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Molecular Targeting of Glioblastoma: Drug Discovery and Therapies

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Abstract

Despite advances of treatment for glioblastoma multiforme (GBM), patient prognosis remains poor. Although there is growing evidence that molecular targeting may translate into better survival for GBM, current clinical data show limited impact on survival. Recent progress in GBM genomics implicate several activated pathways and numerous mutated genes. This molecular diversity may partially explain therapeutic resistance, and several approaches have been postulated to target molecular changes. Furthermore, most drugs are unable to reach effective concentrations within the tumor due to elevated intratumoral pressure, restrictive vasculature and other limiting factors. Here we describe the preclinical and clinical developments in treatment strategies of GBM. We review the current clinical trials for GBM and discuss the challenges and future directions of targeted therapies.

Keywords

Glioblastoma; Molecular Targeting; Drug Discovery; Animal Models; Receptor Tyrosine Kinase; Anti-angiogenesis

An Overview of GBM and Treatments

In the United States, the majority of the estimated 20,000 newly diagnosed primary brain tumors annually are gliomas [1], which are named according to the cell type they most closely resemble and likely originated from. The main histological subtypes of gliomas include astrocytomas, oligodendrogliomas and ependymomas. Astrocytomas are graded from I to IV, with grade I and II as slow growing astrocytomas, grade III as anaplastic astrocytomas and grade IV consisting of glioblastoma multiforme (GBM) - the most common (65%) and malignant form of brain tumors. The prognosis of GBM patients is very poor, largely due to early invasion into the central nervous system, making a surgical cure nearly impossible.

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Only 10 % of those patients including all post-treatment living conditions survive 5 years after diagnosis [1, 4], despite continuous improvement of GBM therapy that currently consists of surgical resection, concurrent or sequential chemo-radiotherapy with temozolomide (TMZ) as the current established first line regimen [1].

GBM is typically characterized by complex chromosome abnormalities and extensive intratumour cytogenetic and histological heterogeneity. Indeed, cytogenetically related or unrelated clones coexist in different regions within the same GBM sample [2, 3]. For example, amplification/overexpression of EGFR and EGFRvIII mutation can be found in scattered cell populations in the same GBM specimen [5, 6]. In addition to the heterogeneity at genomic level, GBMs also present diversely differentiated tumor cells that may have been originated from the cancer stem cells population, which are multipotent and self-renewal immature tumor cells [8-10].

DNA-damaging drugs such as irinotecan (topoisomerase 1 inhibitor), etoposide (topoisomerase 2 inhibitor), doxorubicin (DNA-intercalating agent), BCNU and TMZ (DNA-alkylating agents), have been frequently used or tried for GBM [1]. The lack of clinical success with traditional DNA-damaging chemotherapeutics in systemic use has led to the investigation of targeted therapy that are directed against certain tumoral features including tumor-specific markers, altered signaling or metabolic pathways, tumor vessels and microenvironment. Encouraged by the breakthrough of targeted agents such as imatinib (Gleevec) inhibiting the translocated Abl tyrosine kinase in chronic myelogenous leukemia (CML) [7], multiple concepts were proposed to incorporate targeted agents in GBM treatment. The success of targeted therapy entails several key steps, such as target identification, developing or identifying a small molecule or antibody against the target, relevant preclinical studies and ultimately, clinical trials. This is unfortunately a lengthy and expensive process and compared to other tumors, GBMs are less common, making them less attractive to for-profit entities [11]. In this article, we review some of the steps leading to the drug discovery and development for the treatment of GBM.

Drug Discovery and Preclinical Development

Identification of Targets in GBM

A genome-wide analysis of over 20,000 genes from 22 GBM tumor genomes identified most mutations that likely drive glioblastoma formation [12]. These DNA alterations were point mutations, small insertions/deletions and larger copy-number changes (genomic amplifications and deletions). It is likely that these represent the most common alterations that drive tumor formation. Analysis of the genes mutated showed that they clustered into pathways that drive cell growth, cell cycle control (such as p53 checkpoint) and other key pathways.

DNA alterations drive cancer formation, and their effect is often reflected in changed patterns of gene transcription. There are many ways to comprehensively assay mRNA levels, one of which is serial analysis of gene expression (SAGE), a technique to produce a snapshot of the messenger RNA population in a sample in the form of short sequence tags that correspond to fragments of those transcripts [13]. SAGE has provided the basis of a

widely used online public database, providing a comprehensive and convenient way to obtain expression data and cross-compare gene expression levels in different tumor samples or cell lines of brain tumors and other cancers to normal tissue [14] (http://cgap.nci.nih.gov/SAGE/AnatomicViewer).

The alterations involved in GBM development and growth point to the involvement of several key pathways (Figure 1): 1) the PI3K-Akt and RAS oncogenic pathway with EGFR/PI3K/PTEN/NF1/RAS alterations, 2) the TP53 tumor suppressor pathway with TP53/MDM2/MDM4/$p14^{ARF}$ alterations, 3) RB and cell cycle regulators with RB1/ CDK4/$p16^{INK4A}$/CDKN2B alterations, and 4) the recently discovered metabolic pathway featured by IDH1/IDH2 alterations found in low grade astrocytomas (astrocytoma grade I and II) and secondary GBMs [12, 15, 16].

Further potential targeting areas include DNA repair mechanisms, tumor hypoxia, tumor invasion regulation and microenvironments. The main role of poly ADP ribose polymerase (PARP) family proteins is to detect and signal single strand DNA breaks to the repair mechanism. PARP inhibitors can be used as monotherapy in tumor genetic backgrounds deficient in specific DNA repair pathways such as BCRA 1 and 2 [17]. Frequently, PARP inhibitors have been tried in combination with DNA-damaging agents, like TMZ, topoisomerase inhibitors and radiation that induce PARP-1 activity for the repair of DNA damage, and thereby sensitize tumor cells to these agents [17]. Veliparib and BSI 201 are small molecule PARP inhibitors currently in clinical trials in combination with TMZ for primary GBM (Table 1).

