm for cancer vaccines has since been redirected due to their limited success. Nevertheless, cancer vaccines have provided important insights on immune responses of GBM and are continuously investigated, especially in conjunction with multimodal treatment regimens.

4.2.2.1 Peptide-based vaccines: The most studied peptide-based vaccine, rindopepimut, targets the EGFRvIII variant. Wildtype EGFR of the ErbB family of kinase receptors is activated by EGF signal for cell proliferation, cell migration, and apoptosis inhibition(293). In approximately 20–30% of GBMs, EGFRvIII has a truncated extracellular domain, which prevents binding and leads to constitutive activation. The resulting amino acid sequence spanning across the deleted portion is tumor-specific, not found in any normal tissues(294). This specific epitope led to the development of rindopepimut (CDX-110-KLH), a 14 amino acid peptide, enhanced by the keyhole limpet hemocyanin carrier protein.

Rindopepimut underwent several early clinical trials, ultimately reaching a phase III trial (ACT IV) assessing vaccine efficacy in GBM patients with minimal residual disease. The control arm surpassed expectations and was statistically insignificant from rindopepimut, with a median OS of 20.0 and 20.1 months, respectively(295). The investigators had previously used historical controls during their phase I and II trials, which might have led to an overestimation of rindopepimut's efficacy. Reasons for subsequently noted lower performance of the vaccine in GBM included variable responses to human leukocyte antigen (HLA) haplotype and antigen escape (296). One study reported that 82% of treated GBMs did not express EGFRvIII at the time of tumor recurrence(297). Since then, one small phase II trial (ReACT) combined rindopepimut with bevacizumab for recurrent GBM and reported a small survival benefit of the experimental group (12 months) compared to the control group (8.8 months)(298).

4.2.2.2 Cell-based vaccines: Among cell-based vaccines, the DCVax is possibly the most prominent. In this strategy, dendritic cells (DCs) generated *ex vivo* from patient derived peripheral blood are pulsed with different sources of tumor-associated antigens (TAAs), such as autologous tumor lysates, antigen peptide, and TAA-encoding RNA. These naturally-derived, mature DCs showed stronger antigen presentation potential(299). Moreover, the DCVax enabled customized targeting of multiple tumor antigens for potentially durable response. This approach has been cautiously monitored for the theoretical risk of developing an immune response to normal tissue antigens(300). Multiple early clinical trials were able to identify GBM patients for safe DCVax application, culminating in a phase III study. Although the study has yet to be formally completed, median overall survival has been reported at 23.1 months(301).

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Several challenges unique to DCs have precluded the efficiency of cell-based vaccines. Interestingly, one group described that mature DCs in melanoma may be more motile and capable of migration to lymph nodes(302). Additionally, the immune suppressive environment can hinder a desirable DC-NK cross talk, whereby DC maturation leads to enhancement of NK cytotoxicity and IFN-y production. This has led to consideration of vaccine adjuvants such as, alpha-galactosylceramide or poly(I:C) to enhance NKT and DC activity in GBM treatment(303–305). Current clinical trials continue to incorporate these adjuvants while optimizing the antigen selection with personalized strategies. In addition, combination strategies with checkpoint inhibitors such as anti-PD-1 are also being considered at the preclinical phase in glioma(306).

4.2.3 Virotherapy—Over the past few decades, virotherapy has evolved from a primarily oncolytic to a broader viroimmunotherapeutic approach. Previously, treatment was designed to infiltrate the tumor, mitigate tumor defenses and induce a rapid, large-scale tumor cell death. Now, the focus of the field has shifted from direct oncolysis (payloads of virus) to immunostimulatory effects to induce long-lasting antitumor immune response (307). There are a variety of oncolytic viruses that can be armed with immunoregulatory inserts, like IL14, GMCSF, OX40 ligand or INFbeta to increase safety (308–311).The release of tumor associated antigens or damage-associated molecules can lead to durable and systemic effects, as evidenced in metastatic melanoma(312). Some parallels have been seen in GBM, where the application of an oncolytic adenovirus has led to CD8 effector T cell activation and downregulation of checkpoint inhibitors (313).

A challenge of therapy with oncolytic viruses is to achieve adequate replication of the virus in the tumor and tumor lysis. Viral vectors must avoid triggering an inflammatory response that would invite early elimination(314). Combination regimens have been proposed to avoid this premature viral clearance. Chemotherapies, radiation, and steroids are all post-operative steps that can temporarily suppress the innate immunity and support viral infection and dissemination(315, 316).

Several virotherapies have been investigated in clinical trials for the treatment of GBM, most notably vocimagene amiretrorepvec (Toca 511), which depends on a non-lytic strategy. In this model, a replicating retrovirus encoded with a cytosine deaminase is injected into the tumor bed with the goal to achieve preferential tumor cell infection over several weeks. At that time, the encoded virus converts a prodrug, Toca FC, to 5-fluorouracil which will in turn impair thymidylate synthase and cell replication(317). Several weeks later, when viral infection has been achieved, this prodrug-converting enzyme can convert valaciclovir into a nucleotide analogue and interrupt tumor cell replication(318). Early clinical trials reported a median overall survival of 13.6 months for recurrent or progressive GBM, which is promising when compared to historical controls. Additional viral studies have been performed with alternative vectors as adenovirus, poliovirus, cytomegalovirus and herpes virus(319). ASPECT is one such Phase III clinical trial for patients with operable high-grade glioma currently underway which assesses the use of an adenovirus encoded with herpes-simplexvirus thymidine kinase(320).

