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The mTOR signaling pathway as a treatment target for intracranial neoplasms

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Inhibition of the mammalian target of rapamycin (mTOR) signaling pathway has become an attractive target for human cancer therapy. Hyperactivation of mTOR has been reported in both sporadic and syndromic (hereditary) brain tumors. In contrast to the large number of successful clinical trials employing mTOR inhibitors in different types of epithelial neoplasms, their use to treat intracranial neoplasms is more limited. In this review, we summarize the role of mTOR activation in brain tumor pathogenesis and growth relevant to new human brain tumor trials currently under way using mTOR inhibitors.

Keywords: glioblastoma, meningioma, mTORC1, mTORC2.

Regulation and Function of mTOR Signaling Pathways

In a soil sample from the Easter Island (former Rapa Nui), a bacterial strain was isolated in the 1970s called *Streptomyces hygroscopicus*, which produces a macrolide subsequently found to inhibit the growth of yeast.[1] The macrolide was purified and named *rapamycin* after the place of its discovery. In yeast (*Saccharomyces cerevisiae*), mutations in the target of rapamycin genes (*TOR1* and *TOR2*) contribute to resistance to the growth-inhibitory effects of rapamycin.[2] The eukaryote TOR protein belongs to the phosphatidylinositol kinase–related kinase family, which encodes large proteins (~280 kDa) containing a carboxyl-terminal serine/threonine protein kinase domain.[3] In many mammalian cell types, the mammalian TOR (mTOR) protein forms 2 distinct multimolecular complexes, termed mTORC1 and mTORC2, which differ with respect to their protein composition, substrate specificity, and mechanism of growth regulation. The mTORC1 complex consists of mTOR, regulatory-associated protein of mTOR (RAPTOR), 40 kDa proline-rich Akt substrate (PRAS40), DEP (dishevelled, Egl-10, and Pleckstrin)-domain-containing mTOR (DEPTOR) interacting protein, and mammalian lethal with SEC13 protein 8 (mLST8).[4] The mTORC2 complex is composed of mTOR, rapamycin-insensitive companion of mTOR (RICTOR), proline-rich protein 5, mLST8, mammalian stress activated protein kinase interacting protein 1, and DEPTOR (Fig. 1).[3,5–7]

In a diverse number of distinct cell types, mTORC1 and mTORC2 have been shown to regulate different cellular processes. Rapamycin-sensitive mTORC1 is a nutrient and energy sensor, important for responding to changes in amino acid and nutrient levels, redox states, and growth factor availability as well as regulating ribosomal biogenesis and nutrient transport.[8–11] In contrast, the relatively rapamycin-insensitive mTORC2 is involved in actin cytoskeleton organization and cell survival. In addition, mTORC2 phosphorylates and activates Akt, serum- and glucocorticoid-inducible kinase (SGK1), and protein kinase C alpha (PKCα), which in turn control cell survival, cell cycle progression, and anabolism.[12–14]

Mammalian TORC1 is activated by a plethora of mechanisms. One mechanism involves phosphorylation-driven inactivation of the tuberous sclerosis complex-2 protein (TSC2, tuberin), which functions as a GTPase-activating protein (GAP) for the small GTPase Ras homolog enriched in brain (Rheb). Rheb promotes cell growth in a TOR- and S6 kinase (S6K)–dependent manner.[15,16] Tuberin stimulates the intrinsic GTPase activity of Rheb, thereby accelerating the conversion of active Rheb-GTP to inactive Rheb-GDP.[17] As such, cells lacking the tuberin-hamartin complex function exhibit increased Rheb and mTORC1 activation.[18]

In addition to growth factor–mediated activation via PI3-Kinase (PI3K), phosphoinositolphosphate (PIP) and phosphoinositid-dependend kinase 1 (PDK1), mTORC1 is activated by high amino acid levels, especially leucine and

