Chapter 4
Recent Advances for Targeted Therapies in Glioblastoma

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Abstract Glioblastoma (GBM) is the most common primary brain tumors in adults. Despite aggressive multimodality therapies, GBM unfortunately remains among the most resistant cancers to treatment. In the past, traditional chemotherapy which works by impeding DNA synthesis or cell metabolism has been used to try and slow the progression of GBM with little success. Recently, research has become more focused into the development of targeted therapies in which drugs (small molecules or antibodies) effect specific molecular and genetic alterations in GBM attempting to inhibit and deregulate cell signaling pathways. The Cancer Genome Atlas (TCGA) GBM project has provided an in depth description of the distinct molecular and genetic alterations in GBM stimulating interest in the development of targeted molecular therapies. While the results of targeted therapy studies to date have failed to improve the overall survival of GBM patients, there continues to be enthusiasm in this approach with numerous clinical trials currently underway. Hopefully, knowledge from the previous failed trials will help provide further insight and assist future clinicians in designing new novel targeted treatments to overcome these barriers.

Keywords Glioblastoma • targeted therapy • The Cancer Genome Atlas • Retinoblastoma pathway • p53 pathway • Receptor tyrosine kinase pathway

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4.1 Recent Advances for Targeted Therapies in Glioblastoma

Gliobastoma multiforme (GBM) is the most common malignant primary brain tumor in adults (Davis et al. 2001; Cloughesy et al. 2014). Currently, 10,000 new cases of GBM are diagnosed each year in the United States, and approximately 100,000 new cases are diagnosed yearly worldwide (Davis et al. 2001; Cloughesy et al. 2014; Porter et al. 2010; Ohgaki and Kleihues 2005). Patients often initially undergo surgical resection to provide symptomatic relief and confirm a pathologic diagnosis. However, surgery is not curative as the tumor cells invade surrounding normal brain tissue rendering a complete resection of the tumor impossible (Cloughesy et al. 2014). Following a maximal safe resection, the standard of care treatment for newly diagnosed GBM consists of cytotoxic chemotherapy with daily temozolomide and concurrent radiation therapy for 6 weeks, followed by 6–12 cycles of adjuvant temozolomide (Masui et al. 2012; Stupp et al. 2005). Despite aggressive multimodality therapies, GBM unfortunately remains among the most resistant cancers to treatment leading to a median survival of around 16 months (Stupp et al. 2005). Several potential reasons have been proposed to explain GBMs resistance to treatment including the genetic heterogeneity of the tumor, elaborate signaling pathways, and difficulties with designing drugs capable of crossing the blood brain barrier (Tanaka et al. 2013). In the past, traditional chemotherapy which works by impeding DNA synthesis or cell metabolism has been most often used to try and slow the progression of GBM with little success. Recently, research has become more focused into the development of targeted therapies in which drugs (small molecules or antibodies) effect specific molecular and genetic alterations in GBM attempting to inhibit and deregulate cell signaling pathways. This chapter will explore current targeted therapies and how they relate to the aberrant signaling pathways in GBM.

4.2 The Cancer Genome Atlas

GBM was one of the first cancers studied by The Cancer Genome Atlas (TCGA) Research Network, a collaboration between the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI). The TCGAs key aims were to identify changes in each cancer's genome and understand how these changes interact to drive the disease, thereby laying the foundation for improved cancer prevention, early detection, and treatment (Cancer Genome Atlas Research Network 2008; Bredel et al. 2011; Parsons et al. 2008; Verhaak et al. 2010). The TCGA GBM project was conducted in two phases and developed a genome wide map of the genetic, epigenetic, and transcriptomic changes, as well as proteomic changes in over 500 GBM samples (Cancer Genome Atlas Research Network 2008; Brennan et al. 2013). Based on molecular typing and gene expression profiles, four distinct subtypes of GBM were found which are the classical, mesenchymal, neural, and proneural aubtypes (Freije et al. 2004; Gravendeel et al. 2009; Li et al. 2009; Nigro

et al. 2005; Vitucci et al. 2011; Jue and McDonald 2016). The Classical subtype is associated with Endothelial growth factor receptor (EGFR) amplification, concomitant chromosome 7 amplification and chromosome 10 loss, and focal deletions of 9p encompassing cyclin-dependent kinase Inhibitor 2A (CDKN2A) . Tumor protein p53 (TP53) mutations, while common in GBM, are not seen in the classical subtype (Jue and McDonald 2016). The Mesenchymal subtype is characterized by deletions and mutations in Neurofibromin 1 (NF1) and Phosphatase and tensin homolog (PTEN) genes (Jue and McDonald 2016). The Neural subtype exhibits expression of neuronal markers and displays various mutations and copy number alterations including amplification of EGFR and deletion of PTEN (Jue and McDonald 2016). The Proneural subtype exhibits an oliogodendrocytic expression signature and features mutations of the isocitrate dehydrogenase 1(IDH 1) gene (Jue and McDonald 2016). The proneural subtype is associated with younger age and prolonged survival time, given the IDH1 mutation, as IDH1 mutations are frequently seen in lower grade gliomas and secondary gliomas (Verhaak et al. 2010; Jue and McDonald 2016). The TCGA analysis further identified three key molecular pathways for tumorigenesis: the p53 tumor suppressor and Retinoblastoma (RB) pathways, and the receptor tyrosine kinases (RTKs) signaling pathway (Fig. 4.1).