Tumor hypoxia can provide another highly interesting subject of therapies. Due to limited vasculature, most solid tumors including GBM form intratumoral necrosis with hypoxic condition. This induces either directly or indirectly activation of hypoxia-responding transcription factors, and changes the tumor biology and its microenvironment, leading to increased aggressiveness and the feared resistance to chemotherapy and radiation [18]. In GBM, targeting of hypoxia/necrosis has not been established yet, however, potential targets include the various hypoxia-regulated molecules, among them hypoxia inducible factor-1 (HIF-1), carbonic anhydrase IX and glucose transporter 1 [18].

Although GBM does usually not metastasize to the other organs, it invades into the brain tissue in a highly aggressive manner (Figure 3). Manipulation of microenvironment is required for growth and invasion, and a number of factors related to this can be subjects of GBM therapies. Transforming growth factor β (TGF-β) is a critical component of the GBM microenvironment that drives tumor cells toward more aggressive behaviors and supports their survival while simultaneously limiting suppression by the host [19]. GBM invasion is also promoted by the tumor hypoxia and HIF-1, which upregulates a variety of genes whose products play a well-established role in GBM invasion that include CXCR4, stromal derived factor-1 (SDF-1), VEGF, and matrix metalloproteinase (MMP) [19].

Small Molecule Drugs

In cancer drug development, surface molecules such as receptors are relatively accessible for targeting. Many therapeutic approaches are aimed at EGFR that is overexpressed or

amplified in GBM as well as its variant, EGFRVIII, which is a re-arranged and constitutively activated form of EGFR [20]. Protein kinases including the intracellular kinase domains of growth receptors such as EGFR and PDGFR, are suitable for small molecule inhibitors and have been subject of most anti-GBM drug screening efforts [21]. Most common chemical compounds targeting protein kinases have affinity for ATP binding sites or allosteric sites. Novel kinase inhibitors are typically developed by a combination of methods, including high-throughput screening based on biochemical or cellular assays, *in silico* structure-guided design, analogue synthesis and fragment expansion.

For intracranial tumors, sufficient tumor drug delivery perhaps presents more of a challenge than with other tumors. Several parameters need to be considered including a possibly elevated intratumoral pressure, reduced blood flow to regions of the tumor, a normal or abnormal blood-brain barrier formed by the tumor endothelial cells.

In contrast to the intact blood brain barrier and the close to normal capillaries in the low grade astrocytomas, the blood-brain tumor barrier is usually more permeable in high grade malignant brain tumors. This increased permeability is due to the anatomic defects between the endothelial cells and the tumor barrier in the new tumor microvasculature induced by vascular endothelial growth factor (VEGF), which allow for the transvascular passage of larger molecules with the pore size ranging from 7 to 100 nm depending on the models and studies [22, 23]. Some orthotopic GBM rodent models such as RG-2 and D54 can form highly fenestrated tumor capillaries that measured up to 1 μm [24]. The altered barrier in high grade gliomas not only allows more effective use of small molecule drugs, but it also enables or enhances the delivery of macromolecules, such as nanoparticles and liposomes into the brain tumors. By a so-called enhanced permeability and retention (EPR) effect, tumor vasculature allows macromolecules leaking from the fenestrated vessels to accumulate in the interstitial space in tumor tissues [25].

Drug Screening Assays

Once a molecular target is selected, an *in vitro* enzymatic assay can be setup against a pool of drug candidates or small molecule library, to directly assess the impact of the compounds to the target protein. A hit determined by an *in vitro* screening may display specificity to the target, but its general cellular toxicity cannot be determined. An alternative approach is to use a cell-based assay, such as an isogenic cell screening system, in which the response to small molecules is compared between a cancer cell line with a mutation and a matched cell line without that mutation [26]. Isogenic screening of cell lines with EGFR/PI3K/AKT/PTEN pathway mutations provides a model of this strategy. For example, DLD1 isogenic cell lines carrying wildtype PI3K-CA or a gain-of-function mutation of PIK3-CA were first created by homologous recombination [27], and assayed with a panel of PI3K/AKT inhibitors [28]. A specific AKT1 inhibitor A-443654 was found to selectively inhibit the cell line containing the PI3K-CA mutation and subsequent *in vivo* studies confirmed its efficacy. In another study, D54 GBM cell line was overexpressed with EGFRVIII and transfected with yellow fluorescence protein (YFP), while the parental D54 cells expressing blue fluorescence protein (BFP) served as the reference cell line [29]. D54-EGFRVIII-YFP and D54-BFP cells were mixed and screened against a NCI small molecule diversity library, and the selective

suppression of D54-EGFRVIII-YFP cells was observed with compounds that preferentially targeted on tumor cells harboring the EGFRVIII-induced pathway. Cell-based screening can also be used to find small molecules that target cancer cells with loss of tumor suppressors. In a study of TP53 inactivation in colon cancer cells, isogenic cell lines that differed in TP53 status were exposed to ionizing radiation [30]. Gene-expression analysis revealed a consistent upregulation of polo-like kinase 1 (PLK1) controlling the G2/M transition in the cells whose TP53 genes were inactivated, compared with those with WT TP53 genes. It was subsequently determined that the PLK1 inhibitor BI-2536 specifically inhibited the cells with TP53 inactivation [30]. Similar concepts can be applied to targets not only in aberrant oncogenic pathways, but also in cell populations involved in glioblastomas, such as the tumor stem cells. For example, recent studies used a primitive neural stem cell line as the matched normal cell line against glioblastoma stem cell lines in screening with chemical compounds, to achieve selected killing of tumor stem cells [31].

Preclinical Animal Models

Preclinical animal studies are the make-or-break point in the process of finding innovative drugs for GBM therapy. One major *in vivo* screening strategy involves transplanting various rodent tumor cell lines into the appropriate immunocompetent host (syngeneic) to provide an accurate picture of potential immune responses. Among the various tumor implantation sites (e.g. orthotopic, flank) orthotopic models (i.e. intracranial brain tumor models) offer the most realistic setting to assess the drug delivery aspects because they match the microenvironment in the brain to grow a histologically accurate GBM and offer a realistic tumor vasculature with the blood-brain tumor barrier. However, the use of tumor cell lines in *in vitro* drug testing with the constant selective pressure raises concerns that cells used for transplantation experiments do not represent the heterogeneity of the original tumor. An interesting and highly relevant animal model is therefore the spontaneous high grade gliomas that occur in certain dog breeds such as Boxers and Boston terriers. These breeds are predisposed to develop spontaneous GBMs that closely resemble the pathology of human tumors and can sometimes be used as clinical trial subjects with pet owner consent [32, 33]. However, the adverse factors including the cost, availability and reproducibility make this biologically attractive large animal model a rare choice.

