Published in final edited form as:
Curr Cancer Drug Targets. 2010 December ; 10(8): 840–848.

Targeting Ras-RAF-ERK and its Interactive Pathways as a Novel Therapy for Malignant Gliomas

H.-W. Lo*
Division of Surgical Sciences, Department of Surgery, Duke University School of Medicine, Duke Comprehensive Cancer Center and Duke Brain Tumor Center, 103 Research Drive, Durham, NC 27705, USA

Abstract

Malignant gliomas are the most common and the deadliest brain malignancies in adults. Despite the lack of a complete understanding of the biology of these tumors, significant advances have been made in the past decades. One of the key discoveries made in the area of malignant gliomas is that these tumors can be induced and maintained by aberrant signaling networks. In this context, the Ras pathway has been extensively exploited, from both basic and translational perspectives. Although somatic oncogenic mutations of Ras genes are frequent in several cancer types, early investigations on gliomas revealed disappointing facts that the Ras mutations are nearly absent in malignant gliomas and that the BRAF mutations are present in a very small percentage of gliomas. Therefore, the observed deregulation of the Ras-RAF-ERK signaling pathway in gliomas is attributed to its upstream positive regulators, including, EGFR and PDGFR known to be highly active in the majority of malignant gliomas. In contrast to the initial negative results on the somatic mutations of H-Ras, K-Ras and BRAF, recent breakthrough studies on pediatric low-grade astrocytomas uncovered genetic alterations of the BRAF gene involving copy number gains and rearrangements. The 7q34 rearrangements result in a novel in-frame KIAA1549:BRAF fusion gene that possesses constitutive BRAF kinase activity resembling oncogenic BRAF (V600E). In light of the earlier findings and recent breakthroughs, this review summarizes our current understanding of the Ras-RAF-ERK signaling pathway in gliomas and the outcome of preclinical and clinical studies that evaluated the efficacy of Ras-targeted therapy in malignant gliomas.

Keywords

Akt; Avastin; BRAF; chemotherapy; EGFR; glioma; PDGFR; RAF; Ras

INTRODUCTION

The Ras signaling pathway has been extensively studied since its initial discoveries and characterization in the 1980's [1–10]. Studies over the past three decades have led to many insights into the multi-face nature the Ras pathway and its broad impact not only on oncogenesis and tumor biology, but also on other diseases and normal development [11, 12]. As a result of these efforts, the degrees of importance of the Ras pathway have grown exponentially and the excitement towards the Ras pathway continues to escalate among the scientific society. Notably, the Ras pathway is extremely complex and is consisted of a

*Address correspondence to this author at the Division of Surgical Sciences, Department of Surgery (Box 3156), Duke University School of Medicine, 433A MSRB I, 103 Research Drive, Durham, NC 27705, USA; Tel: 919-668-6792; Fax: 919-684-5483; huiwen.lo@duke.edu.

number of upstream activation factors [13], three Ras proteins that are functionally overlapping, multiple direct downstream effectors (RAF, MEK and ERK) and other peripheral downstream signaling modules (PI3-K-mTOR-Akt, TIAM1-Rac, Ral, and PLC-PKC) [11, 12].

The basic understanding of the Ras-RAF-ERK signaling pathway gained in the past decades has since built strong rationales for developing therapeutic interventions that target the components of this critical pathway [14, 15]. Consequently, inhibitory agents including small molecular weight inhibitors, peptides and anti-sense oligonucleotides have been developed to target (1) various enzymes that catalyze translational modifications of the Ras proteins, (2) the kinase activity of the RAF, MEK and ERK Ser/Thr kinases, and (3) the molecules upstream and downstream of the Ras pathway (EGFR, PDGFR, mTOR and PKC). Several of these agents have shown promising clinical activity towards renal cell carcinoma, hepatocellular carcinoma, non-small cell lung cancer (NSCLC) and colorectal cancer. Many clinical trials are ongoing to evaluate the efficacy of Ras-RAF inhibitors in many different cancer types. One of these agents, sorafenib, has been approved by the FDA in 2005 for the treatment of advanced renal cell carcinoma and hepatocellular carcinoma. In addition to the use as single agent, sorafenib appears to synergize with other targeted therapies in preclinical studies and consequently, sorafenib-based combinational targeted therapy has been evaluated clinically[1] [16]. Combination of sorafenib with chemotherapeutic agents and/or radiation therapy is also actively pursued in several clinical trials [17]. Most recently, genome- wide RNA interference screens have been used to identify genes undergoing interactions with oncogenic K-Ras in cancer cells. *Via* this approach, studies led by Hahn and Gillard [18], Elledge [19] and Settleman [20] identified STK33, PLK1, Syk, Ron kinases and integrin β6 as novel therapeutic targets for cancers harboring the K-Ras oncogene.

Astrocytoma is the most common central nervous system (CNS) tumor in both adults and children. The World Health Organization broadly divides astrocytomas into grades I, II, III and IV tumors based on the histopathologic characteristics observed under light microscopy. Low-grade astrocytomas (grade I and II) are relatively rare in adults but are the predominant type of astrocytoma in children. In particular, pilocytic astrocytoma (also known as juvenile/ pediatric pilocytic astrocytoma and a subclass of grade I astrocytoma) is the most frequent type of astrocytoma in children and young adults. Grade I astrocytoma is recognized as clinically, genetically and histopathologically distinct from the infiltrating astrocytomas." Although low-grade astrocytomas are generally considered benign tumors, adult grade II astrocytomas can progress to malignant grade III astrocytoma. The WHO classification recognizes grades II–IV astrocytomas as infiltrating astrocytoma with increasing histopathological malignancy, and the potential for progression from lower to higher grade.

Grade III astrocytoma (anaplastic astrocytoma; AA), grade IV astrocytoma (glioblastoma multiforme; glioblastoma; GBM), anaplastic oligodendroglioma, anaplastic oligoastrocytoma and anaplastic ependymoma make up the high-grade gliomas and are commonly referred to malignant gliomas. GBM are highly mitotic, necrotic, vascularized and invasive. While the low-grade astrocytomas are relatively rare in adults, GBM is the most frequent astrocytoma as well as the most common brain malignancy in adults. Regardless of age, GBM is the deadliest brain tumor and the most refractory to therapy. Unfortunately, patients with GBM survive only, approximately, one year after diagnosis and less than 10% of them survive beyond two years [21, 22]. This poor prognosis is, in part,

[1]Prados, M.; Gilbert, M.; Kuhn, J.; Lamborn, K.; Cloughesy, T.; Lieberman, F.; Puduvalli, V.; Robins, H. I.; Lassman, A.; Wen, P. Y. Phase I/II study of sorefenib and erlotinib for patients with recurrent glioblastoma (GBM) (NABTC 05-02). *J. Clin. Oncol. (Meeting Abstracts)* **2009,** *27*, 2005.

due to our insufficient understanding of the complex aggressive nature of these tumors and the lack of effective therapy. As such, vigorous efforts are ongoing to either improve current therapy or to identify and strike new molecular targets for GBM therapy. A number of genes and pathways have emerged as attractive therapeutic targets for GBM and AAs, such as, EGFR/EGFRvIII [23–27], Hedgehog [28, 29], PI-3K/mTOR, PDGFR and VEGF [30–33].

Emerging evidence indicates that the Ras-RAF-ERK signaling axis may be an important therapeutic target for malignant gliomas and pediatric pilocytic astrocytomas. The Ras-RAF-ERK signaling pathway is hyperactive in malignant gliomas due to overexpression and/or increased activity attributed to their upstream regulators, such as, EGFR and PDGFR [27], but not somatic mutations of the Ras and BRAF genes [34–36]. Most recent discoveries of the BRAF and RAF-1 gene copy number gains, rearrangements and the resulted constitutive active fusion proteins in pediatric pilocytic astrocytomas have drawn significant attention and provided novel genetic mechanisms by which the Ras-RAF signaling can be permanently activated [35, 37–42]. Importantly, results of the genetic and tissue-specific gene transfer studies indicate that normal astrocytes and neuronal progenitors undergo malignant transformation to malignant gliomas when forced to express oncogenic Ras and BRAF, highlighting a pivotal role of the Ras-RAF abnormality in glioma genesis [43–50]. Additional evidence further indicates that Ras-RAF expression in malignant gliomas is essential for the maintenance of tumor proliferation [51]. As a consequence, a number of preclinical and clinical studies have been conducted to determine the efficacy of Ras/RAF/ERK-targeted therapy in malignant gliomas. Given the highly interactive nature of the Ras pathway, anti-Ras therapy in combination with other targeted therapy, chemotherapy and radiation therapy is being evaluated in clinical trials of malignant gliomas. In light of these recent discoveries and emerging findings, this review will be primarily focused on the nature of the Ras-RAF-ERK signaling axis in gliomas and its potential as a novel therapeutic target in these tumors.