4.3 Tumor Protein P53 Signaling Pathway

Tumor protein p53 is a well-known tumor suppressor gene and transcription factor involved in the coordination of cell responses that are involved in processes such as apoptosis, DNA repair, neovascularization, and metabolism (Bogler et al. 1995; Matlashewski et al. 1984; May and May 1999). p53 has been found mutated in 37.5% and 58% of untreated and treated GBM samples, according to the TCGA (Cancer Genome Atlas Research Network 2008). Disruptions in the p53 pathway are achieved by disruptions in genes that regulate its function, including Mouse double minute homolog (MDM) 2/4 and the tumor suppressor protein alternate reading frame (ARF) in 70% of GBM samples (Cancer Genome Atlas Research Network 2008). A complex that can suppress p53 function is the MDM2-MDM4 heterocomplex through the exertion of degradative control. MDM2-MDM4 protein amplification may represent a possible mechanism that gliomas escape p53 restricted growth (Herman et al. 2011; Reifenberger et al. 1993; Riemenschneider et al. 1999). Inactivation of CDKN2a can also dysregulate the p53 signaling pathway. CDKN2a encodes two proteins (p16INK4a and p14ARF) which are tumor suppressors and are negative regulators of the cell cycle (Ruas and Peters 1998). p16INK4a and p14ARF are deleted in approximately 55% of GBMs (Cancer Genome Atlas Research Network 2008; Schmidt et al. 1994). An encoded protein product, p14ARF, was found to promote degradation of the p53 repressor and lead to stabilization and accumulation of p53. Loss of p14ARF results in suppression of p53 and provides a mechanism for tumorigenesis (Kamijo et al. 1997, 1998; Zhang et al. 1998). CDKN2a also encodes for p16INK4a which is a protein that inhibits CDK4/6