As with most cancers various rodent models remain the most common for brain tumor studies. Compared to mice, rats have larger size of brain (~ 1200mg vs ~ 400mg), which can therefore grow larger brain tumors and allow for more precise stereotactic implantation, facilitating therapeutic and monitoring procedures such as convection enhanced delivery (CED) and micro-dialysis. On the other hand, rats incur more expenses in purchase and maintenance, and increase the cost of drug use due to the approximate 10 times body weight compared to mice (~250 mg vs ~25 mg). Genetic engineering of mouse glioma models has been an ever expanding field promoted by improved understanding of the underlining genetic disorders of the disease [34, 35]. However, considering the genetic and histological heterogeneity that a human GBM displays, our laboratory often chooses syngeneic models induced by mutagens that maintain the genetic complexity and xenograft models with human tumor cells.

The F98 glioma syngeneic model and another similar RG2 glioma model were produced by i.v. administration of N-ethyl-N-nitrosourea (ENU), a highly potent mutagen, to a pregnant Fischer 344 rat [36]. Similar to human GBM, F98 cells carry the deletion of $p16^{INK4A}$ and overexpress PDGF-beta and Ras along with elevated levels of EGFR, cyclin D1 and D2 compared to rat astrocytes as the reference non-tumor population [37, 38]. A fully grown intracerebral F98 tumor shows mixed population of spindle-shaped cells with hemorrhage and necrosis, and displays a highly invasive growth pattern (Fig. 3c) with very low immunogenicity [39, 40]. F98 is also refractory to a number of systemic chemotherapies including paclitaxel and carboplatin and poorly responsive to irradiation alone, suggesting that it closely represents some of the challenges in treating high grade gliomas [36].

The C6 glioma model was produced by administering the mutagen N-nitroso-N-methylurea (MNU) to outbred Wistar rats [41]. C6 cells carry the deletion of $p16^{INK4A}$ with no expression of p16 and p19ARF mRNA and also have increased expression of PDGF-beta, IGF-1, EGFR and Erb3/Her3 [37, 42]. Since produced in outbred Wistar rats and no truly syngeneic host can be found, C6 glioma turned out to be immunogenic in Wistar and other rats and is seriously limited in survival studies [43].

9L gliosarcoma was produced in Fischer 344 rats by i.v. injection of MNU [44]. It has a mutated TP53 gene and elevated EGFR expression [38, 45]. Intracerebral implantation of 9L cells gives rise to rapidly growing tumors with spindle-shaped cells of a sarcomatoid (sarcoma-like) appearance, while the tumor margins are sharply delineated with little invasion into the surrounding brain tissues [36]. 9L gliosarcoma is responsive to radiation and a number of immuno- and chemotherapies, and can be highly immunogenic as revealed by animals immunized by X-irradiated 9L cells, which became subsequently resistant to tumor challenge [36].

GL261 mouse glioma was induced originally by intracranial injection of 3-methylcholantrene into C57BL/6 mice [46]. Another syngeneic mouse glioma of C57BL/6 host, GL26, showed similar overall characteristics and growth patterns [46]. GL261 cells possess a homozygous TP53 mutation, elevated c-myc and no detectable MHCII expression [47]. They are moderately immunogenic as revealed by vaccination experiments with irradiated GL261 cells, in which the simultaneous and post-implantational vaccination did not impair the tumor challenge, while the pre-implantational vaccination did [47]. Intracranial GL261 tumors showed rapid growth and invasive growth pattern (Fig. 2a), and were limitedly responsive to radiation alone [47]. This model has been frequently used in tumor vaccination and immune therapy studies and is a valuable platform given the limited number of available syngeneic mouse glioma models.

In the 1990s, human tumor cell lines entered the field for large scale drug screening. Therefore, xenograft models were routinely employed by implanting and growing a human GBM sample or cell line into the brain of an athymic nude mouse or SCID mouse. Human GBMs can be serially passaged as mouse xenograft in the brain or flank without being subjected to artificial cultural selection, thereby preserving GBM properties such as *EGFR* amplification and CD133-expressing population [48, 49]. Xenograft models of traditional GBM cell lines, grown in serum-containing media, have been used in brain tumor studies.

One example is U87MG, which is used for preclinical tests due to its reliable tumor intake and narrow survival window. However, U87MG and other similar traditional GBM cell lines, form homogeneous and less invasive bulky tumor masses that do not resemble GBM histology and are perfused by leaky vessels, rendering them more accessible for systemic drug delivery than invasive human GBM cells [35]. In contrast, neural stem cell (NSC)-like GBM neurosphere cell lines generated by EGF- and bFGF-containing serum free media have been shown to more closely mirror the phenotype and genotype of primary tumors (Fig. 2) [50]. As example, 060919, one of the GBM neurosphere lines established in our laboratory, can form highly invasive and neo-vascularized xenograft intracranial tumors that well recapitulate the morphology of human GBMs featured by brain tissue infiltrations, heterogenic population, hemorrhages, neoplastic giant cells, necrotic/hypoxic tissues and pseudopalisading cells surrounding the tumor necrosis (Fig. 2 and 3b) [28, 51]. These neurosphere GBM xenograft models or xenografts maintained by serial transplantation may offer more reliable preclinical test ground for GBM therapeutics. The most important caveat to this model is the defects in the immune system of SCID and nude animals, which limit the ability to test the effect of immuno-modulatory agents, and the DNA repair damage that may diminish their capacity to tolerate treatments [52].