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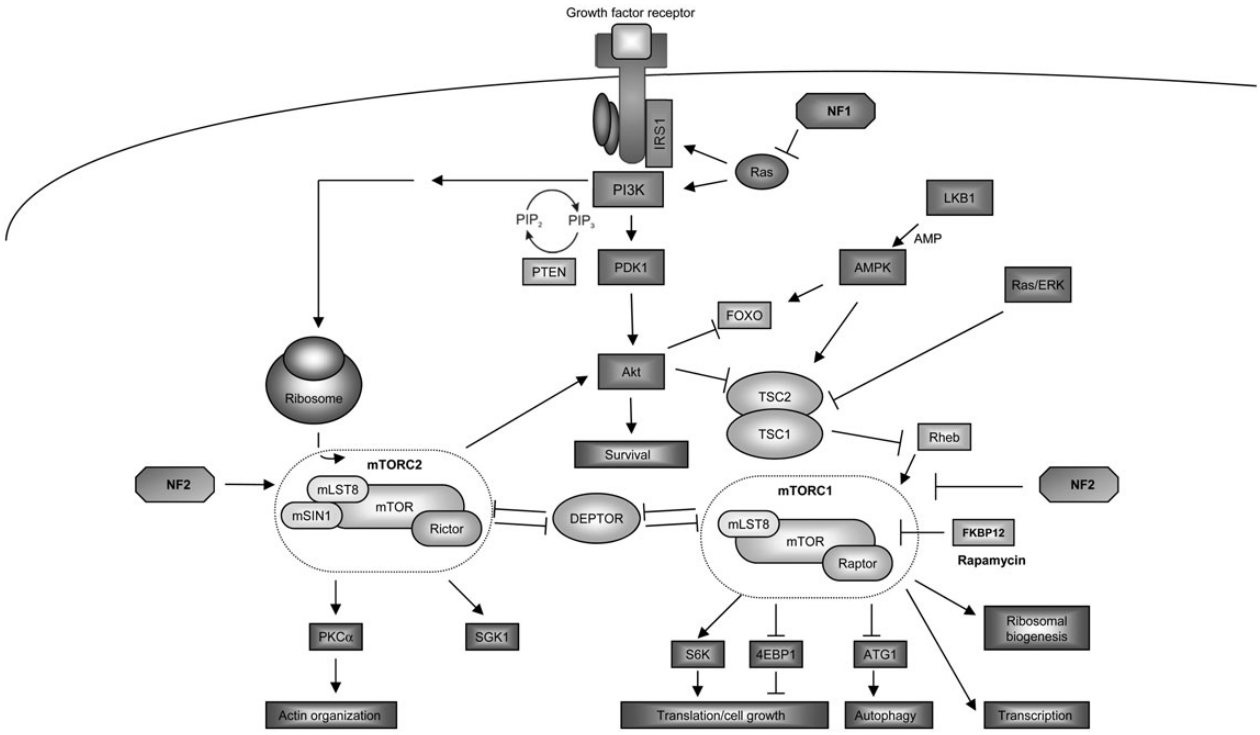

Fig. 1. Intracellular signaling of mTORC1 and mTORC2.

arginine.[19] The GTPase Rag functions as an amino acid–specific regulator of mTORC1, independent of TSC/Rheb function.[20,21] Low intracellular energy levels can inactivate mTORC1 in a manner dependent on activating adenosine monophosphate protein kinase (AMPK) and the transcription factor forkhead box O (FOXO). Activated AMPK controls energy-consuming anabolic pathways and can regulate mTORC1 either directly through phosphorylation of RAPTOR[22] or indirectly by phosphorylating tuberin at residues Thr^{1227} and Ser^{1345}.[23,24]

Following activation, mTORC1 regulates ribosomal translation and protein biosynthesis by phosphorylating key components of the protein synthesis machinery, including ribosomal protein S6K1/2 and 4E-binding protein 1 (4E-BP1). Upon 4E-BP1 phosphorylation, the translation initiation factor eIF4E is released and stimulates cap-dependent RNA translation.[25] S6K1 and S6K2 regulate translation initiation factors during protein biosynthesis and coordinate ribosome biogenesis to drive efficient mRNA translation.[26]

Recent studies have revealed a negative feedback loop in the insulin receptor pathway involving the insulin receptor substrate 1 (IRS-1). Activation of mTORC1 promotes inhibitory IRS-1 phosphorylation, such that Akt can be activated following rapamycin treatment.[27,28] This Akt activation is dose dependent, with lower rapamycin doses potentiating Akt activation, and vice versa.[29,30] The presence of this feedback loop explains the paradoxical Akt activation and increased growth observed in tumor cells following therapeutic rapamycin-mediated mTOR inhibition.