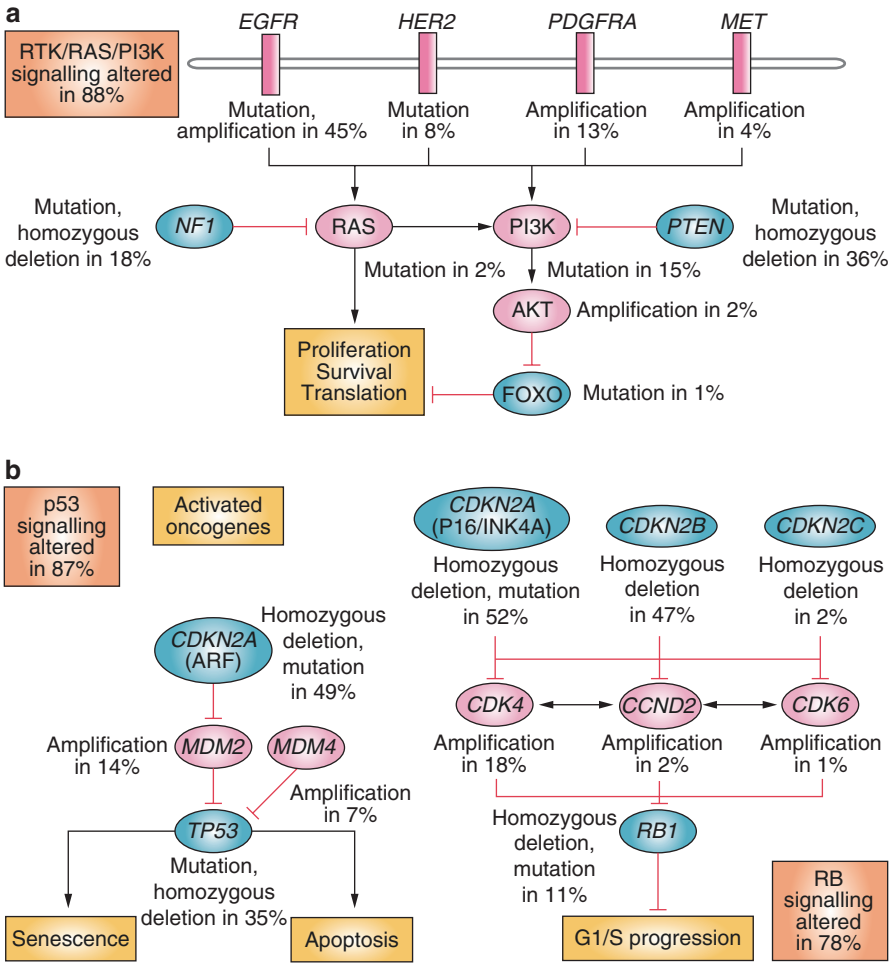

Fig. 4.1 Critical signaling pathways altered in malignant gliomas. Primary sequence alterations and significant copy number changes for components of the (**a**) RTK/RAS/PI3K, (**b**) p53 and (**c**) Rb signalling pathways are shown. *Red* indicates activating genetic alterations. Conversely, *blue* indicates inactivating alterations. For each altered component of a particular pathway, the nature of the alteration and the percentage of tumours affected are indicated. Boxes contain the final percentages of glioblastomas with alterations in at least one known component gene of the designated pathway. Abbreviation: *RTK* receptor tyrosine kinase (Permission obtained from Nature Publishing Group © The Cancer Genome Atlas Research Network (2008))

association with cyclin D. When associated, this forms a complex that promotes G1/S transition through activation of downstream mediators. This process is involved in phosphorylating retinoblastoma protein and facilitating the release of bound E2F, a G1/S transcription factor. If p16INK4a is lost, then CDK4/6 and cyclin D can associate and the G1/S transition occurs freely. In patients with wild-type pRB, CDK4/6 is a target for inhibition (Bastien et al. 2015).

4.4 Retinoblastoma (Rb) Pathway

The Rb protein is encoded by the Rb gene located on chromosome 13q14.1-q14.2. The function of the protein is to prevent unwanted cell growth by inhibiting cell cycle progression until the cell is to undergo mitosis, and at that point the Rb protein becomes phosphorylated by Cyclin D, CDK4, and CDK6, which inactivates it and allows for cell cycle progression (Murphree and Benedict 1984). Typically what occurs is a homozygous deletion of CDKN2A which produces a loss of p16INK4a, a suppressor of CDK4. This leads to a dysregulation of Rb signaling (Murphree and Benedict 1984; Ohgaki and Kleihues 2009; Lin et al. 2013).

4.5 Receptor Tyrosine Kinases Pathway

Recurring molecular alterations have recently been identified in GBM, leading to a better understanding of the pathways that become disrupted in this disease. Frequently seen are gene amplifications and deletions, with deletions most often in chromosomes 1, 9, and 10 and amplifications in chromosomes 7 and 12 (Bello et al. 1994; James et al. 1991; Reifenberger et al. 1995; Rey et al. 1987). Amplifications of a gene can cause an upregulation of various oncogenes while deletions can target tumor suppressors (Purow and Schiff 2009). These are mediated by receptor tyrosine kinases (RTKs), which are also key targets for deregulation in cancers (Zwick et al. 2001). Examples of RTKs in GBM include vascular endothelial growth factor (VEGFR), EGFR, Platelet-derived growth factor receptor (PDGFR), and hepatocyte growth factor receptor (MET) (Blume-Jensen and Hunter 2001). Mutations in these receptors act to relieve auto-inhibitory constraints to prevent degradation (Blume-Jensen and Hunter 2001). Growth factors and RTKs are typically strong candidates for therapeutic targets because mutations here are driver mutations critical for oncogenesis and because kinase receptors are targets for inhibitors that block kinase activation. Moreover, there has been success in other solid tumors (such as erlotinib in lung cancer) and in utilizing RTKs as a target.

4.5.1 VEGFR

A feature of high grade gliomas is angiogenesis, which may be attributed to high levels of VEGF-A in and around the tumor. Bevacizumab is a humanized monoclonal antibody which functions to bind VEGF-A ligand and alter binding to endothelial cells (Ferrara et al. 2005). Studies of bevacizumab have shown high radiographic response rate, prolonged progression-free survival (PFS), and reduced glucocorticoid requirements, all of which led to approval by the Food and Drug administration for patients with GBM (Gilbert et al. 2014). However, several phase III studies have indicated that despite the improved radiographic response, as well as PFS, there is

no significant overall survival benefit (Gilbert et al. 2014; Chinot et al. 2014). Both of these studies showed a prolonged PFS with bevacizumab, however the Avaglio trial indicated that health-related quality of life was stable prior to progression while the RTOG-0825 indicated overall quality of life based on symptom burden was significantly worse in some domains with bevacizumab (Gilbert et al. 2014; Chinot et al. 2014). A post-hoc subgroup analysis of the Avaglio study identified a 4.3 month potential increase in median survival with the addition of bevacizumab for IDH1 wild-type GBM in the proneural subgroup (Sandmann et al. 2015). Phase III studies have not demonstrated a survival benefit in recurrent GBM for bevacizumab. The phase II BELOB trial initially suggested a benefit with combined bevacizumab and lomustine, however the phase III trial EORTC 26101 recently underwent an interim analysis and found no overall survival benefit with the addition of bevacizumab to lomustine compared to lomustine alone for recurrent GBM (Taal et al. 2014). Cediranib, another anti-angiogenic agent whose mechanism of action is as a VEGF receptor tyrosine kinase inhibitor, has been tested in phase III trials with GBM and were found to be ineffective in altering overall outcome (Swartz et al. 2014). Additionally, other VEGF inhibitors such as Pazopanib, Sorafenib, Nintedanib, Sunitib, Vandetanib, Aflibercept, Vatalanib, and Cabozantinib have also failed to improve survival in glioblastoma patients (Table 4.1).