Current Targeted Therapies and Clinical Trials

Inhibitors of Receptor Tyrosine Kinase (RTK) Pathways

Genomic amplification and mutation of the EGFR gene occur in about 40% of GBMs[12, 15]. In addition, PDGFRA and MET showed 8% and 4% genetic aberrations in GBMs, respectively[15]. PTEN phosphatase, the tumor suppressor of RTK/PI3K pathway, is mutated or deleted in 30% of GBMs [12]. Activated RTKs stimulate the RAS/RAF/MAPK and PI3-K/Akt pathways resulting in tumorigenic cell proliferation (Fig. 1). Inhibitors of these pathways have been extensively tested in various clinical trials, in both recurrent GBM and primary GBM in addition to the standard of care. The current standard of care procedures consist of surgical resection and radiation therapy (RT) plus concomitant and adjuvant TMZ treatment as the first line therapy for primary GBM [53]. In addition, Gliadel, a dissolvable polymer wafer, can optionally be used for local delivery of BCNU (carmustine) to GBM after resection and is associated with increased survival [54]. For recurrent GBM, Avastin has been introduced as a common second line therapy [55]. Table 1 and Figure 1 provide an overview of targeted therapeutics in clinical trials for GBM. So far, no clear survival benefits have been demonstrated beyond the accepted standard of care of surgical resection and radiation plus TMZ and the common optional Gliadel® [53, 54].

Anti-angiogenic Drugs

Anti-VEGF therapies have been widely tested in clinical trials and cancer therapies (Table 2). Avastin, a humanized monoclonal antibody against VEGF, is approved as second line treatment for recurrent GBM while its use for treatment for initial GBM is currently undergoing phase III trials. Although in Europe the marketing of Avastin in GBM is pending further demonstration of efficacy, due to its expedited approval and wide use in the US, Avastin becomes an interesting case study of anti-angiogenic strategies in GBM. Avastin has demonstrated improved radiographic response and 6 month progression-free suvival

(PFS6), but with modest or little overall survival benefit, either as a single agent or in combination with irinotecan (CPT-11), a topoisomerase 1 inhibitor mainly used in colon cancer therapy [56-60]. It is possible that similar to dexamethasone, part of the impressive radiographic response after Avastin administration is due to alleviated brain edema without much actual change in tumor mass. While Avastin markedly increased the radiographic PFS6 up to 25–42.6% from 10–15% of prior salvage chemotherapy, a disparity to the overall survival of those patients was noted and attributed to the difficulty of contrast-enhanced MRI in reflecting the real tumor mass, especially as those phase II trials were conducted in single-armed fashions and used various historical records as untreated control [58-61]. Furthermore, the lack of salvage therapies to treat GBM regrowth after Avastin's transient tumor control presents other challenges such as rebound of intracranial edema and changed tumor features as detailed below [58, 62, 63].

The role of Avastin in tumor cell invasion has been controversial. Avastin treatment promoted GBM infiltration in the U87 xenograft model and was associated with diffusing invasive recurrence pattern of some GBM cases [64, 65]. Another anti-angiogenic VEGFR inhibitor, Cediranib, increased tumor infiltration in a phase II trial for recurrent GBM [66]. These come in line with the findings and hypothesis that an angiogenesis-independent tumor population or mechanism in GBM may exist, which can be promoted by anti-angiogenic therapies and responsible for the induced infiltrative tumor phenotype, as reviewed by Miletic et al.[67].

Another question that arises with the use of Avastin is if vascular “normalization” impairs brain penetration of other potential adjuvant therapeutics. Vascular normalization is a hypothesis that certain antiangiogenic agents can transiently “normalize” the abnormal structure and function of tumor vasculature to render it more efficient for blood and oxygen supplies. Blocking VEGF with Avastin or other anti-angiogenic drugs such as cediranib have been shown to induce vascular normalization, leading to a decreased vascular permeability in GBM [68-70]. Although it has also been suggested that the reduced intratumoral pressure and restored vasculature may potentially be beneficial to drug delivery, a definitive answer remains pending with numerous ongoing trials of combination therapies with Avastin. Among the available clinical trial data, a side by side phase II trial of Avastin *vs.* Avastin plus irenotecan in recurrent GBM did not display a significant survival benefit in terms of PFS6 and median overall survival by adding irenotecan [59], while the most recent phase II trial of Avastin in combination with erlotinib failed to show a clear clinical benefit over other Avastin monotherapies [58, 59, 71]. In addition, the latest phase II trial of Avastin plus TMZ during and after RT for primary GBM showed largely unchanged overall survival compared to standard of care with TMZ and RT, but improved PFS based on radiographic evaluation and clinical indications, which might be attributed to the edema reduction by Avastin [72]. Cediranib, an VEGFR inhibitor, alone or in combination with lomustine failed to significantly improve overall survival and PFS in comparison to lomustine alone in recurrent GBM [73]. Thus, clinical evidences so far indicate no overall survival benefit of anti-angiogenic agents such as Avastin in combination with other chemotherapeutics compared to the respective single agent controls in the studies. This may suggest a negative effect of anti-angiogenic drugs in the drug delivery of chemotherapeutics.

With the recent FDA recommendation of revoking approval of Avastin in treating breast cancer, its application in various cancers including GBM will likely be more carefully reviewed in the US. A consensus regarding its efficacy and cost-risk/benefit ratio as single agent or in combination therapies is expected to emerge soon with numerous pre-clinical and clinical studies currently underway.

Challenges and Future Directions

Among the major oncogenic pathways in GBM, TP53/MDM2/MDM4/$p14^{ARF}$ tumor suppressor pathway and cell cycle regulators with RB1/ CDK4/$p16^{INK4A}$/CDKN2B are largely untapped in targeted therapies, mostly due to the difficulties in designing small molecules effective for these mostly intracellular loss-of-function targets. A phase I trial of adenovirus carrying wildtype TP53 gene *via* intratumoral administration found only limited transduction in short distance of injection site [74]. These areas remain a challenge in GBM targeting strategy.

In the preclinical front, drug screening needs to be more focused on the compounds with potentially better tumor delivery. A pre-selection of small molecules based on the molecular weight, polar surface area and lipophilicity would potentially improve the success rate of *in vivo* testing later on. Furthermore, packaging drugs in nano-scale particles, such as long circulating liposomes or liposomes with tumor-targeting surface ligands, can take advantage of the trapping effect of the highly fenestrated vasculature of GBM and greatly increase intratumoral drug concentration.