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4.2.4 Chimeric antigen receptor T (CAR T) cells—Treatment with CAR T cells involves collection of allogeneic T cells from peripheral blood, ex vivo genetic engineering of the cells to express receptors against specific tumor associated antigens, and adoptive transfer of the tumor-targeted T cells. CAR T cells can bind the tumor antigen without antigen processing and independent of HLA-mediated antigen presentation. Immune cell activation signals are derived from CD3 and co-stimulatory receptors, such as CD28 or TNFRSF9/4–1BB (321). Several early phase clinical trials with tumortargeted CAR-T cells have been completed, targeting various tumor antigens such as EGFRvIII, HER2, and IL13R α2(322–324) in GBM. It remains to be seen whether there are significant and durable survival benefits for patients with GBM(322, 323). Several other targets for GBM are also actively being evaluated including CD147, also known as extracellular matrix metalloproteinase inducer, as well as B7-H3, an immune checkpoint transmembrane protein overexpressed in GBM(325). (NCT04045847, NCT04385173)

As with other therapies, CAR T cell therapies are subject to many barriers affecting a robust T cell response such as adequate T cell infiltration and activation. Tumor plasticity, antigen loss and heterogeneity continuously counteract CAR T cell therapies(326). Decreased expression of the target antigen, presence of splice variants and epitope modifications after treatment have all been observed(327). Additionally, not all tumor cells express the targeted antigen at any given time, while current versions of CAR T cells have a limited receptor repertoire. Recent work has described the expansion into bispecific T cell engager (BiTE) and trivalent CAR T cells, enabling recognition of multiple tumor antigens. Unfortunately, immunosuppressive features of the tumor microenvironment, Tregs and TAMs can counteract the efficacy of CAR T cells or promote T cell exhaustion (328). Finally, intrinsic pathways can interrupt sustained therapeutic activity of CAR T cells, such as chronic antigen exposure and tonic CAR signaling, which can also lead to T cell exhaustion.

Overall, many approaches for immunotherapy of GBM rely on activated T cells. Unlike other solid malignancies, GBM treatment is uniquely challenged by its immune-privileged state(326). Intrinsic characteristics of tumor cells can block this activation, leading to a “cold” tumor(329). For example, continuous changes in the tumor cell’s mutational profile can lead to loss of neoantigens that are initial targets for therapeutic T-cells (330). This differs from many hematologic malignances which exhibit more clonal cell populations(331). Meanwhile, extrinsic mechanisms can also hinder immune cell infiltration and coordinate an immunosuppressive tumor microenvironment. This can contribute to tumor-promoting resident immune cell populations and secreted factors in the tissue space. While immunotherapy alone fails to provide significant survival benefits for brain cancer patients, accumulating evidence shows that adjuvant radiation can prime and activate immune cells to tumor-derived antigens, induce presentation of neo-antigens in the tumor, and improve immune-suppressive environment, all of which sensitize brain cancers to immunotherapies by converting the immune environment from “cold” to “hot”(278, 332) (Fig.5). We found that irradiation improves IL-8 secretion by tumors, thus, we constructed IL-8 receptor-modified CD70CAR T cells to migrate into the tumor and induce an enhanced antitumor response in GBM(333). Additionally, Murty et al. found concurrent

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irradiation improved intravenous adoptive T-cell administration in the treatment of GBM in a preclinical immunocompetent GBM model(334).

4.3 Nanotherapy

Conventional therapies for GBM can only marginally prolong the survival of patients with GBM. The vast majority of patients with GBM will die within 1–2 years after their diagnosis. Some challenges to successful treatment include the difficulty of complete tumor resection, the inefficient delivery of chemotherapeutic drugs, and the incomplete eradication of GICs. To overcome these obstacles, the application of nanoparticulate anti-GBM drugs has been proposed.

4.3.1 Passive tumor targeting: EPR effect—Nanoscale drug systems are based on polymeric micelles, liposomes, inorganic nanoparticles, nanotubes, or dendrimers(335, 336) (Fig.6) with attached or internalized chemotherapeutic drugs, sensitizers, or RNA(337). A limited permeability of the blood brain barrier (BBB) is the major obstacle in traditional GBM treatment. As a result of the angiogenesis process, the blood vessels in a tumor may develop a leaky endothelium which allows the entry of macromolecules, such as nanoparticles. After intravenous administration, nanoparticles diffuse into tumor tissue. While small molecular drugs extravasate more easily into tumors, they can also diffuse quickly back into the blood. By contrast, the large size of nanomaterials prevents diffusion of extravasated macromolecules back into the blood. This phenomenon is known as the enhanced permeability and retention (EPR) effect(338–340). The size of macromolecular drugs is critically important to achieve an ideal compromise between vascular extravasation and tumor retention. Xu et al. compared the tumor targeting efficiency of nanoparticles between 3 nm and 30 nm. The study showed that 3 nm nanoparticles exhibited much greater tumor targeting efficiency and penetration compared to the 30 nm nanoparticles(341). Bort et al developed ultrasmall polysiloxane-based nanoparticles with a hydrodynamic diameter of approximately 4 nm, which extravasated into rodent tumors and have recently been further developed towards a phase I clinical trial in patients with brain metastases(342).

4.3.2 Active tumor targeting—The EPR effect is highly dependent on the permeability of blood vessels within a tumor, to improve tumor targeting, nanoparticle carriers have been decorated with specific antibodies, peptides, or aptamers, which can be actively targeted to specific surface markers of tumor cells (Fig.6).

Nanocarriers can transport RNA inhibitors such as siRNAs, miRNAs and LncRNAs(343–347), can be targeted against specific surface markers such as Cx43, CD133, CD44, CD163, CD15, CD49f, CD90 or target signaling pathways such as Hedgehog, Notch, PI3K/Akt/PTEN/mTOR, cAMP-Epac, NF-κB, Jak/STAT(337, 348, 349). Specific tumor targeting is commonly associated with certain nanocarriers. PEG-modified liposomes allow the attachment of functional groups targeting tumor tissues and reduce uptake by the reticuloendothelial system (350). In one case, Pep-1-conjugated PEGylated nanoparticles loaded with paclitaxel (Pep-NP-PTX) improved anti-glioma efficacy with a median survival time of 32 days in mouse models, which was significantly longer than that of control mice treated with PTX-NP (23 days) and Taxol® (22 days)(351). Grafals-Ruiz et al developed

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gold-liposome nanoparticles conjugated with the brain targeting peptides apolipoprotein E (ApoE) and rabies virus glycoprotein (RVG). Spherical nucleic acids (SNAs)-Liposome-ApoE were able to cross the BBB and showed a high accumulation in brain tumor tissues. Furthermore, SNA-Liposome-ApoE specifically targeted a highly abundant miRNA (miR-92b), which is aberrantly overexpressed in GBM (352). Belhadj et al developed a multifunctional liposomal glioma-targeted drug delivery system (c(RGDyK)/pHA-LS) modified with cyclic RGD (c(RGDyK)) and p-hydroxybenzoic acid (pHA). In their study, c(RGDyK)/pHA-LS/DOX showed a median survival time of 35 days, which was 2.31-, 1.76- and 1.5-fold higher than that of LS/DOX, c(RGDyK)-LS/DOX, and pHA-LS/DOX, respectively(353).