The mTORC1 complexes is involved in controlling the cellular responses to changes in nutrient availability (ie, starvation). One of the fundamental processes regulated by mTORC1 following nutrient starvation is autophagy. In this respect, mTORC1 is considered a negative regulator of autophagy.[31] Autophagy is the controlled self-degradation of damaged, supernumerous, or dangerous cellular components in response to starvation. During periods of low extracellular nutrient levels, cellular autophagy provides substrates for energy production. Mammalian TORC1 depends on the Rag and Rheb GTPases for activation and subsequent inhibition of autophagy in response to limiting amino acid availability.[3,21] As such, inhibition of mTORC1 activity induces autophagy.[11,32] In *Saccharomyces cerevisiae*, TOR-dependent phosphorylation of the protein autophagy-related 13 (Atg13) disrupts the Atg1-Atg13-Atg17 complex, which triggers autophagosome formation. ATG13 and Unc-51–like kinase 1 (ULK1) are the mammalian homologs of the yeast proteins Atg13 and Atg1.[33] These proteins bind to the 200-kDa large focal adhesion kinase family kinase-interacting protein and the mammalian-specific homolog ATG101. Mammalian TOR phosphorylates ATG13 and ULK1 to block the initiation of the autophagosome.[34] In addition, the mTORC1- and mTORC2-associated protein DEPTOR can induce autophagy through suppression of mTORC1 activity.[35,36] DEPTOR is negatively regulated by mTORC1 and mTORC2, and depletion of DEPTOR activates mTORC1 and mTORC2 pathways with increased phosphorylation of S6K and Akt, respectively.[4] Mammalian TORC1 has additional important functions as a key regulator of cellular metabolism. It can stimulate glucose uptake, metabolic flux through glycolysis and the oxidative arm of the pentose phosphate pathway, and production of acetyl-CoA with subsequent increase in lipid and sterol synthesis (reviewed in[37]).

While regulators and substrates of mTORC1 are well understood, the function of mTORC2 is less well elucidated.

Mammalian TORC2, similar to yeast TORC2, is involved in actin cytoskeleton reorganization and cell migration. Genetic silencing of expression of mTOR, RICTOR, and mLST8 (but not RAPTOR) results in decreased activation of Ras-related C3 botulinum toxin substrate 1 (Rac1) upon serum restimulation and leads to defective actin reorganization.[38,39] Rac1 belongs to a family of Rho GTPase molecules.[40] Following growth-factor stimulation, Rac1 associates with both mTORC1 and mTORC2. It binds directly to mTORC1/2 independently of the GTP-bound state of Rac1 and mediates the localization of mTOR to specific membrane compartments.[41] The mechanism underlying Rac1 control of actin cytoskeleton reorganization is incompletely understood but may involve recruitment and activation of Rac1 at the plasma membrane to increase synthesis of phosphatidylinositol (3,4,5)-triphosphate and result in actin cytoskeleton rearrangement.[41] Additionally, PKCα is involved in mTORC2-dependent cell migration due to mTORC2 phosphorylation of PKCα.[39] Consistent with a role in cell migration, glioma cell lines with increased RICTOR expression and mTORC2 activity exhibit elevated integrin β1 and β3 expression and enhanced motility. This increased expression of RICTOR correlates with higher levels of PKCα.[42] Moreover, mTORC2 is also required for hydrophobic motif site (Ser^{422}) phosphorylation of SGK1.[14] SGK1 is stimulated by growth factors and osmotic stress.[43] SGK1 phosphorylation requires protor-1 expression, such that cells lacking protor-1 are unable to activate SGK1.[44]

Accumulating evidence also points to a role for mTORC2 in protein synthesis and maturation, processes that have been previously attributed to mTORC1. In this regard, mTORC2 associates with ribosomal proteins,[45] where it directly interacts with the 60S ribosomal subunit. In addition, RICTOR specifically binds to the L23a and L26 ribosomal proteins positioned at the exit tunnel.[45,46] Together, these findings suggest that mTORC2 may control cotranslational processing or the maturation of nascent polypeptides. For example, Akt can be phosphorylated by mTORC2 at Thr^{450} of the turn motif and at Ser^{473} of the hydrophobic motif.[13,47–49] The phosphorylation of the turn motif is a one-shot event, which only occurs during the synthesis of nascent Akt, when the polypeptide is still attached to the ribosome.[45] However, phosphorylation at Ser^{473} of the hydrophobic motif is a posttranslational modification, induced by growth factors and hormones, which allosterically activates Akt to increase its activity toward many substrates.[7,48,50] As such, both mTORC1/2 complexes are involved in the regulation of different members of the family of signal transducer and activator of transcription factors.[51] Finally, mTORC2 may also function as a regulator of the nuclear factor-kappaB transcription factor, thus promoting chemoresistance in epidermal growth factor receptor (EGFR)–mutant glioblastoma.[52]