SOMATIC MUTATIONS OF RAS AND RAF GENES IN GLIOMAS

Discoveries of H-Ras mutations in 1980's [6, 8–10] have led to the accepted notion that the Ras genes are the most common targets for somatic gain-of-function mutations in human cancers. It is estimated that 15–30% of human cancer carries oncogenic Ras. Specifically, mutations in codon 12, 13, or 61 of one of the three Ras proto-oncogenes (H-Ras, K-Ras and N-Ras) transform them into oncogenes. Incidences of these Ras oncogenic mutations, however, vary considerably among different types of cancers [11, 12, 52]. For example, approximately 90% of the pancreatic adenocarcinomas, 50% of the colon and thyroid tumors, and 30% of the lung cancer and myeloid leukemia were reported to carry oncogenic Ras genes [52].

In malignant gliomas, however, Ras somatic mutations are very rare. Bos *et al.* [34] evaluated 30 primary GBM tumors for Ras mutations and did not find any specimen to carry the mutant genes. Knobbe *et al.* [36] screened 94 primary GBMs for mutations in the three Ras genes and failed to find any of the tumors to carry H-Ras and K-Ras mutations and only two GBMs (2.1%) to express mutated N-Ras (G12D). In line with these observations, The Cancer Genome Atlas (TCGA) pilot project reported a 2% mutation rate in the Ras genes in 206 GBM samples [53]. Sharma *et al.* [54] identified one of 21 pilocytic astrocytomas to carry the K-Ras (G13R) mutation but did not detect any H-Ras or N-Ras mutations in the tumor cohort. Similarly, another study led by Janzarik *et al.* [55] identified one pilocytic astrocytoma out of 25 low-grade astrocytomas to contain the somatic G12A K-Ras mutation and did not find any mutations in the H-Ras and N-Ras genes. Despite a low incidence of Ras mutations in GBM, TCGA pilot project found that at least 23% harbored somatic neurofibromatosis 1 (NF1) gene inactivating mutations or deletions [53] albeit earlier studies

reported a lower frequency of NF1 mutations [56]. The NF1 gene product, neurofibromin, is known to antagonize Ras function [57]. Children with the NF1 develop optic pathway glioma [57]. These data, together, indicate that the Ras genes are rarely mutated in malignant and benign gliomas and that its hyperactivation in these tumors is potentially due to activation of NF1, a negative regulator of Ras.

More recently, much attention was drawn to activation mutations of the BRAF gene that encodes for a cytoplasmic Ser/Thr kinase that is regulated by Ras. The BRAF somatic missense mutations are present within the kinase domain in which a single substitution (V599E; also named as V600E) accounts for 80% of all BRAF mutations and transforms NIH3T3 cells [58]. BRAF mutations are frequent in several types of human cancers including the majority of malignant melanomas and melanoma metastases [58, 59]. Analyses of glioma cell lines and primary specimens, however, revealed a lower frequency of BRAF mutation. Davies *et al.* [58] reported that 4/38 (11%) of glioma cell lines carried the BRAF (V600E) mutation whereas 0/15 of primary gliomas contained any BRAF mutations. A study led by Knobbe *et al.* [36] examined 94 GBMs and found only three tumors (3.2%) to carry the oncogenic BRAF (V600E) gene. In line with these observations, Bastro *et al.* [60] reported the presence of BRAF (V600E) hot-spot mutation in 6% of 34 GBMs and 0% of 13 AAs. Results of more recent studies [61, 62] corroborated these earlier observations. Interestingly, Jones *et al.* [41] recently reported the identification of a novel BRAF mutation from a pilocytic astrocytoma that is the result of a three nucleotide insertion at codon 598 of the BRAF gene. Despite its low frequency (1/44), this novel BRAF mutant mimics the hotspot V600E mutation and is constitutively active and transforming [41]. Together, these findings indicate that BRAF activation mutations are not a frequent event in gliomas.

BRAF COPY NUMBER GAIN AND FUSION IN GLIOMAS

Comparative genomic hybridization that detects copy number changes has yielded striking findings that gliomas carry extra copies of the wild-type BRAF gene [35, 61]. In 2007, Jeukin *et al.* [35] reported that 38 out of 87 (44%) of analyzed gliomas contained an increased number of the wild-type BRAF gene at chromosome 7q34 and this gain is more predominant in high-grade gliomas. A significant correlation was found between activated MAPK and BRAF copy number gains. Furthermore, a study by Pfister *et al.* [61] evaluated 66 pediatric low-grade gliomas and found 44% of the tumors to carry BRAF gene duplication. Tumors with BRAF gene duplication also expressed increased BRAF transcripts and its downstream target genes. The high incidences of BRAF copy number gain in pediatric pilocytic astrocytomas were further confirmed by a number of independent studies [40, 63, 64].

In addition to copy number gains, Jones *et al.* [40] recently reported that the tandem duplication of ~2 Mb at 7q34 results in an in-frame fusion gene incorporating the kinase domain of the wild-type BRAF gene but lacking the auto-inhibitory N-terminus. The resulted KIAA1549:BRAF fusion gene encodes for a fusion protein displaying constitutive BRAF kinase activity to the level similar to the oncogenic BRAF (V600E). Strikingly, this novel fusion gene is present in 66% of the 44 pilocytic astrocytomas examined but is absent in a cohort of 244 high-grade astrocytomas [40]. Similar findings were subsequently reported by a number of independent studies [37–39, 42, 65]. Sievet *et al.* [37] identified the majority (17/22, 77%) of pediatric pilocytic astrocytomas to express the KIAA1549:BRAF fusion gene. In a cohort of 70 pilocytic astrocytomas and 50 grade II astrocytomas, Korshunov *et al.* [38] found the KIAA1549:BRAF fusion gene to be present in 76% of the former tumors but is absent in the latter. A study by Forshew *et al.* [39] found the majority of the posterior fossa pilocytic astrocytoma cohort to carry the fusion gene product between

KIAA1549 and BRAF genes. Schiffman *et al.* [65] found 10 of the 10 pediatric pilocytic astrocytomas, but none of the grades II–IV astrocytomas, to express the KIAA1549:BRAF transcript. Yu *et al.* [42] reported that BRAF rearrangements are present in 42 of the 70 sporadic pilocytic astrocytomas analyzed and that they are frequent in pilocytic astrocytomas located in the cerebellum (74%), brainstem (63%) and the 4th ventricle (50%), but is absent in optic nerve and midbrain. This interesting site-specific distribution of BRAF rearrangement is also reported by other studies [64, 66]. Taken, together, these interesting observations warrant future investigations that determine whether BRAF gene duplication and rearrangements are the cause of pilocytic astrocytomas and whether these genetic alterations are also present in other cancer types with increased BRAF activity.

Interestingly, in addition to the KIAA1549:BRAF genetic fusion, two independent studies reported the existence of a novel inframe genetic fusion of the RAF-1 and SRGAP3 (Slit-Robo GTPase-activating protein 3) genes [39, 41]. Both studies identified tandem duplication at chromosome 3p25 which produces an inframe fusion between SRGAP3 and RAF-1. SRGAP3 protein is consisted of an amino-terminal Fes/CIP4 homology domain, a Rho GTPase-activating protein domain and a Src homology 3 domain and is expressed in the brain that is associated with severe mental retardation [67]. It also plays a role in cell movement through interactions with the cytoskeleton [68]. Consistent with the fact that the SRGAP3:RAF-1 fusion protein retains the RAF-1 kinase domain but lacks the N-terminal auto-inhibitory region, the fusion protein contains increased kinase activity compared to wild-type RAF-1. The incidence of this novel fusion, however, is very low in low-grade astrocytomas with reported 1/44 [41] and 1/32 [39] of the low-grade astrocytomas carrying the rearranged gene product.

Taken together, recent discoveries of BRAF and RAF-1 genetic alterations point to a new avenue of research that will shed new light onto the mechanisms by which the Ras signaling is activated in low-grade astrocytomas. These findings also provide a rationale to target RAF proteins as a novel treatment option for low-grade astrocytomas, the most frequent brain tumor in children. Given the consistent high incidences of the rearranged KIAA1549:BRAF fusion gene in pilocytic astrocytomas, it is of importance to conduct *in vivo* animal studies in order to determine whether the rearrangement event is a cause for the formation of these tumors. This investigation is essential given the facts that low-grade astrocytomas are the most frequent CNS tumors in children and its recurrence is common following surgical removal and that in adults, low-grade gliomas can undergo malignant transformation into malignant highgrade gliomas [69]. Furthermore, in light of the fact that BRAF oncogenic mutation predicts sensitivity to MEK inhibition [70], it is worth investigating whether MEK inhibition can be used to treat pilocytic astrocytomas with the BRAF fusion protein that resembles the activity of oncogenic BRAF protein. Also critically important is that these exciting emerging data indeed prompt a need to extend the structural and functional investigations of the BRAF/RAF-1 fusion gene products from astrocytomas to other types of tumors.