4.3.3 Stimuli-responsive drug release—Although various delivery platforms, such as PEGylated nanocarriers, have been developed to prolong circulation and improve drug solubility, the delivery efficacy of most nanocarriers is still quite low(354). Thus, stimuli-responsive nanocarriers are rationally designed to deliver and release drugs by responding to internal stimuli, such as pH, hypoxia, reactive oxygen species, enzyme activity, etc., or external stimuli, including ultrasound, light (e.g., laser), temperature (i.e., thermal) and magnetic field, etc (336, 355) (Fig.6). can specifically delivery cargos to the tumor microenvironment.

GBMs are characterized by extensive tissue hypoxia and various nanocarriers have been designed to target hypoxic tumors (356). Liu et al developed hypoxia-responsive ionizable liposomes, which showed enhanced therapeutic efficacy of small interference RNA (siRNA) anticancer drugs when exposed to low pH and hypoxic tumor microenvironment in glioma(357). GBMs are known for their resistance to radiotherapy due to intratumoral hypoxia. Hua et al have designed angiopep-2-lipid-poly-(metronidazoles)n (ALP-(MIs)n) hypoxic radiosensitizer-polyprodrug nanoparticles (NPs), which enhanced the radiotherapy sensitivity of gliomas(358). Enzyme activity is another internal stimuli to be considered. We developed a cross-linked iron oxide nanoparticle conjugated to azademethylcolchicine (CLIO-ICT), which is specifically activated by the enzyme, matrix-metalloproteinase 14 (MMP-14). Since MMP-14 is highly expressed in GBM, but not in the normal brain or normal visceral organs, it provides cancer-specific therapy with little or no side effects. Upon-cleavage by MMP-14, CLIO-ICT releases azademethylcolchizine in the tumor tissue, which disrupted the tumor vasculature and killed tumor cells and GIC in GBM (359, 360). Our study showed that treatment with CLIO-ICT plus TMZ and irradiation lead to significantly improved tumor growth inhibition and overall survival of mice with GBM when compared to controls that received monotherapy. In addition, the iron core of CLIO-ICT enabled *in vivo* drug tracking with MR imaging, demonstrating in vivo tumor drug accummulation.

External stimuli also have been studied extensively to improve nanodrug delivery in GBM. Although the BBB is disrupted in GBM patients, overwhelming clinical evidence demonstrates the existence of an intact BBB in all GBM, which limit therapeutics delivery and efficacy (361). The combination of focused ultrasound (FUS)-BBB opening and externally applied magnetic field (MT) could deliver nanodrugs more effectively to deepseated GBM tumors(362, 363). Most recently, Curley et al. used

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MRI–FUS and microbubbles to deliver “brainpenetrating” nanoparticle (BPN), which yielded markedly augmented interstitial tumor flow and enhanced BPN penetration in GBM(364). Fan et al. developed boron-containing polyanion [polyethylene glycol-b-poly((closododecaboranyl)thiomethylstyrene) (PEG-b-PMBSH)] nanoparticles (295 ± 2.3 nm in aqueous media) and coupled them with microbubble-assisted FUS for GBM treatment. They found the combination may improve boron neutron capture therapy (BNCT) in GBM(365). Li et al. developed polysorbate 80- modified paclitaxel-loaded PLGA nanoparticles (PS- 80- PTX- NPs, PPNP), which showed significantly stronger anti-tumor efficacy in the tumor- bearing mice when combined with FUS(366). Furthermore, magnetic hyperthermiamediated cancer therapy (MHCT) has shown promising results in the preclinical studies. MHCT has applied magnetic nanoparticles to heat tumor tissues, which is based on an alternating magnetic field (AMF)(367, 368). Gupta et al. have developed manganese-doped magnetic nanoclusters for hyperthermia and photothermal GBM therapy, they observed oxidative stress produced in the cellular environment and confirmed the ROS-dependent apoptosis of GBM cells via the mitochondrial pathway(369). Mamani et al. found magnetic hyperthermia effectively decreased cell viability (about 20% and 100% after 10- and 30-minutes therapy) with a GBM 3D cells culture model(370).

4.3.4 Different administration routes for drug delivery—An intranasal route of drug administration can enable therapeutic compounds to reach GBM tumors in significantly higher doses than with intravenous administration. Sousa et al. developed BCZ-loaded PLGA nanoparticles and intranasally injected them into a GBM mouse model. The drug was found to be efficiently delivered to the brain with very limited off-target delivery to visceral organs, such as the lung and liver(371). Khan et al established nano-lipid chitosan hydrogel formulations for a nose-to-brain delivery of TMZ. After intranasal administration, TMZ release to GBM was 60% within 24 h and Wistar rats showed an increased drug targeting efficiency (DTE) of 326% and a direct transport percentage (DTP) of 93% compared to the intranasal administration of nano-lipid formulation as a control (DTE, 113.36% and DTP, 71.74%)(372). Similarly, the concomitant intranasal administration of chitosan nanoparticles attached with a Gal-1 siRNA enhanced the efficacy of anti-PD-1 antibodies with a median survival of 51.5 days, which is significantly higher than the control groups (17.5 and 30 days for untreated mice and anti-PD-1 antibodies alone, respectively)(373).