The Role of mTOR in Inherited Brain Tumor Predisposition Syndromes

The importance of mTOR in tumorigenesis has been revealed by studies focusing on familial cancer predisposition syndromes characterized by mutations in negative regulators of the mTOR pathway. Germline mutations in the phosphatase and tensin homolog gene (*PTEN*), for example, predispose to several disorders that exhibit diverse overlapping clinical features, collectively classified as PTEN hamartoma tumor syndrome, including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and Proteus syndrome.[53–55] Somatic *PTEN* inactivation with Akt hyperactivation occurs in many human tumors, including glioblastoma.[56,57] Moreover, patients with germline *PTEN* mutations are at risk for the development of numerous cancers, including thyroid and breast cancer.[58] Homozygous *PTEN* deletion in mice results in embryonic lethality, while heterozygous deletion is associated with increased cancer incidence.[59]

Mutations in either the *TSC1* or *TSC2* gene cause TSC hamartomatous syndrome.[60] Loss-of-function *TSC1*/*TSC2* mutations result in mTOR hyperactivation with increased S6K1, 4E-BP1, and ribosomal S6 phosphorylation.[61] Discovering the connection between TSC and the mTOR pathway provided the first link between cancer and increased mTOR activity. In the brain of children with TSC develop subependymal nodules and subependymal giant cell astrocytomas (SEGAs).[62,63] SEGAs are characterized by high expression levels of activated (phosphorylated) S6K,[64] and these tumors are exquisitely responsive to treatment with the mTORC1 inhibitor everolimus.[65–67]

Neurofibromatosis type 1 (NF1) is a common inherited tumor predisposition syndrome affecting 1 in 2500–3000 individuals.[68] Individuals with NF1 are prone to developing both benign and malignant tumors of the peripheral and central nervous systems.[69] Importantly, 15%–20% of children with NF1 develop low-grade gliomas involving the optic pathway,[70] while adults are at increased risk for high-grade gliomas.[71,72] The human *NF1* gene is located on chromosome 17q11.2 and encodes the protein neurofibromin, which functions as a GAP for the Ras small GTPase molecule.[73,74] Loss of neurofibromin expression results in increased Ras activity and cell growth.[75–77] Consistent with increased Ras pathway activity in *NF1*-deficient cancer cells, high levels of activated Akt/mTOR and Raf/mitogen-activated protein kinase (MAPK)–extracellular signal-regulated kinase (ERK) were observed.[78,79] Moreover, studies by The Cancer Genome Atlas have revealed that mutations in the *NF1* gene are among the most frequently occurring mutations found in glioblastoma multiforme (GBM), along with mutations in the *TP53, PTEN, EGFR,* and *RB* genes.[80]

Peutz-Jeghers syndrome is another familiar cancer disorder, which is caused by mutations in the serine/threonine protein kinase 11 (or liver kinase B1 [*LKB1*]) tumor suppressor gene, whose protein product directly activates AMPK.[81] In cells lacking LKB1, mTORC1 remains active due to defective AMPK regulation.[82] In contrast to the above-mentioned inherited cancer conditions, brain tumor development has been only rarely described in this syndrome.[83]

Mammalian TOR Hyperactivation in Gliomas and Nonglial Brain Tumors

Based on a detailed characterization of mTORC1 signaling and function, several reports have demonstrated activation of members of the mTORC1 pathway in brain tumors.

The largest group of brain tumors includes gliomas, a histologically highly heterogeneous group of tumors classified by the World Health Organization (WHO) according to malignancy grade and histological subtype. Among these glial neoplasms,

GBM is the most devastating type.[84] The importance of mTORC1 signaling to brain tumor formation and growth is underscored by the observation that several of the key genetic alterations described in gliomas result in increased mTORC1 activity.