4.5.2 EGFR

In GBM there is evidence EGFR plays an important role in oncogenesis and tumor biology (Swartz et al. 2014). EGFR is amplified in approximately 50% of GBM samples and is associated with an active, mutant form of the EGFRvIIIL receptor, which when overexpressed enhances GBM cell growth and contributes to GBM pathogenesis (Jaros et al. 1992; Nishikawa et al. 1994; Schlegel et al. 1994). EGFRvIII, has been identified in 30% of newly diagnosed GBM and is characterized by deletion of exons 2–7 (Gan et al. 2009). The receptor is rendered active and thereby enhances the tumorigenicity by promoting tumor cell migration, conferring protection from radiation and temozolomide, and secreting EGFRvIII-bound oncosomes onto plasma membranes of neighboring cells (Prados et al. 2015).

Furthermore, EGFR can stimulate increased signaling through the RAS, RAF, MEK, MAP, and mTOR pathways, as well as downregulating cell cycle inhibitor proteins (Mao et al. 2012; Nishikawa et al. 2004). A set of studies found that increased levels of EGFR and EGFRvIII co-activates the RTK MET which leads to ligand-independent activation of the EGFR receptor (Huang et al. 2007; Jo et al. 2000; Stommel et al. 2007). This suggests that a mechanism for tumor cells to reduce dependence on either RTK for downstream signaling exists due to the interaction between the c-MET and EGFR/EGFRvIII signaling pathways. Studies have suggested that RTK MET overexpression may correlate with a shorter median survival time (Kong et al. 2009; Koochekpour et al. 1997; Lamszus et al. 1999).

Table 4.1 Targeted therapies in clinical trials for glioblastoma

Therapy	Target/s	Trial phase	Time of treatment -newly diagnosed (N) or recurrent (R) disease	Mono therapy (M), combination therapy or both (B)	If combination, which other therapies used
Bevacizumab	VEGF-A	II	R	B	Irinotecan (Cohen et al. 2009; Friedman et al. 2009; Kreisl et al. 2009a)
Bevacizumab	VEGF-A	III	N	C	Radiation/Temozolomide, Temozolomide (Gilbert et al. 2014; Chinot et al. 2014)
Bevacizumab	VEGF-A	II,III	R	B	Lomustine (Taal et al. 2014; ClinicalTrialsgov)
Bevacizumab	VEGF-A	II	R	C	Fotemustine (Soffietti et al. 2014)
Bevacizumab	VEGF-A	II	R	C	Carboplatin (Field et al. 2015)
Cediranib	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α/β, FGFR-1, c-Kit	II	R	M (Batchelor et al. 2010)	
Cediranib	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α/β, FGFR-1, c-Kit	III	R	B	Lomustine (Batchelor et al. 2013)
Pazopanib	VEGFR, c-KIT, FGFR, and PDGFR	II	R	M (Iwamoto et al. 2010)	
Pazopanib	VEGFR, c-KIT, FGFR, and PDGFR	I/II	R	C	Lapatinib (Reardon et al. 2013)
Sorafenib	VEGFR-2, Raf, PDGFR, c-KIT, Flt-3	II	N	C	Radiation/Temozolomide, Temozolomide (Hainsworth et al. 2010)
Sorafenib	VEGFR-2, Raf, PDGFR, c-KIT, Flt-3	II	R	C	Temozolomide (Reardon et al. 2011a)
Sorafenib	VEGFR-2, Raf, PDGFR, c-KIT, Flt-3	II	R	C	Bevacizumab (Galanis et al. 2013)

(continued)

Table 4.1 (continued)