RAS-RAF IN GLIOMA GENESIS AND MAINTENANCE

The Ras-RAF-ERK signaling pathway is hyperactive in gliomas due to the increased activity of its upstream regulators, such as, EGFR and PDGFR. To gain a better understanding of the effects of deregulated Ras-RAF-ERK signaling axis on glioma genesis and other glioma-associated phenotypes, a number of studies have utilized the oncogenic Ras genes to create cells with increased Ras activity albeit the Ras genes are rarely mutated in gliomas. In 2000, Holland *et al.* [45] engineered tissue-specific viral expression vectors to specifically express K-Ras (G12D) and Akt in astrocytes (*via* the promoter for the gene encoding glial fibrillary acidic protein, GFAP) and neural progenitors (*via* the nestin

promoter) in mice. Albeit neither K-Ras (G12D) nor Akt alone was sufficient to induce GBM formation, their combination induced the formation of high-grade gliomas with the histological features of human GBMs [45]. These investigators further demonstrated that K-Ras (G12D) expression cooperated with loss of INK4a-Arf locus to induce malignant transformation of astrocytes and neural progenitors into GBMs in mice [49]. These results were in part supported by a more recent study by de Vries *et al.* [71]. More recently in 2008, these researchers subsequently found that constitutive activation of RAF-1 cooperates with Akt activation or loss of INK4a-Arf to induce glioma oncogenesis in mice [46].

In line with these observations, Robinson *et al.* [50] recently reported that combined expression of oncogenic BRAF (V600E) and activated Akt or loss of INK4a-Arf in neuronal progenitors induced GBM formation in nude mice. Similar to the observation with K-Ras (G12D), these investigators found that the expression of activated BRAF alone was not sufficient for glioma genesis [50]. The same study also reported that BRAF (V600E) generated GBMs with characteristics similar to K-Ras (G12D) in the context of Akt but not loss of INK4a/Arf. These findings were subsequently confirmed by a recent study [50]. *Via* a combination of genetically modified human astrocytes and subsequent implantation into nude mice, Sonoda *et al.* [48] demonstrated that H-Ras (G12V) cooperated with expression of human telomerase catalytic component (hTERT) and inactivation of tumor suppressors Rb/p53, thereby transforming normal astrocytes into AAs. When H-Ras (G12V) was replaced by myristoylated Akt or activated EGFR, the genetically modified astrocytes surprisingly did not undergo malignant transformation [48]. Taken together, the findings from the tissue-specific gene transduction model suggest that activation of Ras, RAF-1 and BRAF alone may not be sufficient for glioma genesis, additional activation of other oncogenes or loss of tumor suppressor genes are required for glioma oncogenesis, and that the oncogenic effects of K-Ras (G12D) may be partially transduced through the RAF-1 and BRAF downstream effectors.

In contrast, results from the transgenic mouse model suggest that activated Ras alone is sufficient to transform normal astrocytes and neuronal precursor cells into malignant gliomas [43, 44, 47]. Using a GFAP-driven H-Ras (G12V) transgenic mouse model, Ding *et al.* [44] showed that high levels of H-Ras (G12V) alone in astrocytes yielded multifocal malignant gliomas and the tumor-bearing mice died within two weeks. The mice with moderate levels of H-Ras (G12V) underwent germ-line transmission in which 95% of these mice died from solitary or multifocal low- and high-grade gliomas within 2–6 months. Yielded gliomas are similar to human astrocytomas in their phenotypes, including, high mitotic index, nuclear pleomorphism, infiltration, necrosis, and increased tumor vasculature [44, 47]. Furthermore, Abel *et al.* [43] recently showed that expression of K-Ras (G12D) alone in mouse glioneuronal precursor cells and adult subventricular zone cells (a stem/progenitor cell niche in the mature brain) induced formation of infiltrating gliomas resembling human high-grade gliomas.

In addition to an essential role in glioma genesis, activated Ras-RAF signaling axis may also be important for the maintenance of malignant gliomas. Holmen and Williams [51] developed a viral vector that allows the tumoric expression of activated K-Ras to be controlled *via* a tet-off system. Following tumor formation, inhibition of K-Ras expression (by doxycycline) resulted in tumoric apoptosis and regression. Re-expression of activated K-Ras (by removing doxycycline) reinitiated tumor growth. These results suggest that some progenitor cells may have survived in the absence of activated K-Ras and subsequently developed into gliomas when K-Ras was re-expressed.

Additional evidence further supports the notion that deregulated Ras-RAF pathway, independent of somatic mutations, plays an important role in the biology of malignant

gliomas. For example, the levels of RAF-1 and BRAF proteins and RAF kinase activity were found to be increased in primary GBM specimens [46]. Human malignant gliomas frequently overexpress all three RAF proteins [62]. Increased expression of BRAF due to copy number gains is a frequent event in low-grade pediatric astrocytomas [35, 61, 69]. Moreover, increased Ras-RAF-ERK activity is a common characteristic of malignant gliomas with deregulated EGFR and PDGFR pathways [23, 72]. Conversely, inhibition of Ras-RAF-ERK through transcriptional down-regulation and small molecular inhibitors led to glioma growth reduction (discussed in details in next section). In contrast to these observations, a recent report by Lymbouridou *et al.* [73] showed that primary GBM tumors (N=21) expressed significantly lower levels of K-Ras and H-Ras transcripts compared to normal brain tissues (N=15), albeit all tissue did not express detectable levels of Ras proteins. There was no association between K-Ras/H-Ras expression levels and survival rates in the GBM cohort [73].

THERAPEUTIC TARGETING RAS-RAF-ERK IN MALIGNANT GLIOMAS

Farnesyltransferase Inhibitors (FTIs)

Ras proteins undergo post-translational modifications in which prenylation is the first modification and is required for the transforming activity of Ras [74]. Farnesyltransferase (FTase) catalyzes the transfer of a 15 carbon isoprenyl lipid from farnesyl diphosphate to a cysteine residue in the C-terminal CAAX box (C=Cystein, A=aliphatic amino acid, X= C-terminal amino acid) of various proteins, including the three Ras proteins [75]. In the attempt to inhibit Ras prenylation and thus Ras-mediated transformation, a number of FTIs have been developed and evaluated in preclinical and clinical studies. FTI 276 Ras-CAAX peptidomimetics demonstrated antitumor activity in lung xenografts [76]. Another FTI L-744.832 showed growth-inhibitory activity towards N-Ras-overexpressing transgenic mice with mammary, lymphoid and salivary tumors [77, 78]. An orally active, non-peptidic, small molecule FTI SCH 66336 (lonafarnib; Sarasar; Schering-Plough) competes with the CAAX substrate to inhibit Ras processing in tumor cells both *in vitro* and *in vivo* [79].

A phase I trial of lonafarnib conducted by Kieran *et al.*[2] [80] enrolled 59 children with progressive or recurrent brain tumors (Table 1). The results indicated that lonafarnib appeared to be well-tolerated, able to cross blood-brain barrier and to show modest antitumor effects. The efficacy of another FTI, tipifarnib (Zarnestra, R115777; Johnson & Johnson), was examined in a phase II study [81] in recurrent malignant gliomas. The results showed 6-month progression-free survival (PFS) to be 9% in recurrent AAs (N=22) and 12% in recurrent GBMs (N=67), indicating that tipifarnib had limited effects on these patients. This modest outcome is in line with the results of other clinical trials of FTIs as single agent and in combination with chemotherapy in other cancer types [75, 82]. The lack of clinical efficacy of FTIs is likely due to the fact that unlike H-Ras, N-Ras and K-Ras become geranylgeranylated at the C-terminus following FTI treatments [11]. This additional modification renders N-Ras and K-Ras resistant to FTI-mediated inactivation [83].

Perillyl alcohol (POH), a naturally occurring monoterpene, has been shown to inhibit FTase activity [84]. Intranasal delivery was used to administer POH in both animals and patients with malignant gliomas in order to bypass the blood-brain barrier and thus to effectively enter the brain. Results of *in vitro* studies showed that POH resulted in a transient arrest in the G2/M phase of the cell cycle [85] and induced apoptosis [86] in human GBM cells. POH treatment also sensitized glioma cells to Fas-, cisplatin-, doxorubicin- and radiation-mediated apoptosis [85]. Several Brazilian clinical trials on POH have been conducted in

[2]Kieran, M. W.; Packer, R.; Boyett, J.; Sugrue, M.; Kun, L. Phase I trial of the oral farnesyl protein transferase inhibitor lonafarnib (SCH66336): A Pediatric Brain Tumor Consortium (PBTC) study. *J. Clin. Oncol. (Meeting Abstracts)* **2004,** *22*, 1517.

patients with recurrent malignant gliomas [84, 87, 88]. A phase I/II study [87] on 37 adult patients with recurrent malignant gliomas reported encouraging results with 6-month PFS rates of 48.2% and 60% for GBMs and AAs, respectively (Table 1). No toxicity was noted.