In GBM, alterations of the *EGFR* gene are frequently found.[80] *EGFR* gene amplification in GBM results in activation of phosphatidylinositol-3 kinase (PI3K) in about 45% of cases.[85] Activating mutations or amplification of *PIK3CA*, the gene encoding the p110α subunit of PI3K, or of *PIK3R1*, which encodes for the subunit p85, has been found in ~15% of GBM.[80,86,87] In addition, ~40% of patients with GBM display loss of function of *PTEN* due to mutation, chromosomal deletion, or epigenetic gene silencing, which is associated with poorer overall survival.[88,89] Moreover, S6K has been reported to be activated in GBM[90–92] such that PI3K inhibition in PTEN-deficient GBM suppresses S6K activity and reduces tumor growth.[93]

By comparing primary low-grade tumors and high-grade recurrences, recently it was demonstrated that development of high-grade glioma (ie, glioblastoma) in these cases might be driven by different genetic alterations than the ones responsible for tumor initiation. Using exome sequencing, Johnson et al[94] observed that in 43% of cases half of the mutations present in the original low-grade tumor were undetected at recurrence. Moreover, they found that certain mutations activating the Akt-mTORC1 signaling pathway are closely associated with temozolomide treatment. This suggests that mTORC1 hyperactivation in malignant gliomas might represent a therapy-induced oncogenic transformation.[94]

While less well studied, the role of mTORC2 in gliomas is restricted to analyses of RICTOR and N-myc downstream regulated gene 1 (*NDRG1*). As such, RICTOR is overexpressed in GBM samples compared with normal brain. In addition, NDRG1, a downstream target of mTORC2 activity, is often increased in expression or phosphorylated in GBM.[52] In a *Drosophila* glioma model with constitutive coactivation of EGFR-Ras and PI3K, it was shown that mTORC2-related genes like dSIN1 and dRICTOR are required to generate malignant gliomas.[95]

Similarly, the activation of this pathway by EGFR engagement is an important factor potentially underlying chemotherapy resistance to alkylating agents.[52,96] The molecular mechanism for this negative effect of mTORC2 on GBM therapy is likely mediated by binding and stabilization of O^6-DNA methylguanine-methyltransferase.[96]

Taken together, there is compelling evidence for activation of mTORC1 in human GBM, thus providing a strong rationale for the clinical use of mTORC1 inhibitors as adjuvant therapies for primary or recurrent GBM (Table 1).

In addition to high-grade malignancies, pilocytic astrocytomas (PAs) are WHO grade I glial neoplasms, which occur predominantly in childhood and adolescence.[84] Until recently, very little was known about the genetic alterations underlying this tumor. However, it is now clear that one of the responsible growth control pathways is hyperactivation of MAPK/ERK signaling.[97–100] In this regard, loss of neurofibromin in NF1-associated gliomas leads to Ras- and PI3K-dependent hyperactivation of mTOR signaling.[101–103] Using *NF1* genetically engineered mouse glioma models, rapamycin-mediated inhibition of mTOR hyperactivation resulted in attenuated tumor proliferation. However, the combination of rapamycin with temozolomide in this mouse model did not increase the treatment efficiency.[104] This might be partially caused by rapamycin-dependent Akt activation.[105] Recently, in cell lines derived from pediatric low-grade gliomas, some antitumor effects of the rapalog ridaforolimus were demonstrated.[102]

While most sporadic PA tumors lack *NF1* gene inactivation, they are instead characterized by a signature fusion event in which the *BRAF* kinase domain is fused to the amino terminus of the *KIAA1549* gene.[106] In cerebellar neural stem cells, fusion BRAF expression leads to MAPK-dependent mTOR activation and the formation of glioma-like lesions in vivo.[103] Recent immunohistochemical data have similarly demonstrated activation of mTORC1 and mTORC2 in PAs.[102] Because both NF1-associated and sporadic PAs in children share mTOR hyperactivation, the use of rapalogs as another treatment option than operation for these low-grade pediatric brain tumors is reasonable.