Intratumor administration can also be effective in cases of a solitary tumor which cannot be surgically removed. The injection can be carried out with a syringe or catheter at the tumor location or with the help of convection enhanced delivery (CED). CED is a technique which can precisely control the infusion rates of anti-GBM compound towards GBM tumors by maintaining a hydrostatic pressure gradient(374). CED was first introduced in 1994(375) and applied widely in clinical trials for the non-nano-formulated anti-cancer compounds(376–380). CED involves drilling a small burr hole into the skull and stereotactically positioning catheters to the interstitial space next to the tumor using image guidance. Once in position, the catheter can deliver many different kinds of therapeutic or imaging contrast agents to the site (e.g., small molecules, proteins, viruses, liposomes, nanoparticles, etc.)(381, 382). This technique ensures delivery to the tumor without needing to bypass the blood-brain barrier (BBB), but it failed mostly due to insufficient

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drug distribution(383). Later, it was tested with nano-formulated drugs and showed high retention time in tumors. Polymeric nanoparticles such as poly(lactide-coglycolide) (PLGA) nanoparticles were used for CED. CED of carboplatin nanoparticles conferred greater tumor cytotoxicity and reduced neuronal toxicity in rat and porcine models(374). Finbloom et al found that the CED of virus-like particles (VLPs) and nanophage filamentous rods modified with doxorubicin (DOX) required smaller doses than traditional intravenous routes to achieve comparable survival outcomes(384).

Several potential benefits of anti-GBM nano-drugs have been identified: 1) Improving surgical tumor removal. Most recently, fluorescence guided resection of GBM after intravenous administration of a fluorescent mini nanoimaging agent (NIA) or fluorescent silica coated iron oxide nanoparticles greatly improved the intraoperative delineation of GBM(385, 386). 2) Improving the efficacy of chemotherapy and reducing side effects. As described above, nano-scale drugs have a greater blood circulation half-life and can be predominantly delivered to the tumor via the EPR effect or active delivery systems. Targeting specific markers of tumor cells reduces toxicity to healthy cells and thus decreases side effects(339) 3) Enhancing the efficacy of radiotherapy. Recent studies have shown that nanodrugs that kill GICs can enhance the efficacy of radiotherapy(360, 387). Radiation therapy could, in turn, enhance nanotherapy by affecting the prevalence and polarization of tumor-associated macrophages(388). 4) The nanoparticle carrier can be designed to actively induce an immune response (389, 390). Our group showed that the iron oxide nanoparticle ferumoxytol induced pro-inflammatory macrophage polarization and thus inhibited tumor growth(391). Overall, nanotherapeutics have many advantages for treating GBM, which can enhance the efficacy of traditional therapy.

4.4 Miscellaneous therapies

4.4.1 Laser interstitial thermal therapy (LITT)—LITT is a method for delivering thermal energy to GBM using a stereotaxic device with the goal to ablate the tumor tissue. A small burr hole is drilled into the skull through which an optical fiber is threaded and guided to the center of the tumor. The fiber creates heat which burns and kills the tumor tissue. The heat generated can be monitored using MR-thermometry to ensure safe use and no harm to healthy adjacent tissue. Advantages of LITT over standard surgery include the minimally invasive approach and feasibility to treat inoperable tumors. This technology has been used with other types of brain lesions and was particularly useful for high grade gliomas(392). After treatment with LITT, patients with smaller tumors (<4 cm^3) demonstrated higher survival rates and fewer perioperative complications compared to patients with larger tumors (>4 cm^3)(392, 393). Despite promising results, side effects from LITT can include swelling and inflammation which can cause neurological side effects. Traditional surgery also leads to inflammation; however, the effect is mitigated since there is space left where tissue was removed. With LITT, the dead treated tissue is still present. There is still no clear guidance on who should receive this treatment, but it is a useful tool to supplement existing treatment strategies.

4.4.2 Tumor-treating fields (TTFields)—Tumor treating fields are a new treatment modality for GBM. It was approved by the FDA in 2011 for recurrent GBM and in 2015

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for newly diagnosed GBM. More recently, TTFields has been approved by the FDA for the treatment of patients with pleural malignant mesothelioma(394). The treatment requires the patient to wear a device on their shaved head that delivers low-intensity, intermediate frequency, alternating electric fields(395). The alternating electric fields produce antimitotic effects that are selective for rapidly dividing cells with the maximal benefit occurring when the electric fields are parallel to the axis of cell division(396). Given that cells in a tumor are turned in many different directions, the treatment is most effective when two or more orthogonal pairs of transducer arrays are used. One major benefit to TTFields is the limited toxicity and mild side effects (usually only skin irritation that can be managed with topical treatments). The greatest determinant of treatment success is patient compliance in wearing the device since the treatment is only working when the device is worn and the electric fields are “on”. This technology has been slow to gain traction in the clinic possibly due to the sparse scientific literature and variable compliance of patients to shave their head, wearing the device > 75% of the time, and carrying around a large battery pack. However, the benefits of TTFields are clear. In a phase III multi-center clinical trial, it was shown that in a population of nearly 700 patients, those that received TTField therapy in addition to TMZ had a significantly enhanced progression-free survival (~5 months) and significantly enhanced overall survival(397). Optimization of therapy will come from further examination of the mechanisms of actions of TTFields. For instance, it has been shown that TTFields can disrupt the membrane composition and permeability of cancer cells(398) and this could explain the synergistic effects of combination therapies of TTFields with chemotherapeutics such as Withaferin A (399).

5. Future therapeutic strategies

In 2005, when Stupp et al. demonstrated a major pharmaceutical progress for GBM management by combining radiotherapy and adjuvant TMZ therapy(5). Since then, there have been advances in understanding genetic drivers of tumor development and progression. Many of these molecular characteristics were included in the World Health Organization (WHO) 2016 updated classification of tumors of the central nervous system including *IDH* mutant/wildtype status, *1p/19q* deletions, *EGFR* amplification, and mutations in *TP53, PTEN, ATRX, and TERT* promoters(400). There have been improved 2- and 3- year survival rates, but 5-year survival rates remain poor(401). Advances in precision medicine, surgical techniques, and combination therapies will shape the future directions for GBM treatment.

5.1 Precision medicine

Ideally, a given patient’s brain tumor would be screened for specific biomarkers that could be paired with selective drugs. Only patients having high expression of the biomarker would receive a targeted drug. The treatment plan would likely change over time as the tumor evolves and would also look different for patients with different tumor characteristics. For example, O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation has already been shown to predict tumor response to TMZ therapy (402). Exosomes, extracellular vesicles, and microRNA assays are also being used as biomarkers for GBM (403–406). Intraoperatively, mass spectrometry (MS) can sensitively and specifically

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identify molecules in the tumor(407), aiding the surgeon in defining the tumor margin and determining the most effective systemic therapy.