Therapy	Target/s	Trial phase	Time of treatment -newly diagnosed (N) or recurrent (R) disease	Mono therapy (M), combination therapy or both (B)	If combination, which other therapies used
Sorafenib	VEGFR-2, Raf, PDGFR, c-KIT, Flt-3	II	R	C	Erlotinib (Peereboom et al. 2013)
Sorafenib	VEGFR-2, Raf, PDGFR, c-KIT, Flt-3	I/II	R	C	Temsirolimus (Lee et al. 2012)
Nintedanib	VEGFR 1–3, FGFR 1–3, PDGFR-α/β	II	R	B	Bevacizumab (Muhic et al. 2013)
Vandetanib	VEFGR-2,EGFR	I/II	R	M (Kreisl et al. 2012)	
Vandetanib	VEFGR-2,EGFR	I	R	C	Sirolimus (Chheda et al. 2015)
Sunitinib	VEGFR2, PDGFR-α, and c-KIT	II	R	M (Neyns et al. 2011; Pan et al. 2012; Kreisl et al. 2013)	
Sunitinib	VEGFR2, PDGFR-α, and c-KIT	I	R	C	Irinotecan (Reardon et al. 2011b)
Aflibercept	VEGF and PlGF	II	R	M (de Groot et al. 2011)	
Vatalanib	VEGFR, PDGFR, and c-kit	I	N	C	Radiation/Temozolomide, Temozolomide (Gerstner et al. 2011)
Vatalanib	VEGFR, PDGFR, and c-kit	I	N	C	Imatinib and Hydroxyurea (Reardon et al. 2009b)
Vatalanib	VEGFR, PDGFR, and c-kit	I/II	N	C	Radiation/Temozolomide, Temozolomide (Brandes et al. 2010)
Cabozantinib	VEGFR-2, MET, and RET	II	R	M (Wen et al. 2010)	
Gefitinib	EGFR	II	N	M (Uhm et al. 2011)	
Gefitinib	EGFR	I/II	N	C	Radiation/Temozolomide, Temozolomide (Chakravarti et al. 2013)
Gefitinib	EGFR	II	R	M (Franceschi et al. 2007; Rich et al. 2004)	

Gefitinib	EGFR	I	R	C	Sirolimus (Reardon et al. 2006)
Gefitinib	EGFR	I	R	C	Everolimus (Kreisl et al. 2009b)
Erlotinib	EGFR	II	R	M (Yung et al. 2010)	
Erlotinib	EGFR	II	R,N	M (Raizer et al. 2010)	
Erlotinib	EGFR	II,II,I/II,I	N	C	Radiation/Temozolomide, Temozolomide (Brown et al. 2008; Krishnan et al. 2006; Peereboom et al. 2010; Prados et al. 2009)
Erlotinib	EGFR	I	R	B	Temozolomide (Prados et al. 2006)
Erlotinib	EGFR	II	R	C	Carboplatin (de Groot et al. 2008)
Erlotinib	EGFR	II	R	C	Bevacizumab (Sathornsumetee et al. 2010)
Erlotinib	EGFR	I,II	R	C	Sirolimus (Nghiemphu et al. 2012; Reardon et al. 2010)
Cetuximab	EGFR	II	R	M (Neyns et al. 2009)	
Cetuximab	EGFR	II	R	C	Bevacizumab and Irinotecan (Hasselbalch et al. 2010)
Lapatinib	EGFR and HER2	I/II	R	M (Thiessen et al. 2010)	
Lapatinib	EGFR and HER2	I/II	R	C	Pazopanib (Reardon et al. 2013)
Lapatinib	EGFR and HER2	I	R	C	Temozolomide (Karavasilis et al. 2013)
Nimotuzumab	EGFR	I/II	N	M (Solomon et al. 2013)	
Imatinib	PDGFR, Bcr-Abl, and c-Kit	I/II	R	M (Wen et al. 2006)	
Imatinib	PDGFR, Bcr-Abl, and c-Kit	II	N,R	M (Razis et al. 2009)	
Imatinib	PDGFR, Bcr-Abl, and c-Kit	II	R	C	Hydroxyurea (Reardon et al. 2005, 2009a; Dresemann et al. 2010)
Dasatinib	PDGFR, Src, Bcr-Abl, c-Kit, and EphA2	II	R	M (Lassman et al. 2015)	
Enzastaurin	protein kinase C, PI3K, and Akt	II	N	C	Radiation/Temozolomide, Temozolomide (Butowski et al. 2011; Wick et al. 2013)

(continued)

Table 4.1 (continued)

Therapy	Target/s	Trial phase	Time of treatment -newly diagnosed (N) or recurrent (R) disease	Mono therapy (M), combination therapy or both (B)	If combination, which other therapies used
Enzastaurin	protein kinase C, PI3K, and Akt	II	R	M (Kreisl et al. 2010; Wick et al. 2010)	
Everolimus	mTOR	II	R	M (Cloughesy et al. 2011)	
Everolimus	mTOR	II	N	C	Radiation/Temozolomide, Temozolomide (Ma et al. 2015)
Everolimus	mTOR	II	N	C	Radiation/Temozolomide/ Bevacizumab, Bevacizumab (Hainsworth et al. 2012)
Temsirolimus	mTOR	II	R	M (Chang et al. 2005; Galanis et al. 2005)	
Temsirolimus	mTOR	II	N	C	Radiation/Temozolomide, Temozolomide (Sarkaria et al. 2010)
Temsirolimus	mTOR	II	R	C	Bevacizumab (Lassen et al. 2013)
Sirolimus	mTOR	I	R	C	Vandetanib (Chheda et al. 2015)
Sirolimus	mTOR	I	R	C	Gefitinib (Reardon et al. 2006)
Sirolimus	mTOR	I,II	R	C	Erlotinib (Nghiemphu et al. 2012; Reardon et al. 2010)
Tipifarnib	Ras	I	N	C	Radiation/Temozolomide, Temozolomide (Nghiemphu et al. 2011)
Tipifarnib	Ras	II	R	M (Cloughesy et al. 2006)	
Lonafarnib	Ras	II	R	M (Desjardins et al. 2011)	
Lonafarnib	Ras	I	R	C	Temozolomide (Yust-Katz et al. 2013)