Inhibitors of Other Ras-Processing Enzymes

After FTase-mediated prenylation, the prenylated Ras proteins undergo two additional posttranslational modifications. First, the last three amino acids (AAX) of the Ras proteins are proteolytically cleaved by the protease, Ras-converting enzyme 1 (RCE1). Second, the last cysteine residue undergoes carboxymethylation that is catalyzed by the methyltransferase, protein-S-isoprenylcysteine O-methyltransferase 1 (ICMT1, also named as prenylated protein methyltransferase PPMT). These post-prenylation modifications are important for the association of Ras proteins with membranes. Interestingly, Bergo *et al*. [89] generated mice with a conditional RCE1 allele that is subjected to Cre-mediated excision and found that the loss of RCE1 reduced Ras-induced transformation *in vitro*. Using the same strategy, inactivation of ICMT1 has been shown to inhibit oncogenic K-Ras and BRAF-mediated transformation of normal fibroblasts [90].

Similar to expression knockdown, the ICMT1 inhibitor transfarnesylthiosalicylate (FTS) inhibits Ras activation, results in GBM cell death and suppresses oncogenic ability of U87MG GBM cells [91]. FTS treatment also down-regulates expression of hypoxia-inducible factor-1α and its downstream target genes, including, VEGF and the Glut-1 glucose transporter. Consequently, glycolysis was shut down resulting in significant GBM cell death [91]. FTS appears to also negatively impact cell cycle progression and promote apoptosis by inhibiting expression of E2F1 and survivin, respectively [92, 93]. Corroborating these observations, Amos *et al*. [94] reported that FTS increased the activities of both caspase-3 and -9, leading to significant apoptosis in GBM cells. FTS-mediated apoptosis is also observed in GBM cells with high EGFR expression, suggesting that inhibition of Ras protein methylation may provide a new therapeutic option for GBMs overexpressing EGFR [94].

Inhibitors of RAF-1

A number of inhibitors including small molecular kinase inhibitors and anti-sense oligonucleotides have been developed to target RAF [14, 95]. In GBM cells, an orally active RAF-1/VEGFR kinase inhibitor AAL881 has been shown to block the growth of malignant gliomas *in vitro* and *in vivo* [96]. AAL881 inhibited proliferation and induced apoptosis of cultured GBM cells. It also decreased the proliferation of bovine aortic endothelial cells and the ability of GBM cells to secret VEGF, as well as, inhibited GBM cell invasion. AAL881 treatment also extended the survival of orthotopic glioma xenografts-bearing nude mice. Another RAF-1 inhibitor GW5074 has been shown to synergize with MEK inhibitor U0126 and other anti-cancer drugs to target GBM cells [97].

Sorafenib (Nexavar; BAY 43–9006)

Sorafenib is a multikinase inhibitor that targets RAF-1, PDGFR, and VEGFR [98, 99]. It has been approved by the FDA for the treatment of advanced renal cell carcinoma and hepatocellular carcinoma. It is probably by far the most promising and most extensively studied Ras-RAF inhibitor. Efficacy of sorafenib as single agent and in combination with other targeted therapy or chemotherapeutic agents, is being evaluated in many clinical trials in several different cancer types, including, malignant gliomas. *In vitro* studies showed that combination of sorafenib with rottlerin (PKC-δ inhibitor) potently inhibits proliferation and migration of human malignant glioma cells [100]. This combination is based on the fact that RAF-1 is phosphorylated and activated by PKC [101, 102]. Furthermore, sorafenib and bortezomib (protease inhibitor; MG132) synergistically induced GBM apoptosis [103]. A

phase I sorafenib trial (Trial number NABTT 0401) was conducted by Nabors *et al*.[3] in a total of 35 patients with malignant gliomas. Sorafenib was reported to be well tolerated with limited toxicities in these patients (Table 1). Another phase I trial of sorafenib is ongoing for patients with recurrent or progressive malignant gliomas (Trial number NCT00093613).

Several sorafenib-based combinations have been evaluated in clinical trials in patients with malignant gliomas. For example, sorafenib was combined with small molecule EGFR kinase inhibitors. It is worth noting that three small molecular weight EGFR kinase inhibitors and two monoclonal anti-EGFR antibodies have been approved by the FDA for treating cancer patients [27, 104]. (1) Gefitinib (ZD1839; Iressa) is a small molecular weight EGFR kinase inhibitor approved for locally advanced and metastatic non-small cell lung cancer, NSCLC. (2) Erlotinib (OSI-774; Tarceva), a small molecule EGFR kinase inhibitor, was approved to treat metastatic NSCLC as single agent and in combination with gemcitabine for pancreatic cancer that is unable to be removed by surgery or has metastasized. (3) Lapatinib (GW572016; Tykerb/Tyverb) is an EGFR/Her-2-dual targeting small molecule inhibitor approved to be combined with other anti-cancer drugs to treat advanced or metastatic breast cancer [105]. It is used in patients whose cancer is Her-2 positive and has failed to respond to other drugs. (4) Cetuximab (C225; Erbitux) is a humanized monoclonal antibody that recognizes the extracellular domain of both EGFR [105] and EGFRvIII [106] and has been approved for squamous cell carcinoma of the head and neck that has metastasized or recurred after other chemotherapy. Cetuximab is also approved for treating metastatic colorectal cancer that has metastasized after other chemotherapy has failed and for combined used with irinotecan for metastatic colorectal cancer patients who have not responded to irinotecan alone. (5) Panitumumab (ABX-EGF; Vectibix) is a human monoclonal antibody raised against the extracellular domain of EGFR [107]. All but panitumumab have been evaluated in clinical trials for patients with malignant gliomas.

A phase I trial by Kuhn *et al*.[4] evaluated the pharmacological interaction between sorafenib and erlotinib. The results indicate that although the pharmcokinetic (PK) for sorafenib was not affected by erlotinib, there was an apparent effect of sorafenib on that of erlotinib. This adverse effect was similarly reported with the co-administration of sorafenib with gefitinib in a phase I non-small cell lung cancer trial [16]. A more recent phase I/II study of sorafenib and erlotinib for patients with recurrent GBM (Trial number NABTC 05–02) just reported their phase I results and preparing those of the phase II study[1]. PK results from the phase I study showed that although there was no alterations in sorafenib PK, accumulation of erlotinib was very low, suggesting a drug-drug interaction with sorafenib altering erlotinib metabolism or clearance. Another phase II trial (Trial number NCT00445588) of sorafenib plus erlotinib for patients with progressive or recurrent GBM has been recently completed and the results are forthcoming. Based on these observations, future efforts are required to minize the negative impact of sorafenib on the metabolism of erlotinib and gefitinib.

In addition to combining with EGFR inhibitors (erlotinib and gefitinib), sorafenib has been tested in combination with temsirolimus (mTOR inhibitor) for treating GBM patients. A recently completed phase I/II study led by Wen *et al*.[5] reported that although PK data indicated no significant interaction between the two drugs, no patient remained progression

[3]Nabors, L. B.; Rosenfeld, M.; Chamberlain, M.; Phuphanich, S.; Batchelor, T.; Supko, J.; Desideri, S.; Xiaobu, Y.; Wright, J.; Grossman, S. A phase I trial of sorafenib (BAY 43-9006) for patients with recurrent or progressive malignant glioma (NABTT 0401). *J. Clin. Oncol. (Meeting Abstracts)* **2007,** *25*, 2058.

[4]Kuhn, J. G.; Gilbert, M.; Wen, P.; Cloughesy, T.; Cooper, J.; Puduvalli, V.; DeAngelis, L.; Lieberman, F.; Lamborn, K.; Prados, M. Interaction between sorafenib and erlotinib. *J. Clin. Oncol. (Meeting Abstracts)* **2009,** *27*, 2500.

[5]Wen, P. Y.; Cloughesy, T.; Kuhn, J.; Lamborn, K.; Abrey, L. E.; Lieberman, F.; Robins, H. I.; Wright, J.; Prados, M. D.; Gilbert, M. Phase I/II study of sorafenib and temsirolimus for patients with recurrent glioblastoma (GBM) (NABTC 05-02). *J. Clin. Oncol. (Meeting Abstracts)* **2009,** *27*, 2006.

free at 6 months. As such, the study was terminated and did not proceed to the second stage of the phase II study. Another phase I/II trial with the sorafenib-temsirolimus combination is ongoing in treating patients with recurrent GBM (Trial number NCT00329719). An active phase I/II trail is evaluating the effects of sorafenib in combination with erlotinib, tipifarnib, or temsirolimus in patients with recurrent GBM or gliosarcoma (Trial number NCT00335764).

A major characteristic of malignant gliomas is the prominent microvascular proliferation. Consequently, targeting GBM vasculature is an attractive approach to reduce GBM growth. An anti-VEGF monoclonal antibody, bevacizumab (Avastin; Genentech), has been proven to be effective in targeting GBM in preclinical studies. In May 2009, bevacizumab was approved by the FDA to treat GBM that have progressed. This approval was based on the promising results of two clinical trials, NCT00345163 conducted by Friedman *et al.* [32] with 167 patients and a NCI study 06-C-0064E (56 patients). Overall results from the two studies showed that responses were observed in 20–26% of patients and the median duration of response was approximately 4 months. In light of these encouraging clinical results, a number of clinical trials are ongoing to examine the efficacy of bevacizumab in combination with other anti-cancer drugs, including, sorafenib. An active phase II trial is examining the bevacizumab-sorafenib combination in patients with recurrent GBMs (Trial number NCT00621686-55415).