GBMs are known of complex heterogeneity at the genomic and differentiontion levels. Not surprisingly, perhaps with the exception of Avastin, so far the targeted therapies with single agents have disappointed in delivering clear survival benefit in GBM patients most likely due to multiple driver mutations in the various cell populations within a tumor. Combinations of multiple RTK inhibitors has been proposed for GBM similar to many other tumors [75], and have been under various clinical trials.

In dealing with the major challenges of GBM, namely intratumoral heterogeneity and invasive growth pattern, two concepts of targeted therapy emerge as possible future directions: go personal *versus* go universal. Personalized tumor therapy has been proposed for GBM; for example, determining the O-6-methylguanine methyltransferase (MGMT) status to determine response to TMZ [76, 77]. A recent study of personalized tumor markers using personalized analysis of rearranged ends (PARE) offered a clinically feasible approach in profiling certain types of genetic aberrations individually as well as accurately tracking recurrence [78]. A specific molecular profile of individual tumors could eventually be beneficial in designing a tailored therapeutic approach to maximize therapeutic efficacy of existing targeted drugs in GBM patients. However, there is still a lack of effective therapies for GBM and no present incentive for parsing GBMs into different treatment groups.

The ultimate quest of the search for tumor markers in benefit of therapies would be finding a targetable molecular feature that reflects the fundamental differences between tumor and normal cells. In contrast to the diagnostic tumor markers, such as the circulating tumor DNA and tumor antigens, an effective universal therapeutic marker needs to be accessible and

technically targetable, as well as presented in all tumor cells capable of tumor propagation. With CD133 as the prominent surface marker, glioma stem cells have been proposed as the primary population of GBM initiation and a major culprit conveying resistance to radiotherapy [79, 80]. Considerable efforts have been made to target this multipotent and self-renewal population during last few years, which have not delivered convincing results yet [8]. Another possible venue of searching for targetable tumor properties would be tumor metabolic pathways. Since tumors use altered metabolic arrangements, such as high glucose uptake, elevated aerobic glycolysis and reduced oxidative phosphorylation (Warbug effect), compared with those of normal differentiated cells in the body [81], tumor metabolic presents a widely open field with very promising potential. For example, recent data showed somatic mutations in isocitrate dehydrogenase 1 (IDH1) in low grade astrocytomas and secondary GBMs [12]. This enzyme converts isocitrate to α-ketoglutarate and its gain-of-function mutations produced instead 2-hydoxyglutarate, an oncometabolite [82]. This altered metabolic pattern can be exploited for potential targeted therapeutic intervention [83]. Besides the scientific and technical difficulties discussed above, development of a GBM therapy also faces challenges in high cost of clinical trials and extensive regulatory procedures. In order to initiate a first-in-human drug in human trial, an application of investigational new drug (IND) needs to be submitted to FDA, which requires the information including mechanism of action and pharmacodynamics, pharmacokinetics, safety pharmacology, general pharmacology and toxicology studies, and determination of a safe starting dose based on rodent data for the first-inhuman phase I trial [84]. There are three types (investigator, emergency use and treatment), and two categories (commercial and research) of IND [85]. Pharmaceutical sponsors can pursue full “traditional” IND or expedited IND. In 2005, FDA introduced a new category of expedited IND, namely the exploratory IND, with the purpose of allowing for early clinical testing of one or several new chemical entities based on a reduced pre-clinical package. Once a suitable candidate is determined, a full traditional IND has to be submitted [85]. Designed for the limited dosing and duration (microdosing), an exploratory IND study could improve the quality of internal decision making by sponsors based on the exploratory human data obtained early, before the substantial investments are made for a traditional phase I trial [85].

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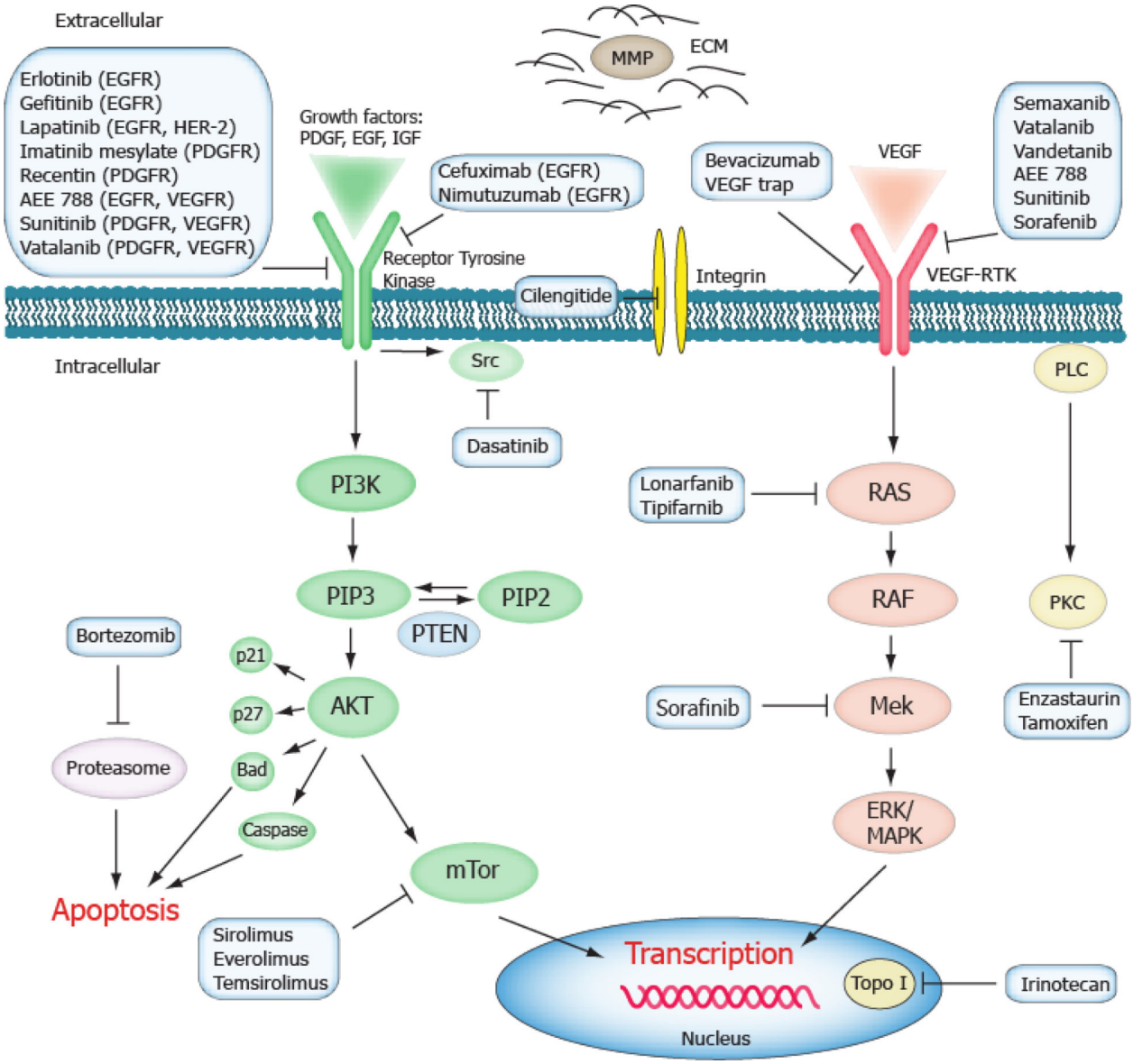