EGFR remains an elusive target for therapy in GBM as initial studies have suggested activity of some EGFR inhibitors in molecular subsets of GBM but larger studies failed to replicate these results (Haas-Kogan et al. 2005; Mellinghoff et al. 2005; Reardon et al. 2014).

Rindopepimut is a tumor vaccine which is a conjugate of peptides that span the EGFRvIII mutation site with an immunogenic carrier protein keyhole limpet hemocyanin. Phase I and II trials in newly diagnosed GBM patients treated with rindopepimut along with temozolomide indicated a PFS of 10–15 months and overall survival of 22–26 months, which was improved compared to historical controls of 6 and 15 months respectively (Swartz et al. 2014). Another multicenter trial with rindopepimut in newly diagnosed GBM, the ACTIVATE trial, demonstrated an immune response after vaccination. In ACTIVATE, 43% of those treated had a positive humoral response, and the majority of patients with relapse had lost all of the EGFRvIII expression, a phenomenon known as antigen escape which suggested that the immune system successfully targeted EGFRvIII-expressing cells (Swartz et al. 2014). Furthermore a similar response in recurrent disease was noted in the ReACT trial, where rindopepimut reportedly caused an immune response and significantly prolonged survival when administered with bevacizumab (Phillips et al. 2016). However, in the phase III study ACT IV, rindopepimut combined with temozolomide did not increase overall survival in newly diagnosed EGFRvIII-positive GBM (Reardon et al. 2015; Inman 2016).

Therapy remains ongoing to identify new targets and mechanisms, such as ABT-414, which is a conjugate of a potent microtubule inhibitor and a monoclonal antibody against a tumor-selective EGFR epitope found in EGFR wild-type-overexpressing tumors and EGFRvIII mutant-expressing tumors. Preclinical studies have indicated that ABT-414 is selective and a phase I study, as well as a phase III study are ongoing (Gan et al. 2015; Phillips et al. 2016). Unfortunately, other EGFR inhibitors such as Gefitinib, Erlotinib, Cetuximab, Nimotuzumab, and Lapatinib, have similarly failed to improve survival in glioblastoma patients (Table 4.1).

4.5.3 PDGFR

Platelet derived growth factor receptors (PDGFR) are cell surface receptors for members of the platelet derived growth factor family and signal through the alpha and beta platelet derived growth factor receptor tyrosine kinases (Matsui et al. 1989). Chromosome 7p22 contains the PDGFR alpha gene which is amplified in approximately 13% of GBM samples (Cancer Genome Atlas Research Network 2008; Stenman et al. 1992). Multiple aberrations in expression of PDGFR have been observed, including overexpression, amplification, mutations, and truncations, however point mutations are exclusively seen in GBM (Alentorn et al. 2012).

An inhibitor of PDGFR is Imatinib mesylate, which also functions to inhibit bcr-abl and c-kit tyrosine kinases and is beneficial in the treatment of CML and in GI stromal tumors (Buchdunger et al. 2000; Druker et al. 2001; Demetri et al. 2002). Imatinib however has shown minimal activity in recurrent gliomas as well as newly diagnosed GBM (Wen et al. 2006; Razis et al. 2009). Others studies have been performed looking at the addition of hydroxyurea to imatinib in recurrent malignant gliomas and found that it failed to show any meaningful anti-tumor activity (Reardon et al. 2005, 2009a; Dresemann et al. 2010; Desjardins et al. 2007).

Another drug that has been studied is dastinib, which is an inhibitor of PDGFR, SRC, bcr-abl, c-Kit, and EphA2 receptors. The study was conducted in patients with recurrent GBM and found that dastinib was ineffective with no radiographic responses (Lassman et al. 2015). Another phase 1 trial of dastinib in combination with CCNU found hematological toxicities, which limited the amount of exposure available for both therapeutic agents, in contrast to a trial with dastinib and erlotinib which was much better tolerated (Reardon et al. 2012; Franceschi et al. 2012).