Radiation therapy and use of temozolomide, a DNA-damaging alkylating agent, are standard therapies for patients with malignant gliomas. A phase II trial of radiation therapy/temozolomide followed by sorafenib was conducted as the first-line treatment in GBM[6]. While the full results are being collected and computed, preliminary results showed median PFS for all patients with and without sorafenib to be six months. Median PFS for patients who received at least one dose of sorafenib was also six months. Although the addition of sorafenib to standard treatments (radiation therapy/temozolomide) is feasible and well tolerated by most patients, sorafenib did not demonstrate increased benefit compared to standard therapy. Several other trials are actively investigating the efficacy of sorafenib in combination with temozolomide (Trial numbers NCT00597493 and NCT00544817) in recurrent GBMs and with radiation therapy/temozolomide in newly diagnosed GBM patients (Trial numbers NCT00884416 and NCT00734526).

CONCLUDING REMARKS

Patients with malignant gliomas have dismal prognosis who are in an urgent need of more effective therapy. In light of encouraging preclinical results, anti-Ras/RAF therapy is being actively exploited as mono and combination therapies for patients with these aggressive tumors. Based on the FDA clinical trial database (http://clinicaltrials.gov), as least eight phases I and II clinical trials are being conducted to evaluate the effects of sorafenib in patients with malignant gliomas. Several recently completed clinical trials with sorafenib are expected to report their results in the near future. Complementary to translational and clinical studies, genetic analyses have yielded breakthrough discoveries on BRAF gene duplication and rearrangements that are drawing additional enthusiasm to this already exciting field and pointing to a new direction of Ras-RAF-ERK research in brain tumors and potentially in other malignancies and developmental disorders.

[6]Lamar, R. E.; Spigel, D. R.; Burris, H. A.; Markus, T. M.; Kuzur, M.; Ervin, T.; Fichtel, L.; Greco, F. A.; Hainsworth, J. D. Phase II trial of radiation therapy/temozolomide followed by temozolomide/sorafenib in the first-line treatment of glioblastoma multiforme (GBM). *J. Clin. Oncol. (Meeting Abstracts)* **2009,** *27*, 2018.

Acknowledgments

This study was supported by NIH grants (5K01-CA118423 and 2P50-NS020023-26), the Pediatric Brain Tumor Foundation and the Beez Foundation (to H.-W. L).

ABBREVIATIONS

AA	anaplastic astrocytoma
AO	anaplastic oligodendroglioma
CNS	central nervous system
EGFR	epidermal growth factor receptor
EGFRvIII	epidermal growth factor receptor variant III
FTase	farnesyltransferase
FTI	farnesyltransferase inhibitor
GBM	glioblastoma multiforme
ICMT1	protein-S-isoprenylcysteine O-methyltransferase 1
mTOR	mammalian target of rapamycin
NF1	neurofibromatosis 1
NSCLC	non-small cell lung cancer
PDGFR	platelet-derived growth factor receptor
PFS	progression-free survival
PKC	protein kinase C
POH	perillyl alcohol
PPMT	prenylated protein methyltransferase
RCE1	Ras-converting enzyme 1
SRGAP3	Slit-Robo GTPase-activating protein 3
TMZ	temozolomide
VEGF	vascular endothelial growth factor
WHO	World Health Organization

REFERENCES

1. DeFeo D, Gonda MA, Young HA, Chang EH, Lowy DR, Scolnick EM, Ellis RW. Analysis of two divergent rat genomic clones homologous to the transforming gene of Harvey murine sarcoma virus. Proc. Natl. Acad. Sci. USA. 1981; 78:3328–3332. [PubMed: 6267583]
2. Chang EH, Gonda MA, Ellis RW, Scolnick EM, Lowy DR. Human genome contains four genes homologous to transforming genes of Harvey and Kirsten murine sarcoma viruses. Proc. Natl. Acad. Sci. USA. 1982; 79:4848–4852. [PubMed: 6289320]
3. Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. Proc. Natl. Acad. Sci. USA. 1982; 79:3637–3640. [PubMed: 6285355]
4. Ellis RW, DeFeo D, Furth ME, Scolnick EM. Mouse cells contain two distinct ras gene mRNA species that can be translated into a p21 onc protein. Mo.l Cell. Biol. 1982; 2:1339–1345.
5. Parada LF, Tabin CJ, Shih C, Weinberg RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. Nature. 1982; 297:474–478. [PubMed: 6283357]

6. Reddy EP, Reynolds RK, Santos E, Barbacid M. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. Nature. 1982; 300:149–152. [PubMed: 7133135]
7. Santos E, Tronick SR, Aaronson SA, Pulciani S, Barbacid M. T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes. Nature. 1982; 298:343–347. [PubMed: 6283384]
8. Tabin CJ, Bradley SM, Bargmann CI, Weinberg RA, Papageorge AG, Scolnick EM, Dhar R, Lowy DR, Chang EH. Mechanism of activation of a human oncogene. Nature. 1982; 300:143–149. [PubMed: 6290897]
9. Taparowsky E, Suard Y, Fasano O, Shimizu K, Goldfarb M, Wigler M. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. Nature. 1982; 300:762–765. [PubMed: 7177195]
10. Capon DJ, Chen EY, Levinson AD, Seeburg PH, Goeddel DV. Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. Nature. 1983; 302:33–37. [PubMed: 6298635]
11. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. Nat. Rev. Mol. Cell Biol. 2008; 9:517–531. [PubMed: 18568040]
12. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat. Rev. Cancer. 2007; 7:295–308. [PubMed: 17384584]
13. Buday L, Downward J. Many faces of Ras activation. Biochim. Biophys. Acta. 2008; 1786:178–187. [PubMed: 18541156]
14. Beeram M, Patnaik A, Rowinsky EK. Raf: A strategic target for therapeutic development against cancer. J. Clin. Oncol. 2005; 23:6771–6790. [PubMed: 16170185]
15. Saxena N, Lahiri SS, Hambarde S, Tripathi RP. RAS: target for cancer therapy. Cancer Invest. 2008; 26:948–955. [PubMed: 18798058]
16. Adjei AA, Molina JR, Mandrekar SJ, Marks R, Reid JR, Croghan G, Hanson LJ, Jett JR, Xia C, Lathia C, Simantov R. Phase I trial of sorafenib in combination with gefitinib in patients with refractory or recurrent non-small cell lung cancer. Clin. Cancer Res. 2007; 13:2684–2691. [PubMed: 17473200]
17. Loriot Y, Mordant P, Deutsch E, Olaussen KA, Soria JC. Are RAS mutations predictive markers of resistance to standard chemotherapy? Nat. Rev. Clin. Oncol. 2009; 6:528–534. [PubMed: 19597509]
18. Scholl C, Frohling S, Dunn IF, Schinzel AC, Barbie DA, Kim SY, Silver SJ, Tamayo P, Wadlow RC, Ramaswamy S, Dohner K, Bullinger L, Sandy P, Boehm JS, Root DE, Jacks T, Hahn WC, Gilliland DG. Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. Cell. 2009; 137:821–834. [PubMed: 19490892]
19. Luo J, Emanuele MJ, Li D, Creighton CJ, Schlabach MR, Westbrook TF, Wong KK, Elledge SJ. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell. 2009; 137:835–848. [PubMed: 19490893]
20. Singh A, Greninger P, Rhodes D, Koopman L, Violette S, Bardeesy N, Settleman J. A gene expression signature associated with "K-Ras addiction" reveals regulators of EMT and tumor cell survival. Cancer Cell. 2009; 15:489–500. [PubMed: 19477428]
21. Selznick LA, Shamji MF, Fecci P, Gromeier M, Friedman AH, Sampson J. Molecular strategies for the treatment of malignant glioma--genes, viruses, and vaccines. Neurosurg. Rev. 2008; 31:141–155. [PubMed: 18259789]
22. Schwartzbaum JA, Fisher JL, Aldape KD, Wrensch M. Epidemiology and molecular pathology of glioma. Nat. Clin. Pract. Neurol. 2006; 2:494–503. quiz 491 p following 516. [PubMed: 16932614]
23. Lo HW, Cao X, Zhu H, Ali-Osman F. Constitutively activated STAT3 frequently coexpresses with epidermal growth factor receptor in high-grade gliomas and targeting STAT3 sensitizes them to Iressa and alkylators. Clin. Cancer Res. 2008; 14:6042–6054. [PubMed: 18829483]
24. Mellinghoff IK, Cloughesy TF, Mischel PS. PTEN-mediated resistance to epidermal growth factor receptor kinase inhibitors. Clin. Cancer Res. 2007; 13:378–381. [PubMed: 17255257]