Figure 1.

Schematic overview of current molecular targeted therapies of GBM. Aberrant oncogenic RTK pathways are frequent therapeutic targets in GBM. The PI3K-Akt (green) and RAS (pink) oncogenic pathways are often targeted intracellularly with small molecules inhibitors. EGF, VEGF and PDGF as well as their receptors can be blocked by small molecules and monoclonal antibodies. Items in blue boxes include examples of drugs targeting on the respective pathways. Abbreviations: ECM: extracellular matrix, MMP: matrix metalloproteinase, Topo I: topoisomerase I.

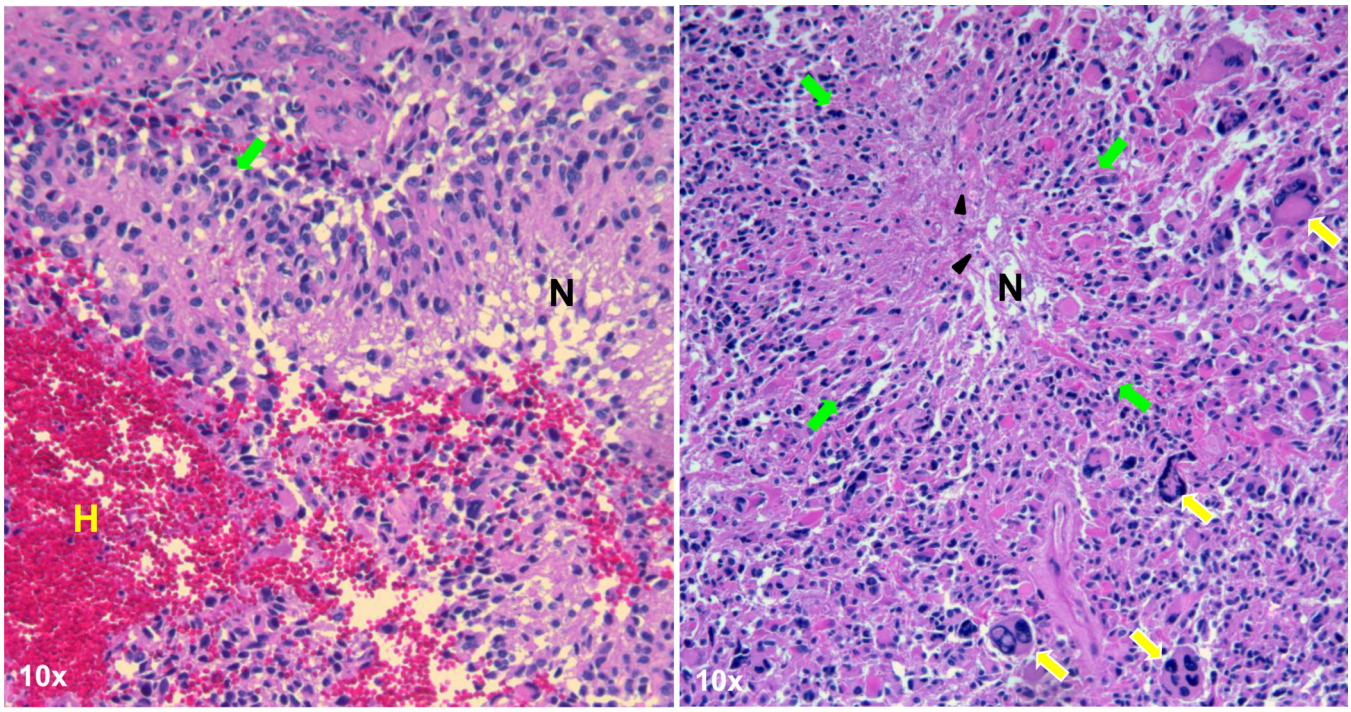

Figure 2.

Histological features of human GBMs. Paraffin-embeded human GBM samples were stained with H&E. Human GBMs are characterized by pseudopalisading necrosis (N) in a garlandlike arrangement of hypercellular tumor nuclei (pseudopalisades: green arrows) lining up around tumor necrosis (N) containing pyknotic nuclei (black arrowheads). Further features include hemorrhage (H) and multi-nucleated giant cells (yellow arrows).