Primary CNS lymphoma is an aggressive brain tumor, the majority of which are diffuse B-cell lymphomas.[107] In most B-cell lymphomas, the PI3K pathway is hyperactivated.[108,109] Although the expression and activation of the mTORC1/2 pathway has not been studied in tumor samples, rapamycin and temsirolimus exhibit potent antitumor activity against a variety of lymphoma cell lines in vitro.[109] Clinical trials using mTOR inhibition as a strategy to treat CNS lymphoma have been recently initiated.

Meningiomas are the second most common adult brain tumors, originating from the meningeal coverings of the brain and the spinal cord.[110] While the majority of tumors are benign WHO grade I meningiomas, ~20% of meningiomas are atypical (grade II) or anaplastic (grade III) tumors with significantly increased morbidity and mortality.[111] One of the most common genetic alterations observed in meningioma is inactivation of the *NF2* tumor suppressor gene. The *NF2* gene encodes a protein called merlin or schwannomin, a member of the ezrin, radixin, moesin family of membrane-cytoskeleton linker proteins.[112] Merlin regulates cytoskeleton remodeling, cell motility, and cell proliferation in response to extracellular signals.[113] Of the many intracellular signaling pathways regulated by merlin, constitutively activated mTORC1 has been identified in merlin-deficient meningioma cells.[114] Tumors from *NF2* patients, as well as from *NF2*-deficient mouse embryonic fibroblasts, display elevated mTORC1 activation, which is consistent with a role for merlin in the regulation of mTORC2 function.[115] As such, meningioma samples have been shown to express high levels of mTORC1 and S6K, implicating mTORC1 as a relevant signaling pathway in meningiomas.[116,117]

Vestibular schwannomas are another type of primary benign intracranial tumor characterized by frequent *NF2* alterations. Surprisingly, data regarding the expression of mTOR-related proteins in schwannomas are rare. Only one paper described expression and phosphorylation of mTOR proteins in schwannomas, but vestibular schwannomas were not included.[118]

Clinical Trials Using mTOR Inhibitors to Treat Brain Tumors

The ability of rapamycin and its analogs to inhibit mTORC1 function has prompted the initiation of several clinical trials

Table 1. Current clinical studies using mTOR inhibitors for the treatment of common brain tumors

Substance	Tumor Type	Phase	Combination	Study Number	Status	Results	Ref.
Everolimus	Low-grade glioma (P)/NF1	II	–	NCT01158651	Recruiting		–
	Low-grade glioma (R)	II	–	NCT00823459	Recruiting		–
	Low-grade glioma (R/P)	II	–	NCT00782626	Completed	?	–
	Low-grade glioma (R/P)	II	–	NCT00831324	Recruiting		–
	Glioblastoma (R)	II	–	NCT00515086	Completed	Terminated	–
	Giant cell astrocytoma (TSC)	I/II	–	NCT00411619	Active		–
	Glioblastoma (P/R/N)	I	Temozolomide	NCT00387400	Completed	?	–
	Glioblastoma	I	Temozolomide	NCT00553150	Ongoing		[132]
	Malignant glioma (R)	I/II	Sorafenib	NCT01434602	Recruiting		–
	Glioblastoma (P)	I/II	Gefitinib	NCT00085566	Completed	?	–
	Glioblastoma	I/II	Temozolomide	NCT01062399	Ongoing		–
	Glioblastoma (R)	I/II	AEE788	NCT00107237	Completed	?	–
Temsirolimus	Malignant glioma	I/II	–	NCT00022724	Completed	?	–
	Glioblastoma (MGMT unmethylated)	II	–	NCT01019434	Ongoing		–
	Glioblastoma (R)	II	Bevacizumab	NCT00800917	Completed	No effect	[137]
	Glioblastoma	I	Temozolomide	NCT00316849	Completed	?	–
	Glioblastoma (R)	I/II	Sorafenib	NCT00335764	Ongoing		–
	Glioblastoma (R/P)	I/II	Perifosine	NCT01051557	Ongoing		–
	Malignant glioma (R)	I/II	Erlotinib	NCT00112736	Completed	No effect	[138]
	Glioblastoma (R)	I/II	Sorafenib	NCT00329719	Ongoing		–
	Glioblastoma (R)	I/II	Sorafenib	NCT00335764	Completed	No effect	[136]
Sirolimus	Glioblastoma	I/II	–	NCT00047073	Completed		–
	Malignant glioma (R)	I/II	Erlotinib	NCT00509431	Completed	?	[139]
	Glioblastoma (R)	I	Vandetanib	NCT00821080	Ongoing		–
AZD8055	Malignant glioma (R)	I	–	NCT01316809	Ongoing		–