25. Friedman HS, Bigner DD. Glioblastoma multiforme and the epidermal growth factor receptor. N. Engl. J. Med. 2005; 353:1997–1999. [PubMed: 16282174]
26. Karpel-Massler G, Schmidt U, Unterberg A, Halatsch M-E. Therapeutic inhibition of the epidermal growth factor receptor in high-grade gliomas: where do we stand? Mol. Cancer Res. 2009; 7:1000–1012. [PubMed: 19584260]
27. Lo HW. EGFR-targeted therapy in malignant glioma: novel aspects and mechanisms of drug resistance. Curr. Mol. Pharmacol. 2010; 3:37–52. [PubMed: 20030624]
28. Lo HW, Zhu H, Cao X, Aldrich A, Ali-Osman F. A novel splice variant of GLI1 that promotes glioblastoma cell migration and invasion. Cancer Res. 2009; 69:6790–6798. [PubMed: 19706761]
29. Zhu H, Lo H-W. The human glioma-associated oncogene homolog 1 (GLI1) family of transcription factors in gene regulation and diseases. Curr. Genomics. 2010; 11:238–245. [PubMed: 21119888]
30. Rao RD, Uhm JH, Krishnan S, James CD. Genetic and signaling pathway alterations in glioblastoma: relevance to novel targeted therapies. Front. Biosci. 2003; 8:e270–e280. [PubMed: 12700121]
31. Sandsmark DK, Pelletier C, Weber JD, Gutmann DH. Mammalian target of rapamycin: master regulator of cell growth in the nervous system. Histol. Histopathol. 2007; 22:895–903. [PubMed: 17503347]
32. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, Yung WK, Paleologos N, Nicholas MK, Jensen R, Vredenburgh J, Huang J, Zheng M, Cloughesy T. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J. Clin. Oncol. 2009; 27:4733–4740. [PubMed: 19720927]
33. Omuro AM. Exploring multi-targeting strategies for the treatment of gliomas. Curr. Opin. Investig. Drugs. 2008; 9:1287–1295.
34. Bos JL. The ras gene family and human carcinogenesis. Mutat. Res. 1988; 195:255–271. [PubMed: 3283542]
35. Jeuken J, van den Broecke C, Gijsen S, Boots-Sprenger S, Wesseling P. RAS/RAF pathway activation in gliomas: the result of copy number gains rather than activating mutations. Acta Neuropathol. 2007; 114:121–133. [PubMed: 17588166]
36. Knobbe CB, Reifenberger J, Reifenberger G. Mutation analysis of the Ras pathway genes NRAS, HRAS, KRAS and BRAF in glioblastomas. Acta Neuropathol. 2004; 108:467–470. [PubMed: 15517309]
37. Sievert AJ, Jackson EM, Gai X, Hakonarson H, Judkins AR, Resnick AC, Sutton LN, Storm PB, Shaikh TH, Biegel JA. Duplication of 7q34 in pediatric low-grade astrocytomas detected by high-density single-nucleotide polymorphism-based genotype arrays results in a novel BRAF fusion gene. Brain Pathol. 2009; 19:449–458. [PubMed: 19016743]
38. Korshunov A, Meyer J, Capper D, Christians A, Remke M, Witt H, Pfister S, von Deimling A, Hartmann C. Combined molecular analysis of BRAF and IDH1 distinguishes pilocytic astrocytoma from diffuse astrocytoma. Acta Neuropathol. 2009; 118:401–405. [PubMed: 19543740]
39. Forshew T, Tatevossian RG, Lawson AR, Ma J, Neale G, Ogunkolade BW, Jones TA, Aarum J, Dalton J, Bailey S, Chaplin T, Carter RL, Gajjar A, Broniscer A, Young BD, Ellison DW, Sheer D. Activation of the ERK/MAPK pathway: a signature genetic defect in posterior fossa pilocytic astrocytomas. J. Pathol. 2009; 218:172–181. [PubMed: 19373855]
40. Jones DT, Kocialkowski S, Liu L, Pearson DM, Backlund LM, Ichimura K, Collins VP. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res. 2008; 68:8673–8677. [PubMed: 18974108]
41. Jones DT, Kocialkowski S, Liu L, Pearson DM, Ichimura K, Collins VP. Oncogenic RAF1 rearrangement and a novel BRAF mutation as alternatives to KIAA1549:BRAF fusion in activating the MAPK pathway in pilocytic astrocytoma. Oncogene. 2009; 28:2119–2123. [PubMed: 19363522]
42. Yu J, Deshmukh H, Gutmann RJ, Emnett RJ, Rodriguez FJ, Watson MA, Nagarajan R, Gutmann DH. Alterations of BRAF and HIPK2 loci predominate in sporadic pilocytic astrocytoma. Neurology. 2009; 73:1526–1531. [PubMed: 19794125]

43. Abel TW, Clark C, Bierie B, Chytil A, Aakre M, Gorska A, Moses HL. GFAP-Cre-mediated activation of oncogenic K-ras results in expansion of the subventricular zone and infiltrating glioma. Mol. Cancer Res. 2009; 7:645–653. [PubMed: 19435821]
44. Ding H, Roncari L, Shannon P, Wu X, Lau N, Karaskova J, Gutmann DH, Squire JA, Nagy A, Guha A. Astrocyte-specific expression of activated p21-ras results in malignant astrocytoma formation in a transgenic mouse model of human gliomas. Cancer Res. 2001; 61:3826–3836. [PubMed: 11325859]
45. Holland EC, Celestino J, Dai C, Schaefer L, Sawaya RE, Fuller GN. Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. Nat. Genet. 2000; 25:55–57. [PubMed: 10802656]
46. Lyustikman Y, Momota H, Pao W, Holland EC. Constitutive activation of Raf-1 induces glioma formation in mice. Neoplasia. 2008; 10:501–510. [PubMed: 18472967]
47. Shannon P, Sabha N, Lau N, Kamnasaran D, Gutmann DH, Guha A. Pathological and molecular progression of astrocytomas in a GFAP: 12 V-Ha-Ras mouse astrocytoma model. Am. J. Pathol. 2005; 167:859–867. [PubMed: 16127163]
48. Sonoda Y, Ozawa T, Hirose Y, Aldape KD, McMahon M, Berger MS, Pieper RO. Formation of intracranial tumors by genetically modified human astrocytes defines four pathways critical in the development of human anaplastic astrocytoma. Cancer Res. 2001; 61:4956–4960. [PubMed: 11431323]
49. Uhrbom L, Dai C, Celestino JC, Rosenblum MK, Fuller GN, Holland EC. Ink4a-Arf loss cooperates with KRas activation in astrocytes and neural progenitors to generate glioblastomas of various morphologies depending on activated Akt. Cancer Res. 2002; 62:5551–5558. [PubMed: 12359767]
50. Robinson JP, VanBrocklin MW, Guilbeault AR, Signorelli DL, Brandner S, Holmen SL. Activated BRAF induces gliomas in mice when combined with Ink4a/Arf loss or Akt activation. Oncogene. 2010; 29:335–344. [PubMed: 19855433]
51. Holmen SL, Williams BO. Essential role for Ras signaling in glioblastoma maintenance. Cancer Res. 2005; 65:8250–8255. [PubMed: 16166301]
52. Bos JL. ras oncogenes in human cancer: a review. Cancer Res. 1989; 49:4682–4689. [PubMed: 2547513]
53. Network CGAR. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008; 455:1061–1068. [PubMed: 18772890]
54. Sharma MK, Zehnbauer BA, Watson MA, Gutmann DH. RAS pathway activation and an oncogenic RAS mutation in sporadic pilocytic astrocytoma. Neurology. 2005; 65:1335–1336. [PubMed: 16247081]
55. Janzarik WG, Kratz CP, Loges NT, Olbrich H, Klein C, Schafer T, Scheurlen W, Roggendorf W, Weiller C, Niemeyer C, Korinthenberg R, Pfister S, Omran H. Further evidence for a somatic KRAS mutation in a pilocytic astrocytoma. Neuropediatrics. 2007; 38:61–63. [PubMed: 17712732]
56. Thiel G, Marczinek K, Neumann R, Witkowski R, Marchuk DA, Nurnberg P. Somatic mutations in the neurofibromatosis 1 gene in gliomas and primitive neuroectodermal tumours. Anticancer Res. 1995; 15:2495–2499. [PubMed: 8669813]
57. Dasgupta B, Li W, Perry A, Gutmann DH. Glioma formation in neurofibromatosis 1 reflects preferential activation of K-RAS in astrocytes. Cancer Res. 2005; 65:236–245. [PubMed: 15665300]
58. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. Nature. 2002; 417:949–954. [PubMed: 12068308]