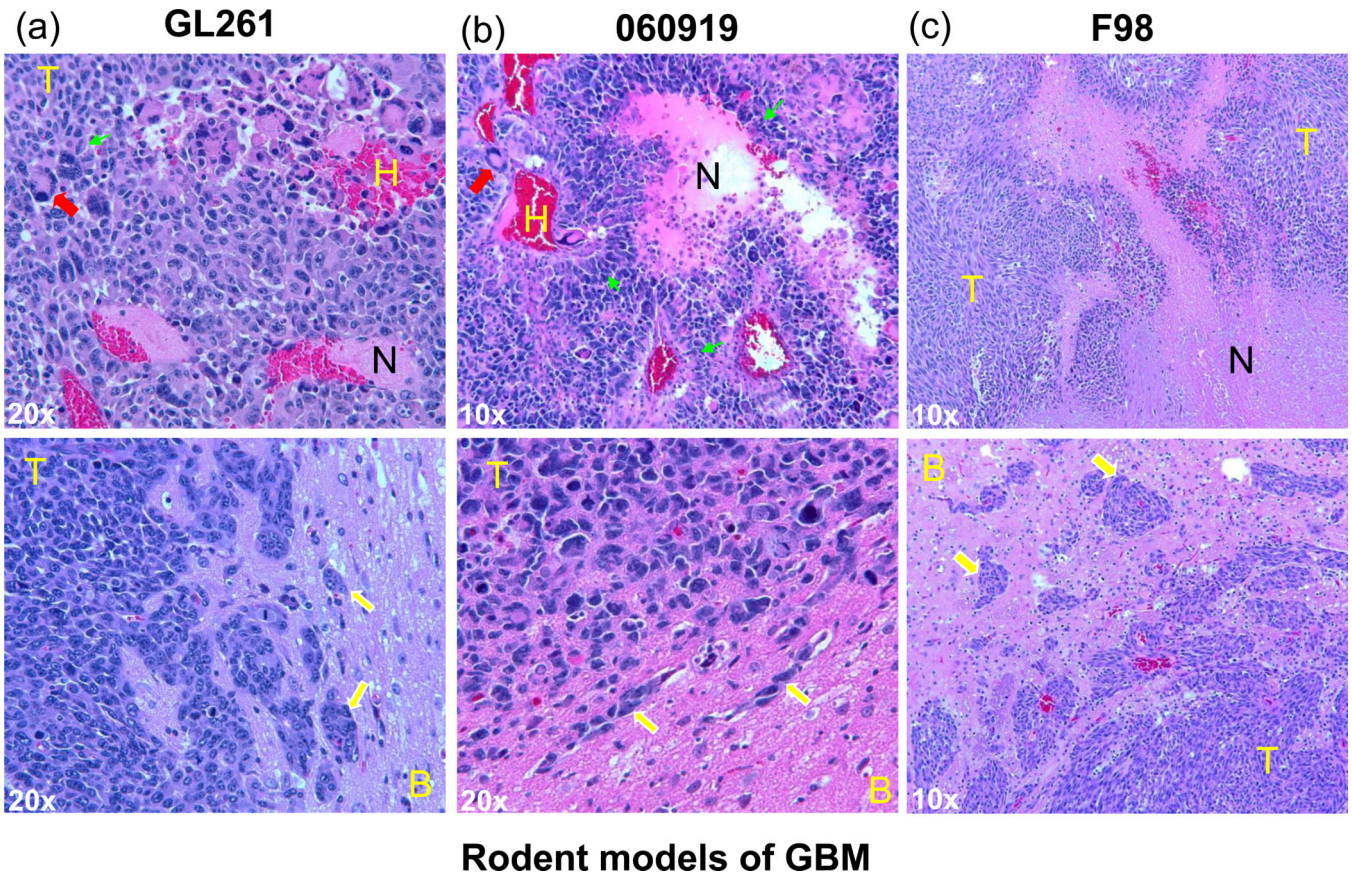

Figure 3.

Histological features of select rodent GBMs. (a) GL261, (b) 060919 and (c) F98 tumors were grown in the frontal lobe of C57BL6 mouse, athymic nude rat or F344 Fischer rat, respectively. Paraffin-embedded brain samples were stained with H&E. In 060919, the pseudopalisading necrosis is especially pronounced and histological features of necrosis, giant cells, hemorrhage and invasive growth closely resemble those in human GBM. Abbreviations: T: tumor, B: brain, N: necrosis, H: hemorrhage. Symbols: yellow arrow: invasion, red arrow: giant cell, green arrow: pseudopalisades.

Table 1

Select targeted drugs of GBM in clinical trials.

Drug	Class / MW	Target	Most recent GBM Trial / Initial or Recurrent GBM	Comments	References
Erlotinib (Tarceva, OSI-774)	Small molecule / 393 Da	EGFR	Phase II / initial & recurrent	Minimal efficacy as single agent; modest survival benefit with TMZ & radiation; ongoing trials in combination with other drugs; so far no significant efficacy has been reported in completed combination therapies.	**9, 70, 71, 86, 87**
Gefetinib (Iressa, ZD1839)	Small molecule / 447 Da	EGFR	Phase II / recurrent	Minimal efficacy as monotherapy compared to current standard RT/ TMZ; combination therapies not effective either.	**88-90**
Lapatinib (Tykerb, GW572016)	Small molecule / 581 Da	EGFR, ErbB2	Phase II / recurrent	No efficacy in a trial with small number of recurrent GBMs; one phase II trial is ongoing.	**91**
Sunitinib (Sutent, SU11248)	Small molecule / 398 Da w/o malate	PDGFR, VEGFR, c-Kit	Phase II / recurrent	Phase II trials under way.	
Sorafenib (Nexavar)	Small molecule / 465 Da	Raf, VEGFR, PDGFR	Phase II / initial & recurrent	Minimal efficacy compared to standard RT/ TMZ; ongoing phase II trials in combination with other drugs	**92, 93**
Dasatinib (Sprycel)	Small molecule/ 488 Da	BCR-ABL, SRC family kinases	Phase I, II / initial & recurrent	SRC family kinases might promote the invasion of GBM cells; among 7 phase I/II trials of	**94**