4.5.4 PI3K/AKT/PTEN/mTOR

Stimulation of the PI3K/AKT/PTEN/mTOR pathway enhances growth via activation of receptor tyrosine kinases. This occurs via the regulation of cell division, proliferation, differentiation, metabolism, and survival (Carnero et al. 2008; Morgensztern and McLeod 2005). Several genomic alterations in GBM activate this pathway, most often of which is the amplification of EGFR (Peraud et al. 1997; Watanabe et al. 1996; Wong et al. 1992). Other alterations leading to activation of this pathway include lesions in PIK3R1/PIK3CA and mutations or deletions of AKT, which occur in 84% of GBM cell lines and/or PTEN mutations, which occur in 30–44% of high-grade gliomas (Wang et al. 1997, 2004; Tohma et al. 1998; Teng et al. 1997; Koul 2008). PTEN typically inhibits the AKT pathway, therefore deletion of PTEN leads to activation of this pathway (Liu et al. 1997; Li et al. 1997; Stambolic et al. 1998). PTEN also facilitates the degradation of EGFR, which leads to termination of EGFR signaling, leading to an explanation for why PTEN confers resistance to epidermal growth factor inhibitors in vitro (Vivanco et al. 2010; Bianco et al. 2003).

mTOR is a master nutrient and energy sensor which regulates processes including transcription, protein synthesis, as well as other cellular functions including proliferation, cell motility survival, and anabolism (Hay and Sonenberg 2004; Kim et al. 2002; Sarbassov et al. 2004, 2005; Facchinetti et al. 2008; Ikenoue et al. 2008). mTOR is made of two different complexes—mammalian target of rapamycin complex 1 mTORC1 and mTORC2—and acts as a regulator of PI3K upstream and as its effector downstream (Akhavan et al. 2010). Inhibitors of mTOR have been developed, including Sirolimus, a mTORC1 inhibitor, which leads to reduced expression of neural stem cell progenitor markers and neurosphere formation in GBM (Sami and Karsy 2013; Sunayama et al. 2010). Another mTOR inhibitor, Dactolisib, a potent dual PI3K-mTOR inhibitor has shown potential benefit as a radiosensitizer for

GBMs in preclinical studies (Fan et al. 2010; Mukherjee et al. 2012). Regrettably, mTOR inhibitors examined to date including Sirolimus, Everolimus and Temsirolimus have not prolonged overall survival in glioblastoma patients (Table 4.1).

4.5.5 RAS/RAF/MEK/MAP (ERK) Kinase

The RAS/RAF/MEK/MAP kinase pathway mediates cellular responses via growth, migration, apoptosis, proliferation, differentiation, and cell survival. This pathway can be activated by EGFR and PDGFR via signal transmission through indirect associations with cytosolic mediator proteins growth factor receptor-bound protein 2 (GRB2) and son of sevenless (SOS) to RAS. RAS then stimulates RAF, which activates MAPK and MEK (Moodie et al. 1993; Thomas et al. 1992).

RAS has been found to be upregulated in GBM samples, and activation is typically through loss of the NF-1 gene, which encodes a tumor suppressor protein and is a negative regulator of RAS and mTOR signaling in astrocytes (Banerjee et al. 2011; Nissan et al. 2014; Dasgupta et al. 2005). NF-1 mutation has been implicated in prior studies in glioma tumorigenesis, specifically noting that the homozygous loss of NF-1 in glial cells has been shown to develop fully malignant astrocytomas with a p53-null background (Alcantara Llaguno et al. 2009; Zhu et al. 2005; Kwon et al. 2008). Hyper activation of protein kinase C also causes increased degradation of NF-1, which also puts patients at an increased risk of developing gliomas (McGillicuddy et al. 2009). NF-1 mutations are a defining feature of the mesenchymal GBM subtype, and therefore this subgroup may potentially be good candidates for agents that target NF-1 driven pathways (Cancer Genome Atlas Research Network 2008; Phillips et al. 2006). Loss of NF-1 function leads to enhanced RAS activity, which will lead to increased RAS/RAF/MEK/MAPK pathway activation, leading to the hypothesis that MEK inhibitors were good therapeutic targets. Two MEK inhibitors, PD0325901 and AZD6244, both appeared effective against NF-1 deficient GBM cells dependent on RAF/MEK signaling in preclinical studies (See et al. 2012). Furthermore, this study showed that MEK inhibitor-resistant NF-1 deficient cells could be re-sensitized to MEK inhibitors with the co-application of dual PI3K-mOTR inhibitor PI-103, also suggesting that NF-1 deficient GBM patients may respond to a MEK inhibitor based chemotherapy (See et al. 2012). Drugs that have been tested that target RAS specifically (Tipifarnib, Lonafarnib) have not shown any improvement in overall survival for GBM patients (Table 4.1).