59. Pollock PM, Harper UL, Hansen KS, Yudt LM, Stark M, Robbins CM, Moses TY, Hostetter G, Wagner U, Kakareka J, Salem G, Pohida T, Heenan P, Duray P, Kallioniemi O, Hayward NK, Trent JM, Meltzer PS. High frequency of BRAF mutations in nevi. Nat. Genet. 2003; 33:19–20. [PubMed: 12447372]
60. Basto D, Trovisco V, Lopes JM, Martins A, Pardal F, Soares P, Reis RM. Mutation analysis of B-RAF gene in human gliomas. Acta Neuropathol. 2005; 109:207–210. [PubMed: 15791479]
61. Pfister S, Janzarik WG, Remke M, Ernst A, Werft W, Becker N, Toedt G, Wittmann A, Kratz C, Olbrich H, Ahmadi R, Thieme B, Joos S, Radlwimmer B, Kulozik A, Pietsch T, Herold-Mende C, Gnekow A, Reifenberger G, Korshunov A, Scheurlen W, Omran H, Lichter P. BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. J. Clin. Invest. 2008; 118:1739–1749. [PubMed: 18398503]
62. Hagemann C, Gloger J, Anacker J, Said HM, Gerngras S, Kuhnel S, Meyer C, Rapp UR, Kammerer U, Vordermark D, Flentje M, Roosen K, Vince GH. RAF expression in human astrocytic tumors. Int. J. Mol. Med. 2009; 23:17–31. [PubMed: 19082503]
63. Bar EE, Lin A, Tihan T, Burger PC, Eberhart CG. Frequent gains at chromosome 7q34 involving BRAF in pilocytic astrocytoma. J. Neuropathol. Exp. Neurol. 2008; 67:878–887. [PubMed: 18716556]
64. Jacob K, Albrecht S, Sollier C, Faury D, Sader E, Montpetit A, Serre D, Hauser P, Garami M, Bognar L, Hanzely Z, Montes JL, Atkinson J, Farmer JP, Bouffet E, Hawkins C, Tabori U, Jabado N. Duplication of 7q34 is specific to juvenile pilocytic astrocytomas and a hallmark of cerebellar and optic pathway tumours. Br. J. Cancer. 2009; 101:722–733. [PubMed: 19603027]
65. Schiffman JD, Hodgson JG, VandenBerg SR, Flaherty P, Polley MY, Yu M, Fisher PG, Rowitch DH, Ford JM, Berger MS, Ji H, Gutmann DH, James CD. Oncogenic BRAF mutation with CDKN2A inactivation is characteristic of a subset of pediatric malignant astrocytomas. Cancer Res. 2010; 70:512–519. [PubMed: 20068183]
66. Horbinski C, Hamilton RL, Nikiforov Y, Pollack IF. Association of molecular alterations, including BRAF, with biology and outcome in pilocytic astrocytomas. Acta Neuropathol. 2010; 119:641–649. [PubMed: 20044755]
67. Endris V, Wogatzky B, Leimer U, Bartsch D, Zatyka M, Latif F, Maher ER, Tariverdian G, Kirsch S, Karch D, Rappold GA. The novel Rho-GTPase activating gene MEGAP/ srGAP3 has a putative role in severe mental retardation. Proc. Natl. Acad. Sci. USA. 2002; 99:11754–11759. [PubMed: 12195014]
68. Yang Y, Marcello M, Endris V, Saffrich R, Fischer R, Trendelenburg MF, Sprengel R, Rappold G. MEGAP impedes cell migration. *via* regulating actin and microtubule dynamics and focal complex formation. Exp. Cell Res. 2006; 312:2379–2393. [PubMed: 16730001]
69. Hargrave D. Paediatric high and low grade glioma: the impact of tumour biology on current and future therapy. Br. J. Neurosurg. 2009; 23:351–363. [PubMed: 19637006]
70. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N. BRAF mutation predicts sensitivity to MEK inhibition. Nature. 2006; 439:358–362. [PubMed: 16273091]
71. de Vries NA, Bruggeman SW, Hulsman D, de Vries HI, Zevenhoven J, Buckle T, Hamans BC, Leenders WP, Beijnen JH, van Lohuizen M, Berns AJ, van Tellingen O. Rapid and robust transgenic high-grade glioma mouse models for therapy intervention studies. Clin. Cancer Res. 2010; 16:3431–3441. [PubMed: 20472681]
72. Lo HW, Hung MC. Nuclear EGFR signalling network in cancers: linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. Br. J. Cancer. 2006; 94:184–188. [PubMed: 16434982]
73. Lymbouridou R, Soufla G, Chatzinikola AM, Vakis A, Spandidos DA. Down-regulation of K-ras and H-ras in human brain gliomas. Eur. J. Cancer. 2009; 45:1294–1303. [PubMed: 19179066]
74. Jackson JH, Cochrane CG, Bourne JR, Solski PA, Buss JE, Der CJ. Farnesol modification of Kirsten-ras exon 4B protein is essential for transformation. Proc. Natl. Acad. Sci. USA. 1990; 87:3042–3046. [PubMed: 2183224]
75. Basso AD, Kirschmeier P, Bishop WR. Lipid posttranslational modifications. Farnesyl transferase inhibitors. J. Lipid Res. 2006; 47:15–31. [PubMed: 16278491]

76. Sun J, Qian Y, Hamilton AD, Sebti SM. Ras CAAX peptidomimetic FTI 276 selectively blocks tumor growth in nude mice of a human lung carcinoma with K-Ras mutation and p53 deletion. Cancer Res. 1995; 55:4243–4247. [PubMed: 7671229]
77. Mangues R, Corral T, Kohl NE, Symmans WF, Lu S, Malumbres M, Gibbs JB, Oliff A, Pellicer A. Antitumor effect of a farnesyl protein transferase inhibitor in mammary and lymphoid tumors overexpressing N-ras in transgenic mice. Cancer Res. 1998; 58:1253–1259. [PubMed: 9515813]
78. Kohl NE, Omer CA, Conner MW, Anthony NJ, Davide JP, deSolms SJ, Giuliani EA, Gomez RP, Graham SL, Hamilton K, et al. Inhibition of farnesyltransferase induces regression of mammary and salivary carcinomas in ras transgenic mice. Nat. Med. 1995; 1:792–797. [PubMed: 7585182]
79. Liu M, Bryant MS, Chen J, Lee S, Yaremko B, Lipari P, Malkowski M, Ferrari E, Nielsen L, Prioli N, Dell J, Sinha D, Syed J, Korfmacher WA, Nomeir AA, Lin CC, Wang L, Taveras AG, Doll RJ, Njoroge FG, Mallams AK, Remiszewski S, Catino JJ, Girijavallabhan VM, Bishop WR. Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice. Cancer Res. 1998; 58:4947–4956. [PubMed: 9810004]
80. Kieran MW, Packer RJ, Onar A, Blaney SM, Phillips P, Pollack IF, Geyer JR, Gururangan S, Banerjee A, Goldman S, Turner CD, Belasco JB, Broniscer A, Zhu Y, Frank E, Kirschmeier P, Statkevich P, Yver A, Boyett JM, Kun LE. Phase I and pharmacokinetic study of the oral farnesyltransferase inhibitor lonafarnib administered twice daily to pediatric patients with advanced central nervous system tumors using a modified continuous reassessment method: a pediatric brain tumor consortium study. J. Clin. Oncol. 2007; 25:3137–3143. [PubMed: 17634493]
81. Cloughesy TF, Wen PY, Robins HI, Chang SM, Groves MD, Fink KL, Junck L, Schiff D, Abrey L, Gilbert MR, Lieberman F, Kuhn J, DeAngelis LM, Mehta M, Raizer JJ, Yung WK, Aldape K, Wright J, Lamborn KR, Prados MD. Phase II trial of tipifarnib in patients with recurrent malignant glioma either receiving or not receiving enzyme-inducing antiepileptic drugs: a North American Brain Tumor Consortium Study. J. Clin. Oncol. 2006; 24:3651–3656. [PubMed: 16877733]
82. Van Cutsem E, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, Safran H, Humblet Y, Perez Ruixo J, Ma Y, Von Hoff D. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J. Clin. Oncol. 2004; 22:1430–1438. [PubMed: 15084616]
83. James GL, Goldstein JL, Brown MS. Polylysine and CVIM sequences of K-RasB dictate specificity of prenylation and confer resistance to benzodiazepine peptidomimetic *in vitro*. J. Biol. Chem. 1995; 270:6221–6226. [PubMed: 7890759]
84. da Fonseca CO, Landeiro JA, Clark SS, Quirico-Santos T, da Costa Carvalho Mda G, Gattass CR. Recent advances in the molecular genetics of malignant gliomas disclose targets for antitumor agent perillyl alcohol. Surg. Neurol. 2006; 65(Suppl. 1):S1:2, S1:8. discussion S1:8-1:9. [PubMed: 16427438]
85. Rajesh D, Stenzel RA, Howard SP. Perillyl alcohol as a radio-/chemosensitizer in malignant glioma. J. Biol. Chem. 2003; 278:35968–35978. [PubMed: 12805388]
86. Fernandes J, da Fonseca CO, Teixeira A, Gattass CR. Perillyl alcohol induces apoptosis in human glioblastoma multiforme cells. Oncol. Rep. 2005; 13:943–947. [PubMed: 15809762]
87. da Fonseca CO, Schwartsmann G, Fischer J, Nagel J, Futuro D, Quirico-Santos T, Gattass CR. Preliminary results from a phase I/II study of perillyl alcohol intranasal administration in adults with recurrent malignant gliomas. Surg. Neurol. 2008; 70:259–266. discussion 266-257. [PubMed: 18295834]
88. Da Fonseca CO, Silva JT, Lins IR, Simao M, Arnobio A, Futuro D, Quirico-Santos T. Correlation of tumor topography and peritumoral edema of recurrent malignant gliomas with therapeutic response to intranasal administration of perillyl alcohol. Invest. New Drugs. 2009 Jan 13. [Epub ahead of print].
89. Bergo MO, Ambroziak P, Gregory C, George A, Otto JC, Kim E, Nagase H, Casey PJ, Balmain A, Young SG. Absence of the CAAX endoprotease Rce1: effects on cell growth and transformation. Mol. Cell. Biol. 2002; 22:171–181. [PubMed: 11739732]
90. Bergo MO, Gavino BJ, Hong C, Beigneux AP, McMahon M, Casey PJ, Young SG. Inactivation of Icmt inhibits transformation by oncogenic K-Ras and B-Raf. J. Clin. Invest. 2004; 113:539–550. [PubMed: 14966563]