GBM is marked by extensive inter- and intra-individual heterogeneity in driver mutations and molecular subtypes (408). Identification of new biomarkers is also important for treatment of recurrent GBM as 90% of druggable targets are differentially expressed in a recurrent tumor compared to the tumor at initial diagnosis(409). Patients may need to receive new therapies over the course of their disease. Le Rhun et al. provided an in depth summary of known GBM-specific biomarkers(410). Many GIC markers are also being explored, including CD133, CD44, CD15, CD70, S100A4, ALDH1A3, NANOG, OCT-4, SOX-2, and NESTIN(411–414).

Tools that aid discovery and understanding of biochemical pathways will likely include new genetic screening techniques(415, 416), next generation sequencing and epigenetic studies(417). In addition, machine learning algorithms and bioinformatics analyses of tumor omics data will enable correlations of complex multi-factorial clinical information with tailored treatment options(418, 419). Machine learning algorithms could be designed to predict responses to treatment and support treatment decisions(420). Furthermore, organoids gain more and more attention in recent years. Organoids possess greater predictive power compared to assays of single cell monolayers and can be used to test patient-specific therapies(421, 422). They are especially useful in situations where patient samples are difficult to obtain(423, 424). Some organoid models have even been shown to exhibit stage-specific neural development and yield pathology results similar to resected patient tumors(425).

Unfortunately, targeted therapies in clinical trials have had limited success thus far, partly due to the heterogeneity and plasticity of GBM but also due to practical limitations(193). A future clinical trial would likely have the following elements: i) strong evidence of anti-tumor activity in relevant pre-clinical models, ii) selection of patients with molecular enrichment for the drug target ideally with a non-invasive diagnostic technique, iii) a drug that by design or by delivery route accounts for the restrictive nature of the BBB, iv) inclusion of tissue collection or advanced molecular imaging *during* the treatment for analysis of pharmacodynamic markers, and v) close integration of advanced imaging technologies and treatment, including advanced imaging biomarkers for improved tumor detection, characterization and treatment monitoring.

5.2 Advances in surgical techniques

Given the importance of complete tumor resection toward positive patient outcomes, advances in surgical techniques and image guidance will be critical for the future of GBM treatment. LITT has shown efficacy in improving quality of life, cognition, and stabilization of Karnofsky Performance Status (KPS) score for patients suffering from radiation necrosis(426). Appropriate patient selection is important. LITT may not be appropriate for patients who are significantly functionally compromised(393). The development of novel fluorescent imaging agents and trelated equipment may improve the surgeon’s ability to see and remove the entire tumor. Only 5-aminolevulinic acid (5-ALA) is approved for intraoperative use in GBM resection(49, 427, 428). Unfortunately, the US was a full decade

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behind Europe in approving this clinical imaging agent(49). In addition, diffusion tensor imaging (DTI)-MRI has also shown promise to visually help locate important motor fibers which can be spared during tumor resection(429). In addition to improving tumor resection, emphasis should be placed equally on improving safety as new deficits after surgery can eliminate the survival benefits of surgery(430).

5.3 Combination therapies

Unfortunately, current combination therapies for the treatment of GBM are often rendered ineffective because the tumor can find redundant compensatory mechanisms. This was likely the reason why isolated studies that targeted PI3K/AKT/mTOR, p53/RB pathways, or EGFR gene amplification or mutation, have failed to improve outcomes(410). Therefore, to be successful, novel GBM therapies target distinct biological pathways and include novel methodologies such as electric field, viral, or cell-based therapeutic strategies. Tumor treating fields (TTFields) may be particularly suited for combination therapy(431). TTFields elicit a therapeutic effect by applying alternating electric fields that alter DNA repair, membrane permeability, and immunological responses(432).

Major considerations for combination therapies are the method of delivery, time of administration, and the order of therapy(433). Nanocarriers hold promise for delivery of multiple chemical therapeutics and/or a high payload to GBM tumors in a single dose. Nanoparticles are then often phagocytosed by tumor-associated macrophages which can create both limitations and opportunities (434). Encapsulating several agents in a single nanocarrier can deliver them together (435). For radiation therapy, fractionated doses are important for normal cell recovery and reoxygenation of acutely hypoxic tissues(436). In addition to DNA damage induced by radiation, the “abscopal effect” can prime and activate the innate immune system (437–439). The order of combination therapies also needs to be carefully evaluated. For example, a first therapy could be used to “prime” the tumor microenvironment and make it more receptive for a subsequent second therapy(440). “Priming” may include normalizing the vasculature, reducing stress associated with the large solid tumor mass, or degrading the extracellular matrix for better drug penetration (441).

6. Conclusion

GBM continues to be the most lethal tumor type with limited treatment options. Given the genomic complexity and multiple signaling pathways of GBM, a monotherapy will likely not be effective. An effective treatment approach for GBM will require combination therapies targeting distinct oncogenic pathways. A comprehensive understanding of the molecular biology of GBM, along with mechanisms of therapy resistance and effective integration of different therapy approaches will be key for the development of effective therapies. While histology and the identification of chromosomal deletions are important to estimate prognosis and plan targeted therapies, imaging can help in guiding surgeries and monitoring treatment response.

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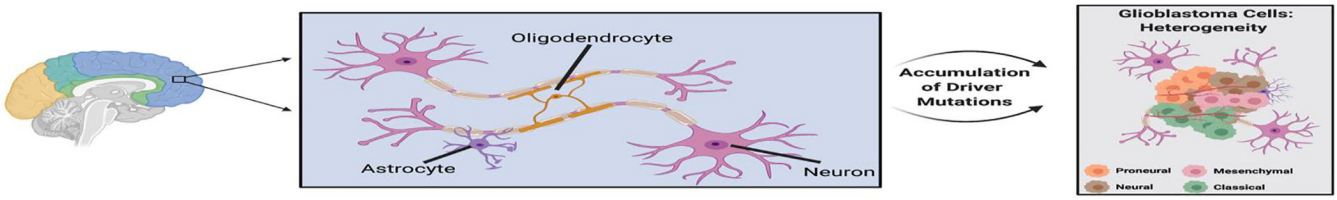

Fig.1.