4.6 IDH1/IDH2 Mutations

The identifications of mutations in the metabolic enzymes IDH 1 and 2 is one of the most important discoveries that has led to a remodeling of our understanding of gliomas, including GBMS (Parsons et al. 2008). The majority of tumor samples in

this study bore this mutation and were classified as secondary GBMs, suggesting that IDH 1 and IDH 2 mutations can serve as a genetic marker for this type of GBM. When IDH 1 and 2 were mutated, the result was enzymes with a neomorphic function, meaning that the mutant enzymes acquired the ability to catalyze the NADPH-dependent reduction of alpha-KG to the R-enantiomer of 2-hydroxyglutarate (2-HG) (Dang et al. 2010). This study showed that IDH 1 and 2 mutants had high levels of 2-HG, which is also found in primary IDH 1 mutant gliomas, and is also found in AML patients (Gross et al. 2010; Ward et al. 2010). IDH 1 and 2 mutant expression results in inhibition of alpha-KG-dependent dioxygenases by 2-HG. The enzymes that are dependent on this regulate physiological processes including hypoxia sensing, histone demethylation, and changes in DNA methylation (Loenarz and Schofield 2008). The glioma CpG island methylator phenotype (C-CIMP) is a distinctive and nearly invariable feature of IDH 1 and 2 mutant gliomas that has been studied to indicate that gliomas with this mutant expression are correlated with better prognosis (Baysan et al. 2012; Noushmehr et al. 2010). IDH 1 and 2 mutant gliomas are detected with the IDH-R132H antibody as well as with DNA sequencing of antibody-negative cases, which provides more accurate diagnosis and prediction of patient outcomes and prognosis.

IDH 1 and 2 mutational status also provides a prognostic marker in patients with lower grade gliomas and GBMs, as well as providing insights about the origin of gliomas (Parsons et al. 2008; Hartmann et al. 2009; Sanson et al. 2009; Weller et al. 2009; Yan et al. 2009). IDH 1 and 2 wild-type GBMs typically exhibit a characteristic pattern of genetic changes associated with primary GBMs such as a gain of chromosome 7, loss of chromosome 10, and amplification of EGFR which are not seen in IDH 1 and 2 mutant GBMs.

The importance of IDH mutation is eminent as the new 2016 WHO Classification of tumors of the central nervous system now defines glioblastoma based upon IDH status (Louis et al. 2016). Currently, several phase I studies examining IDH mutation inhibitors in advanced malignancies including glioblastoma are underway. However, as IDH is thought to be an early driver mutation in glioma, it remains unknown the potential impact of its inhibition in recurrent glioblastoma (Watanabe et al. 2009; Mandel et al. 2016).

4.7 Discussion

The Cancer Genome Atlas analysis revealed multiple molecular pathways and potential therapeutic genetic targets ushering in a new era for glioblastoma treatment. Bevacizumab, an anti-VEGF agent, has displayed an increased time to progression and improved imaging response but disappointingly has been unsuccessful in increasing patient overall survival. Furthermore, it remains an area of debate whether bevacizumab has any anti-tumor benefit (Kruser et al. 2016). Despite our increased knowledge of these tumors, our ability to divide them into molecular subgroups, and several promising therapeutic targets, every targeted

therapy examined to date unfortunately has failed to demonstrate any benefit in overall survival.

Several possible explanations for this lack of success have been proposed including lack of tumor dependence on the pathway targeted, inadequate CNS penetration of the drug, intratumoral heterogeneity, and clonal evolution.

In regards to lack of pathway dependence, evidence suggests that tumors may be able upregulate a different pathway when another pathway is inhibited. This has been seen in the use of EGFR inhibitors, where treatment has demonstrated the lack of ability to change targets downstream like Akt and can even upregulate the PI3K/Akt pathway (Hegi et al. 2011; Chakravarti et al. 2002). Another possible area of concern is that many of the mutations targeted are essential for early tumor development and may be subsequently superseded by secondary pathways of tumor growth (Lee et al. 2012). Additionally, mutations present in a tumor on initial presentation may change or no longer be expressed on disease recurrence (van den Bent et al. 2015).

Drug penetrance is also major concern as it is frequently difficult to determine how well therapeutic agents cross the blood brain barrier in brain tumor patients. Due to the eloquent location, it is often unfeasible to obtain tissue samples at recurrence making it impossible to assess how much of a chemotherapeutic agent is being delivered to its intended target. It is also possible that a targeted agent may fail, not due to being a poor genetic target, but rather because the drug is not reaching that target in adequate quantity to cause the desired or intended treatment effect.

Additionally, tumor heterogeneity and clonal evolution is an issue that may affect targeted therapies. GBM's are heterogenous in nature, and it is possible that when one cell group is inhibited via a targeted therapy to one pathway, another is left to proliferate unabated as their development is uninhibited.

While the results of targeted therapy studies to date in glioblastoma have been disappointing, there continues to be enthusiasm in this approach with numerous clinical trials currently underway. Hopefully, knowledge from the previous failed trials will help provide further insight and assist future clinicians in designing new novel targeted treatments to overcome these barriers.

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