91. Blum R, Jacob-Hirsch J, Amariglio N, Rechavi G, Kloog Y. Ras inhibition in glioblastoma down-regulates hypoxia-inducible factor-1alpha, causing glycolysis shutdown and cell death. Cancer Res. 2005; 65:999–1006. [PubMed: 15705901]
92. Blum R, Jacob-Hirsch J, Rechavi G, Kloog Y. Suppression of survivin expression in glioblastoma cells by the Ras inhibitor farnesylthiosalicylic acid promotes caspase-dependent apoptosis. Mol. Cancer Ther. 2006; 5:2337–2347. [PubMed: 16985068]
93. Blum R, Nakdimon I, Goldberg L, Elkon R, Shamir R, Rechavi G, Kloog Y. E2F1 identified by promoter and biochemical analysis as a central target of glioblastoma cell-cycle arrest in response to Ras inhibition. Int. J. Cancer. 2006; 119:527–538. [PubMed: 16496386]
94. Amos S, Redpath GT, Polar G, McPheson R, Schiff D, Hussaini IM. Farnesylthiosalicylic acid induces caspase activation and apoptosis in glioblastoma cells. Cell Death Differ. 2006; 13:642–651. [PubMed: 16239932]
95. Bollag G, Freeman S, Lyons JF, Post LE. Raf pathway inhibitors in oncology. Curr. Opin. Investig. Drugs. 2003; 4:1436–1441.
96. Sathornsumetee S, Hjelmeland AB, Keir ST, McLendon RE, Batt D, Ramsey T, Yusuff N, Rasheed BK, Kieran MW, Laforme A, Bigner DD, Friedman HS, Rich JN. AAL881, a novel small molecule inhibitor of RAF and vascular endothelial growth factor receptor activities, blocks the growth of malignant glioma. Cancer Res. 2006; 66:8722–8730. [PubMed: 16951188]
97. Edwards LA, Verreault M, Thiessen B, Dragowska WH, Hu Y, Yeung JH, Dedhar S, Bally MB. Combined inhibition of the phosphatidylinositol 3-kinase/Akt and Ras/mitogen-activated protein kinase pathways results in synergistic effects in glioblastoma cells. Mol. Cancer Ther. 2006; 5:645–654. [PubMed: 16546979]
98. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004; 64:7099–7109. [PubMed: 15466206]
99. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, Wilhelm S, Lynch M, Carter C. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. Cancer Res. 2006; 66:11851–11858. [PubMed: 17178882]
100. Jane EP, Premkumar DR, Pollack IF. Coadministration of sorafenib with rottlerin potently inhibits cell proliferation and migration in human malignant glioma cells. J. Pharmacol. Exp. Ther. 2006; 319:1070–1080. [PubMed: 16959960]
101. Kolch W, Heidecker G, Kochs G, Hummel R, Vahidi H, Mischak H, Finkenzeller G, Marme D, Rapp UR. Protein kinase C alpha activates RAF-1 by direct phosphorylation. Nature. 1993; 364:249–252. [PubMed: 8321321]
102. Cai H, Smola U, Wixler V, Eisenmann-Tappe I, Diaz-Meco MT, Moscat J, Rapp U, Cooper GM. Role of diacylglycerol-regulated protein kinase C isotypes in growth factor activation of the Raf-1 protein kinase. Mol. Cell. Biol. 1997; 17:732–741. [PubMed: 9001227]
103. Yu C, Friday BB, Lai JP, Yang L, Sarkaria J, Kay NE, Carter CA, Roberts LR, Kaufmann SH, Adjei AA. Cytotoxic synergy between the multikinase inhibitor sorafenib and the proteasome inhibitor bortezomib *in vitro*: induction of apoptosis through Akt and c-Jun NH2-terminal kinase pathways. Mol. Cancer Ther. 2006; 5:2378–2387. [PubMed: 16985072]
104. Lo HW. Nuclear mode of the EGFR signaling network: biology, prognostic value, and therapeutic implications. Discov. Med. 2010; 10:44–51. [PubMed: 20670598]
105. Moy B, Kirkpatrick P, Kar S, Goss P. Lapatinib. Nat. Rev. Drug Discov. 2007; 6:431–432. [PubMed: 17633789]
106. Patel D, Lahiji A, Patel S, Franklin M, Jimenez X, Hicklin DJ, Kang X. Monoclonal antibody cetuximab binds to and down-regulates constitutively activated epidermal growth factor receptor vIII on the cell surface. Anticancer Res. 2007; 27:3355–3366. [PubMed: 17970081]
107. Rivera F, Vega-Villegas ME, Lopez-Brea MF, Marquez R. Current situation of Panitumumab, Matuzumab, Nimotuzumab and Zalutumumab. Acta Oncol. 2008; 47:9–19. [PubMed: 18097777]

Table 1

Summary of Malignant Glioma Clinical Trials with Pharmacological Agents that Target the Ras Pathway

Agent	Targets	Phase	Study Design	Outcomes	Trial ID and References
Completed Trials					
Lonafarnib (SCH 66336/ Sarasar)	FTase	I	single agent 53 children with brain tumors	Recommendations for phase-2 doses	Pediatric Brain Tumor Consortium Study Kieran 2007 [80]
Tipifarnib (R115777/ Zarnestra)	FTase	II	single agent 67 recurrent GBM 22 recurrent AA	6-month PFS: 9% in AA 12% in GBM	North American Brain Tumor Consortium Study Cloughesy 2006 [81]
Perillyl alcohol	FTase 37 recurrent AA, GBM &AO	I/II	single agent	6-month PFS: 48.2% in GBM 60% in AA 66.6% in AO	Brazilian trials de Fonseca 2008 [87]
Sorafenib (BAY 43-9006/ Nexavar)	RAF-1 PDGFR VEGFR	I	single agent 35 recurrent AA, GBM &AO	MTD determined	NABTT 0401 Nabors 2007[3]
		I/II	sorafenib+ sirolimus (mTOR inhibitor) 32 recurrent GBM phase I: 13 recurrent GBM phase II: 19 recurrent GBM	6-month PFS: 0%	NABTC 05–02 Wen 2009[5]
		I/II	sorafenib+ erlotinib (EGFR inhibitor) phase I: 17 recurrent GBM phase II: 19 recurrent GBM	sorafenib altering erlotinib metabolism or clearancephase II outcome to be prepared	NABTC 05–02 Prados 2009[1]
		II	radiation+TMZ+ sorafenib 45 GBM	median PFS: 6 months	Lamar 2009[3]
Ongoing Trials					
Sorafenib PDGFR VEGFR	RAF-1	I	single agent recurrent AA & GBM		NCT00093613
		I	radiation+TMZ+sorafenib first-line treatment for AA & GBM		NCT00884416
		II	TMZ+sorafenib recurrent AA & GBM		NCT00597493
		I/II	radiation+TMZ+sorafenib 4 study arms newly diagnosed GBM & gliosarcoma		NCT00734526
		II	radiation+TMZ+sorafenib		NCT00544817

Agent	Targets	Phase	Study Design	Outcomes	Trial ID and References
			first-line treatment for GBM		
		I/II	sorefenib+temsirolimus (mTOR inhibitor) recurrent GBM		NCT00329719
		II	sorefenib+ bevacizumab/Avastin (VEGF antibody) recurrent GBM		NCT00621686
Tipifamib Sorafenib	FTase RAF-1 PDGFR VEGFR	I/II	Arm 1: sorefenib+erlotinib (EGFR inhibitor) Arm 2: sorefenib+temsirolimus (mTOR inhibitor) Arm 3: sorefenib+tipifarnib recurrent GBM & gliosarcoma		NCT00335764

PFS: progression-free survival, MTD: maximum tolerance dose, AO: anaplastic oligodendrogliomas, TMZ: temozolomide.