Cellular origin and heterogeneity of glioblastoma multiform (GBM). GBM tumors originate from three types of cells in the brain parenchyma: neural stem cells (NSCs), NSC-derived astrocytes, and oligodendrocyte precursor cells (OPCs). GBM is characterized by extensive intertumor and intratumor heterogeneity, and has, therefore, been divided into four sub-groups: mesenchymal, classical, proneural, and neural.

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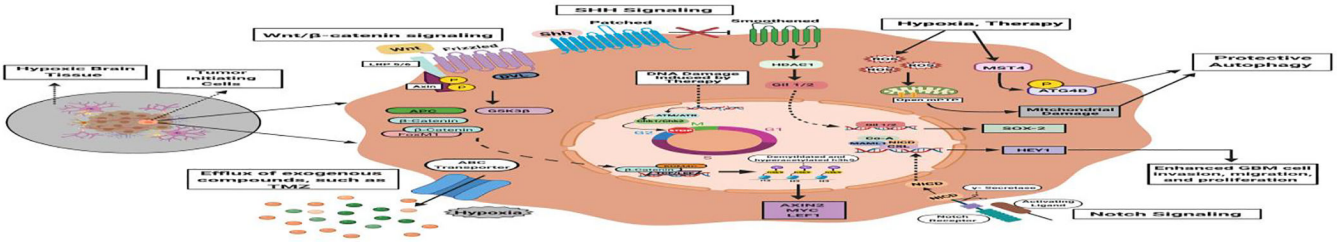

Fig. 2.

The molecular mechanisms of resistance in glioma initiating cells (GICs). GBM is characterized by extensive intratumoral hypoxia. GIC niches are most commonly to be found in tumor core regions that are lessoxygenated. GICs are generally resistant to therapies and mostly due to following mechanisms: 1) Enhanced DNA repair capacity. Cell cycle arrest at G2 phase in GICs allows the DNA repair and further enter mitotic phase. 2) GICs express higher level of ABC transporters which promote efflux of therapeutic compounds. 3) The poorly oxygenated tumor tissue creates perfect GIC niches, which induce autophagy to maintain cellular homeostasis. Protective autophagy can also be triggered in GICs when challenged by cytotoxic therapies. 4) Epigenetic modifications contribute to functional heterogeneity and maintenance of GIC hierarchies. Multiple GICs-related signaling pathways (Wnt/β-catenin, Sonic Hedgehog (SHH), Notch) can be epigenetically regulated to gain self-renewing capabilities and drug resistance properties(179, 191, 192).

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Fig. 3.
New therapies described in this review.

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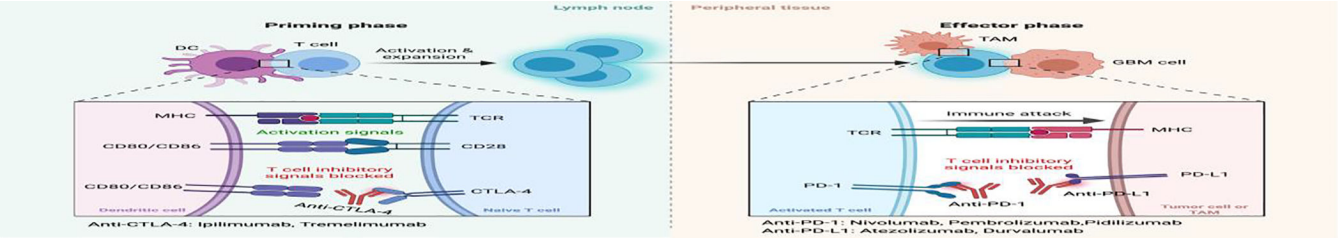

Fig.4.

The inhibition of CTLA-4 and PD-1 in GBM immunotherapy. Dendritic Cells (DCs) traffic between CNS tumors and the cervical lymph nodes to prime T cells against tumor neo-antigens. T cells receive effective activation signals with the engagement of two T cell receptors, antigen-specific T-cell receptor (TCR) and CD28, simultaneously. TCR binds to tumor-associated antigens (TAA) presented on major histocompatibility complex (MHC) molecule while CD28 interact with CD80/CD86(B7–1/2 receptors) costimulatory molecules on the surface of DCs. T cell activation leads to upregulation of checkpoint molecule CTLA-4(cytotoxic Tlymphocyte-associated protein 4). The interaction of CTLA-4 and CD80/CD86 of DCs results in blockage of T cell activation. The effector T cells can proliferate and migrate to the tumor microenvironment, leading to tumor eradication via MHC-I/TCR interaction. Some GBM cells and TAM-BMDMs express high levels of checkpoint molecules including transmembrane protein PD-L1. PD-L1 binds to PD-1 receptors on T cells, which leads to attenuation of TCR and CD28 signals, and subsequently promotes T cell apoptosis and functional exhaustion. Cytotoxic T cell responses are further inhibited by immune-suppressive cytokines by Tregs, astrocytes and neurons(277, 278). Immune checkpoint blockade (ICB) is based on a range of monoclonal antibody-based therapies, especially checkpoint inhibitors that block CTLA-4, PD-1, or PD-L1. Anti-CTLA-4 antibodies restore T cell activation in the lymph nodes, and PD-1/PD-L1 antibodies enhance the functional properties of effector T cells at the tumor site(279, 280). TAM: tumor-associated macrophage, BMDMs: bone marrow–derived macrophages, TCR: antigen-specific T-cell receptor.

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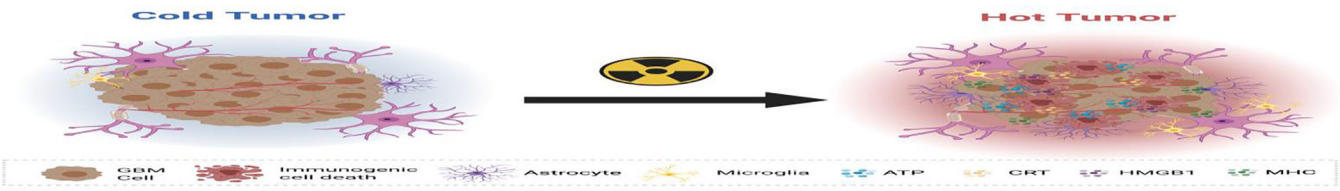

Fig.5.