Abbreviations: P, progressive; R, recurrent; ?, no results published; MGMT, O^6-DNA methylguanine-methyltransferase.

that aim to block the progression of tumors characterized by mTOR hyperactivation. Unlike kinase inhibitors that bind to the catalytic ATP-binding side, rapamycin and its derivatives are relatively specific for mTORC1, because they target FK506-binding protein 12 (FKBP12).[119] When rapamycin enters the cell, it binds to the intracellular receptor FKBP12, which binds the FKBP-rapamycin binding domain in mTOR to abrogate mTOR kinase activity of mTORC1 in vitro and in vivo[3] allosterically.

The development of rapamycin analogs with more favorable pharmacokinetic profiles than the parental molecule has provided new opportunities for anticancer clinical trials. These "rapalogs" include temsirolimus (Pfizer), everolimus (Novartis), and ridaforolimus (Ariad), which are slightly different in terms of their metabolism, formulation, and administration schedules. Temsirolimus is administered in a once-weekly schedule intravenously, similar to ridaforolimus.[120,121] Everolimus is an orally available mTOR inhibitor, typically administered on a continuous daily schedule.[122] Phase III temsirolimus trials for patients with solid tumors showed that weekly infusions of the drug in doses of 7.5 to 220 mg/m^2 in patients with advanced cancer resulted in mild toxicity and evidence of antitumor activity.[123–125] Rapalogs also exhibit efficacy in the treatment of TSC-associated SEGAs in patients who are not candidates for surgical intervention.[126] A randomized, placebo-controlled phase III trial demonstrated a 50% reduction of tumor volume in 35% of the treated SEGA patients.[66] Even long-term treatment with everolimus of TSC patients suffering from SEGAs has been proven to be safe and effective.[127] Furthermore, treatment with everolimus effectively reduces seizure frequency.[67] Currently, a phase III trial is testing everolimus as adjunctive therapy in patients with TSC and refractory partial-onset seizure (NCT01713946). These data convincingly demonstrate that everolimus treatment in general represents a useful therapy option for slowly growing benign intracranial tumors.

Treatment of high-grade gliomas with rapalogs has recently been more intensely studied. A phase II trial for patients with recurrent GBM revealed that 36% of the subjects treated had evidence of radiographic improvement following temsirolimus administration.[128] In addition, the majority of the patients showed an improvement of symptom status. However, another study reported only short-term stabilization of disease in 50% of patients with recurrent glioblastoma treated with temsirolimus.[129] In a phase I trial it was proven that short-term treatment with rapamycin in patients with recurrent PTEN-deficient glioblastomas reduced tumor cell proliferation in a substantial number of cases. Moreover, the inhibition of tumor cell proliferation correlated well with the magnitude of mTOR inhibition. An activation of Akt was found in some rapamycin-treated patients, and this feedback loop was associated with shorter time to progression during postsurgical maintenance rapamycin therapy.[130] In a phase II trial using temsirolimus in children

and adolescents with high-grade glioma, neuroblastoma, and rhabdomyosarcoma, in some patients a prolonged stable disease following weekly administered temsirolimus 75 mg/m^2 was observed.[131] Another phase I trial studied the combination of radiotherapy and temozolomide with everolimus in newly diagnosed glioblastoma.[132] This scheme was well tolerated, and a subsequent phase II trial is still ongoing (NCT00553150). Other studies for newly diagnosed glioblastomas show similar beneficial results (RTOG0913,[133] NCIC CTG[134]). However, it should be mentioned that combination of radiotherapy with temozolomide and temsirolimus revealed increased risk for infections.[135]

Other combined treatment schemes with temsirolimus have been evaluated, but the combination with neither sorafenib,[136] bevacizumab,[137] nor erlotinib[138,139] has been proven to be effective in recurrent GBM. For newly diagnosed glioblastoma, a large phase II trial was initiated by the European Organisation for Research and Treatment of Cancer (EORTC). In this trial, patients lacking hypermethylation of the O^6-methylguanine-methyltransferase promoter were treated with radiation therapy combined with temsirolimus (experimental arm) or temozolomide (control arm). The study is closed, and results are expected to be published soon.