Drug	Class / MW	Target	Most recent GBM Trial / Initial or Recurrent GBM	Comments	References
				GBM, one was withdrawn and three were suspended.	
Nimotuzumab	Humanized extracellular-binding antibody	EGFR	Phase II, III / initial & recurrent	Well tolerated in patients, modest (17.47 mo *vs* 14.6 mo) survival benefit in small subgroup of GBM or no survival benefit of GBM patients in Cuban patients compared to standard RT/ TMZ.	**95,96**
Cetuximab (Erbitux)	Chimeric extracellular-binding antibody	EGFR	Phase I, II / initial & recurrent	Phase II trials ongoing; a small group of GBM patient responded in a phase II study; little additional efficacy in combination with irrenotecan and bevacizumab in a phase II trial.	**97, 98**
AMG 102	Human HGF antibody	Hepatocyte growth factor (HGF)	Phase II / recurrent	Phase II trials ongoing.	**99**
Imatinib (Gleevec)	Small molecule / 494 Da	PDGFR, c-KIT, BCR-ABL	Phase I, II / recurrent	Minimal efficacy as single agent; after an initially promising phase II trial of imatinib in combination with hydoxyurea, a multicenter study and further trials failed to show meaningful anti-tumor efficacy;	**100-104**

Drug	Class / MW	Target	Most recent GBM Trial / Initial or Recurrent GBM	Comments	References
				further trials of combination therapies are ongoing.	
Tandutinib (MLN518)	Small molecule / 562 Da	PDGFR, FLT3, c-KIT	Phase II / recurrent	Phase II trials as single agent and in combination with Avastin are underway.	
Enzastaurin (LY317615)	Small molecule / 516 Da	PKC, PI3K/AKT pathway inhibitor	Phase I, II, III / initial & recurrent	Limited efficacy in recurrent GBM as monotherapy; in a phase III trial with recurrent GBM, it failed to show superior efficacy compared with lomustine.	**105, 106**
Sirolimus (Rapamycin)	Small molecule / 914 Da	mTOR inhibitor	Phase II / initial & recurrent	Not effective as single agent; other phase II trials in combination with EGFR/ PI3K pathway inhibitors ongoing; limited efficacy in phase II trial in combination with erlotinib.	**87, 107**
Temsirolimus (Toricel, CCI-779)	Small molecule / 1030 Da	mTOR inhibitor, ester analog of sirolimus	Phase I, II / initial & recurrent	Limited or inclusive efficacy as single agent in recurrent GBM; Ongoing trials of combination therapies with EGFR/ PI3K pathway inhibitors or Avastin.	**108, 109**
Everolimus (RAD-001, Zortress)	Small molecule / 958 Da	mTOR inhibitor, derivative of sirolimus	Phase II / initial & recurrent	No clear clinical benefit in	**90**

Drug	Class / MW	Target	Most recent GBM Trial / Initial or Recurrent GBM	Comments	References
				combination with gefitinib in a pilot trial of recurrent GBM; multiple phase II trials of combination therapies ongoing.	
Veliparib (ABT-888)	Small molecule / 244 Da	Poly ADP ribose polymerase (PARP) inhibitor	Phase II / initial & recurrent	Currently phase II trials ongoing.	
Iniparib (BSI 201)	Small molecule / 292 Da	PARP1 inhibitor	Phase I, II / primary	Currently phase I & II trial recruiting.	
Bortezomib (Velcade)	Small peptide / 384 Da	Proteasome inhibitor	Phase II / initial & recurrent	Phase I trials established the safe doses and showed low response rate in recurrent GBM but favorable tendency in initial GBM with standard RT/TMZ.	**110, 111**
Cilengitide	Cyclic peptide / 589 Da	α_v integrins inhibitor, anti-angiogenesis	Phase II, III / initial & recurrent	Phase I trials found the drug well tolerated also with TMZ; modest efficacy as single agent in recurrent GBM; encouraging results of combining cilengitide with standard TMZ/RT in initial GBM with methylated *MGMT* promoter, on which a phase III trial is ongoing.	**76, 112**

Table 2

Select anti-agiogenic drugs of GBM in clinical trials.

Drug	Class / MW	Target	Most Recent GBM Trial / Initial or Recurrent GBM	Comments	References
Bevacizumab (Avastin)	Anti-VEGF antibody	VEGF	Phase II, III / initial & recurrent	FDA approved for treating recurrent GBM due to high response rates; modest survival benefit as mono-therapy; many phase II trials underway as combination therapies; phase III trials treating initial GBM with standard RT/TMZ ongoing.	113, 114
Vatalanib (PTK787, PTK/ZK)	Small molecule / 347 Da	VEGFR, c- KIT, PDGFR	Phase I, II / initial & recurrent	Well tolerated in treating initial and recurrent GBM; a phase II trial with intial GBM was discontinued due to industrial decision, showing limited efficacy with a small number of patients; multiple phase II trials also as combination therapies ongoing.	115, 116
Cediranib (Recentin, AZD2171)	Small molecule kinase inhibitor / 451 Da	VEGFR, PDGFR, FGFR1, c- KIT	Phase I, II / initial & recurrent	Initial human trial showed normalization of tumor vessels and reduction of brain edema; increased tumor infiltration was detected; multiple phase II trials ongoing also as combination therapies.	66, 69
Pazopanib (Votrient)	Small molecule kinase inhibitor / 438 Da	VEGFR, PDGFR, c- KIT	Phase II / recurrent	No survival benefit as single agent in recurrent GBM, while showing MRI responses.	117
Vandetanib (Zactima, ZD6474)	Small molecule kinase inhibitor / 475 Da	VEGFR, EGFR	Phase I, II / initial & recurrent	Safe to use with standard RT/TMZ in initial GBMs in a phase I study; multiple phase I and II trials underway as mono and combination therapies.	118
Aflibercept	Protein / 97 kD	VEGF trap	Phase I, II / initial & recurrent	Working as a decoy receptor of VEGF; a phase I trial with standard RT/TMZ of initial GBMs and a	119

Drug	Class / MW	Target	Most Recent GBM Trial / Initial or Recurrent GBM	Comments	References
				phase II trial with recurrent GBMs,ongoing.	
AEE-788		VEDGR, EGFR/ErbB 2	Phase I, II / recurrent	Completed phase I/II trial of AEE788 as single agent in recurrent GBM; ongoing phase I/II trial in combination with everolimus in recurrent GBM.	