Immune modulatory effects of radiotherapy. The localized cytotoxic effects of radiation have been shown to cause immunogenic cell death (ICD). Radiation can induce all three arms of ICD: upregulation of the release of adenosine triphosphate (ATP), the extracellular release of “danger signal” high motility group box 1 (HMGB1) and translocation of “eat me” signal calreticulin (CRT) to the cell surface. Additionally, radiation regimens unmask the tumor by upregulating major histocompatibility complex (MHC) on the tumor cell, which enhances neo-antigen presentation in tumor cells for recognition by cytotoxic T-cells. Besides the effects on tumor cells, irradiation also affects tumor-associated stromal cells, such as reactive astrocytes, and the recruitment of microglia, which further contribute to the establishment of radiation-induced immune responses.

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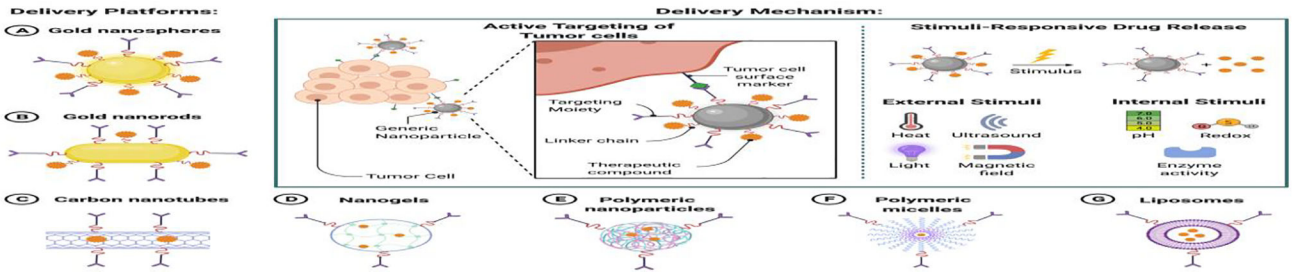

Fig.6.

Advanced delivery platforms and delivery mechanisms of nanoparticulate anti-GBM drugs. Nanocarriers have been proposed based on various materials and principles as shown in the figure (a-g). Ligand-installed nanocarriers achieve therapeutic effects by actively targeting the surface marker or signaling pathway of cancer cells. Stimuli-responsive nanocarriers can release drug by responding to internal/external stimuli, which enable specific delivery cargos into the tumor microenvironment.

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Table 1.

Summary of therapeutic strategies to treat GBM.

Treatment	Biological Action	Notes
Image-guided Surgery		
Intraoperative ultrasonography	tumor removal	Routine clinical use. Used in ORs for maximal safe resection of brain tumors since the 1980s.
Intraoperative MRI	tumor removal	Routine clinical use. The first iMRI system was installed in 1994 at Brigham and Women's Hospital.
Intraoperative fluorescence imaging	tumor removal	New clinical use. In 2017, Gleolan® (5-aminolevulinic acid) was FDA-approved for intraoperative fluorescence imaging.
Chemotherapy (small Molecules)		
Temozolomide	alkylates DNA base pairs	Routine clinical use. Approved by the FDA in 1999 as a monotherapy and again in 2005 for use in newly diagnosed GBM concomitantly with radiotherapy and as maintenance treatment.
Carmustine (BCNU)	alkylates DNA base pairs	Routine clinical use. Gliadel® wafers were approved by the FDA for recurrent GBM in 1997 and for the newly diagnosed high-grade gliomas (III and IV) in 2003.
Lomustine (CCNU)	alkylates DNA base pairs	Routine clinical use. FDA approved in 2014 for patients with brain tumors following surgery and/or radiotherapy.
Fotemustine	alkylates DNA base pairs	Approved in Europe but not in the US. Phase II clinical trials have shown therapeutic benefit in recurrent GBM.
Radiation therapy (RT)		
2D conventional RT	creates DNA double-strand breaks and ROS	Routine clinical use. Largely being phased out for brain RT. Still used in some instances of uncomplicated bone metastases.
3D conformal RT	creates DNA double-strand breaks and ROS	Routine clinical use. Good for advanced and inoperable tumors; used post-operatively.
Intensity-modulated RT	creates DNA double-strand breaks and ROS	Routine clinical use as adjuvant therapy after surgical tumor resection.
Stereotactic radiosurgery (SRS)	creates DNA double-strand breaks and ROS	Routine clinical use with either a gamma emitter (e.g., Gamma Knife) or a linear accelerator (e.g., Cyber Knife).
Brachytherapy	creates DNA double-strand breaks and ROS	Clinical adoption is slow due to adverse events and risk of exposure to people in close proximity to the patient.
Particle RT (Proton therapy)	creates DNA double-strand breaks and ROS	FDA approved but reimbursement for the procedure is low. Phase II clinical trials are underway to evaluate the efficacy of proton versus photon irradiation (NCT02179086, NCT01854554).
Inhibitor Therapy		
Bevacizumab (mAb)	inhibits VEGF-A	Routine clinical use. FDA approved for recurrent GBM in 2009.
Irinotecan (CPT-11) (small molecule)	inhibits topoisomerase I	Phase I/II trials for recurrent GBM showed mixed results. Phase II clinical trials for combination therapies are under way for recurrent and pediatric GBM (NCT04267978, NCT02192359).
Veliparib (ABT-888) (small molecule)	inhibits PARP	Phase II trials in adults with recurrent (NCT01026493) and new (NCT00770471, NCT02152982) GBM showed limited benefit. A phase I/II study of veliparib with RT and TMZ in children with diffuse intrinsic pontine glioma reported little to no survival benefit (NCT01514201).
Olaparib (AZD-2281, MK-7339) (small molecule)	inhibits PARP	Phase II trials in recurrent GBM are ongoing (NCT03212274). Olaparib exacerbated hematological toxicities when used with TMZ in patients with recurrent GBM (NCT01390571). Further studies are warranted to understand potential clinical benefit.
Niraparib (MK-4827) (small molecule)	inhibits PARP	A phase I trial evaluated niraparib and TMZ in advanced cancer but with few GBM patients (NCT01294735). A phase II trial evaluating niraparib with TTFs in recurrent GBM is underway (NCT04221503). May be good treatment option for tumors over-expressing EGFR.