Besides mTORC1, mTORC2 has been increasingly recognized as a promising candidate target for therapeutic inhibition in human cancer. Consequently, a number of FKBP12-independent adenosine triphosphate (ATP)–competitive mTOR kinase inhibitors (eg, Torin1, PP242, PP30) have been generated that target both mTOR complexes at similarly low half-maximal inhibitory concentration values. In contrast to rapamycin and its derivatives, newly developed ATP-binding inhibitors target mTOR-kinase activity by competing with ATP to the kinase domain in mTOR. Similarly, mTORC2-specific inhibitors are currently under development.[119] Based on studies in prostate cancer models with reduced PTEN expression,[140] brain tumors with elevated PI3K activity might be reasonable candidates for mTORC2 inhibitors or dual mTOR/PI3K inhibitors. Dual mTOR/PI3K inhibitors were originally developed in programs screening for new PI3K inhibitors. However, they were found to be effective inhibitors of mTORC1 and mTORC2 as well.[141,142] When the half-maximal inhibitory concentration for mTORC1 and mTORC2 inhibition is significantly lower than that for PI3K inhibition, they are called pan-mTOR-inhibitors.[143] Currently, there is one interventional study listed in the National Institutes of Health clinical trial database that explores the value of XL765, a dual PI3K/mTOR inhibitor, but no results have been published (NCT01240460). The same drug has been tested in a phase I study in combination with temozolomide (NCT00704080), but no study results are available.

No other malignant brain tumors (eg, primary CNS lymphoma, anaplastic oligodendroglioma) have been treated with mTORC1 inhibitors thus far. Regarding low-grade tumors, the efficacy of temsirolimus in SEGAs is well established (see above). Limited data are available for vestibular schwannomas from recent studies. It has been reported that treatment with temsirolimus in *NF2*-deficient vestibular schwannomas might have only limited effects,[144] while another group showed at least in animal models that mTORC1 inhibition can be effective.[145] Moreover, a study of a single patient with recurrent ependymoma and remarkable response to temsirolimus was published.[146] No meningioma studies have been reported so far, but preclinical data indicate that meningiomas might represent a suitable target.[117]

Certain cancer types are at high risk to develop brain metastases during the course of the disease. Malignant melanoma, breast, lung, kidney, and gastrointestinal cancers are especially prone to spread to the brain.[147] Rapalogs are able to cross the blood–brain barrier[148–150] and have been designed for long-term use,[151] qualifying them as interesting candidates for brain metastases treatment.

In triple-negative breast cancer metastatic to the brain, considerable effects of rapamycin and temsirolimus treatment have been recently demonstrated in vitro and in vivo.[152] Interestingly, low-dosage rapamycin showed good efficacy in reducing the invasion of brain metastatic cells, while high-dosage treatment was less effective due to activation of the MAPK signaling pathway. However, combination of temsirolimus with the MAP/ERK inhibitor SL325 was able to overrun MAPK activation, with prominent inhibition of perivascular tumor cell invasion. In a currently recruiting phase II trial, patients with Her2+ brain-metastatic breast cancer will be treated with everolimus in combination with trastuzumab and vinorelbine (NCT01305941). The mTOR pathway is also activated in malignant melanoma.[153] Brain metastases are frequent in melanoma, but so far only preclinical data are available. Combined treatment of brain-metastatic melanoma cell lines with the BRAF inhibitor vemurafenib and temsirolimus showed encouraging results in at least one melanoma cell line.[154] Unfortunately, a phase II trial exploring the combination of temsirolimus with sorafenib and tipifarnib in untreated metastatic melanoma did not include patients with brain metastases.[155] No clinical data are available regarding lung cancer metastatic to the brain, while mTOR signaling is activated in non–small cell lung cancer and may present a mechanism of acquired resistance to EGFR–tyrosine kinase inhibitors.[156] Moreover, no clinical studies are under way treating brain metastases from kidney or gastrointestinal cancer.

In summary, despite the obvious activation of mTORC1 in malignant gliomas, currently the clinical value of single or combined treatment of primary or recurrent glioblastoma is unclear. In contrast, mTORC1 seems to be a reasonable target in benign intracranial tumors such as SEGA and meningioma.

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