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Treatment	Biological Action	Notes
Pamiparib (BGB-290) (small molecule)	inhibits PARP	Phase I/II trials are underway studying pamiparib with TMZ in new and recurrent GBM (NCT03914742, NCT03150862).
Cediranib (AZD-2171) (small molecule)	inhibits VEGFR	Phase II-III trials in recurrent GBM showed little to no benefit. Some studies may be underpowered or lack proper patient selection (NCT00777153, NCT01310855, NCT00305656). A phase II trial is comparing cediranib and olaparib to bevacizumab for recurrent GBM (NCT02974621).
Gossypol (AT-101) (small molecule)	binds with and inhibits Bcl-2, Bcl-xL and Mcl-1	Phase II trials were performed in recurrent and new GBM (NCT00390403, NCT00540722). Very little follow-up data exists.
Cabozantinimb (XL-184) (small molecule)	tyrosine kinase inhibitor	Phase II trial in adult patients with recurrent GBM showed modest clinical activity (NCT00704288). Phase II trial in pediatric patients with recurrent or progressive high grade gliomas is ongoing (NCT02885324).
Erlotinib	EGFR inhibitor	Phase II studies in recurrent GBM as monotherapy (NCT00337883,) and in combination with other therapies (NCT00039494, NCT00445588, NCT00525525 NCT00335764). Also evaluated in new GBM (NCT00187486, NCT00720356). Clinical results have not confirmed benefit.
Gefitinib	EGFR inhibitor	Phase II trials in new and recurrent GBM for both adult and pediatric patients (NCT00052208, NCT00014170, NCT00025675, NCT00042991).
Depatuxizumab mafodotin (ABT-414)	Ab targets EGFR and drug inhibits tubulin	Phase III trial was halted when no survival benefit for new GBM patients could be demonstrated over placebo plus TMZ/radiation (NCT02573324).
Imatinib	multi-targeted tyrosine kinase inhibitor	Phase II trials in new and recurrent GBM showed no clinical activity. Drug has poor BBB penetration.
Dasatinib	multi-targeted tyrosine kinase inhibitor	Phase I/II clinical trials in recurrent GBM failed to show treatment efficacy and have been limited by toxicity, especially when used in combination with a second chemotherapy (NCT00948389, NCT00423735).
Sorafenib	multi-targeted protein kinase inhibitor	Clinical trials to date have not been promising. Ongoing phase I/II trial evaluating sorafenib + everolimus (NCT01434602) and sorafenib, valproic acid, and sildenafil (NCT01817751) in recurrent high grade gliomas.
Sunitinib	multi-targeted protein kinase inhibitor	Several phase II clinical trials have not shown anti-glioma effects. There are no ongoing clinical trials known currently.
Temsirolimus (CCI-779)	inhibits mTOR	Phase I/II trials as a mono- and co-therapy mostly for recurrent GBM (NCT00329719, NCT00112736, NCT00022724). Little added benefit.
Everolimus	inhibits mTOR	Underway: Phase II trial evaluating combination with sorafenib (NCT01434602), Phase I trial evaluating combination with ribociclib in children (NCT03355794).
Liposomes		
2B3–101 PEGylated liposomes	Target GSH/GSH transporters	Phase I/IIa trial to explore the preliminary antitumor activity of 2B3-101 in brain metastases or recurrent malignant glioma (NCT01386580).
SGT-53 Cationic liposomes	Target Scfv/TfR	Phase II trial of combined temozolomide and SGT-53 for treatment of recurrent glioblastoma (NCT02340156).
Liposomal irinotecan	convection enhanced delivery (CED)	Phase II trial of convection-enhanced, image-assisted delivery of liposomal-irinotecan in recurrent high grade glioma (NCT02022644).
Immunotherapy		
Cemiplimab	checkpoint inhibitor that binds to PD-1	Phase II trials are underway for recurrent (NCT04006119) and newly diagnosed (NCT03491683) GBM.
Nivolumab	checkpoint inhibitor that binds to PD-1	Phase III trials are underway for recurrent (NCT02017717) and newly diagnosed (NCT02617589, NCT02667587) GBM.
Rindopepimut peptide vaccine	targets EGFR deletion mutation EGFRvIII	Phase III clinical trial is ongoing (NCT01480479).
DCVax®-L	DCs are primed to recognize tumor-specific antigens	Ongoing phase III clinical trial (NCT00045968).
VB-111 (Ofranergene obadenovec) gene therapy	Virus carries a trans-gene for chimeric death receptor	Phase I/II trials showed statistically significant improvement for VB-111 monotherapy in recurrent GBM (NCT01260506). Phase III trial using dual

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Treatment	Biological Action	Notes
using an adenovirus type 5 vector	that connects Fas to hTNF receptor 1.	administration of VB-111 and bevacizumab failed to improve outcomes in recurrent GBM. (NCT02511405).
CAR T cell therapy	T cells are engineered to express receptors against specific tumor markers	There are currently 19 clinical trials listed under clinicaltrials.gov, including ongoing studies NCT04385173, NCT04077866, NCT04045847, NCT04045847, NCT04214392, NCT04003649, NCT02937844, NCT03392545, NCT02208362, NCT0338923
Misc. Therapies		
Laser interstitial thermal therapy (LITT)	thermal ablation of tumor tissue	Studied for its applications toward tumor therapy and treatment of radiation necrosis. Current clinical trials: NCT02970448 (Phase I: LITT + chemoradiation for new HGGs), NCT03341806 (Phase 1: LITT + avelumab for recurrent GBM), NCT04699773/ NCT04181684 (LITT + hypofractionated RT for new/recurrent GBM)
Tumor Treating Fields (TTF)	disrupts mitotic cell division	May be good for recurrent GBM, inoperable tumors, and/or effective supplement to chemo/radiotherapy