Dual PI3K/mTOR Inhibitor, XL765, suppresses glioblastoma growth by inducing ER stress-dependent apoptosis

Background: Deregulated phosphoinositide 3-kinase (PI3K)/mTOR signaling commonly exists in glioblastoma (GBM), making this axis an attractive target for therapeutic manipulation. A recent dual inhibitor of PI3K/mTOR pathway, XL765, exhibited an attractive suppression effect on GBM tumor growth. However, the exact functional mechanisms of tumor suppression mediated by XL765 have not yet been fully characterized.

Purpose: In this study, we took efforts to assess the effects of PI3K/mTOR blockade by XL765 on GBM growth in vitro and in vivo.

Methods: We analyzed the cytotoxicity of XL765 in three different GBM cell lines, A172, U87MG, and T98G, by using Hoechst 33258 (Invitrogen), Annexin V/propidium iodide (PI), as well as Cell Counting Kit -8 (CCK-8) assay. We also used A172 xenograft model to study the effect of XL765 in vivo.

Results: We found that XL765 inhibits GBM viability with a wide range of potencies. Importantly, XL765 suppressed GBM cell growth by inducing endoplasmic reticulum (ER) stress dependent apoptosis. The activation of CHOP/DR5 pathway by XL765 induced ER stress is responsible for the induction of apoptosis. Moreover, the inhibition of mTOR signal by XL765 is the major source of ER stress, rather than inhibition of PI3K. At last, we demonstrated that combination of XL765 with GMB chemotherapeutic drug, temozolomide (TMZ), can achieved better therapy effect in vitro and in vivo.

Conclusion: Overall, our data show that targeting PI3K/mTOR by XL765 is a promising therapeutic strategy to relieve tumor burden in GBM patients.

Keywords: XL765, glioblastoma, apoptosis, ER stress

Introduction
Glioma, as the most frequent primary brain tumor, accounts for 81% of malignant tumors in central nervous system, which has been increasing as a severe threat to human life.1 Currently, the standard therapy strategy is surgery, radiotherapy, and adjuvant temozolomide (TMZ) chemotherapy.2 However, the glioblastoma (GBM) tumors usually possess mixed cytological subtypes in phenotype, multiple mutations and gene amplifications in genotype, which make any conventional or pathway-specific therapy less effective.3-5 With the development of molecular biology, it has been identified several molecular pathway abnormal expression, which are related to the pathogenesis and prognosis of GBM, including TP53 mutations, TERT promoter region mutations, EGFR amplification, PTEN deletions, and 1p/19q combined deletions.6
As its potential function in cancer, the phosphoinositide-3-kinase (PI3K)/mTOR pathway signaling axis is receiving more and more attention in recent years.7 Database analysis by the Cancer Genome Atlas shows that the PI3K/mTOR pathway is activated in around 88% in GBM patients.6,8 This critical pathway acts as upstream effector of multiple signal pathways, and in turn stimulates numerous downstream effectors, which are involved in cellular survival, growth, protein synthesis, motility, and other functions of pro-tumorigenic effects.5 The PI3K pathway is manipulated by different signaling cascades, including loss function of the phosphatase and tensin homologue (PTEN) protein, amplification and/or mutation of the EGFR, which occurs in 40% and 50% of GBM cases, respectively.8 These perspectives led to the development of novel drugs targeting single (eg, mTORC1 inhibitors) or multiple (eg, dual PI3K/mTOR inhibitors) components of this pathway.9 Currently, several drugs targeting this signaling pathway are in Phase I and II clinical trials, either in combination with other chemotherapeutic agents such as TMZ, or as single agents.10–12 Although first-generation PI3K pathway inhibitors have effects on the most downstream node of mTOR, an enhanced PI3K signal by feedback loops was observed, which has led to the development of second-generation drugs.13–15

XL765 (SAR245409) is a potent class I dual inhibitor of PI3Ks and mTOR. In cellular assays, treatment with XL765 suppresses phosphorylation of PI3K and mTOR downstream effectors in multiple tumor cell lines, such as AKT and ribosomal protein S6 (S6RP).16 In multiple tumor cell lines, XL765 exhibits wide range of inhibitory potencies.14 It was also reported that XL765 treatment in GBM tumor in mice model is associated with enhanced survival,17 but without clear mechanisms. The goal of the current study was to reveal the underlying mechanism of XL765 suppression of GBM tumor growth, which will provide more insights about PI3K/mTOR axis as a potential target for anti-GBM therapy.

Materials and methods

Cell lines and reagents

A172, U-87MG, and T98G GBM cell lines were obtained from ATCC (Manassas, VA, USA). The cells were cultured with DMEM consisting of Ham’s F12 medium (1:1) (Invitrogen) was mixed with 10% FBS (HyClone, Logan, UT, USA), 100 units/mL penicillin, and 100 μg/mL streptomycin. All of the cells were cultured at 37°C and 5% CO2.

Chemicals, including TMZ, XL765, Z-VAD-FMK (z-VAD), salubrinal were purchased from Sigma-Aldrich (Shanghai, People’s Republic of China).

Cell survival and viability assay

Cell survival was determined by using Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich). A172, U-87MG, and T98G cells were seeded at 2×103–4×103 cells/well in 96-well plates. On the following day, cells were treated with different concentrations of drugs. After 24 and 48 hrs, viable cells were quantified by using a CCK-8 assay according to the manufacturer’s protocol.

The cell viability was determined by crystal violet staining. Briefly, 1×105 A172, U-87MG, and T98G cells were seed in 12-well plates, and treated with different concentrations of drugs for 24 hrs. The attached cells were washed with PBS and stained with a 0.05% crystal violet solution (containing 3.7% paraformaldehyde prepared in distilled water) at room temperature.

Apoptosis analysis

The apoptosis of GBM cells was analyzed by nuclear staining with Hoechst 33,258 (Invitrogen), or Annexin V/propidium iodide (Invitrogen) followed by flow cytometry as described previously.18 For the nuclear Hoechst 33,258 staining, the treated GBM cells were stained with Hoechst 33,258 (3.7% formaldehyde, 0.5% Nonidet P-40, and 10 μg/mL Hoechst 33,258 (Invitrogen)), and the condensed chromatin and micronucleation was counted under microscopic visualization.

Intracellular Ca2+ detection

The levels of intracellular Ca2+ were determined using Fluo-3 AM (S1056, Beyotime, Shanghai, People’s Republic of China) staining followed by flow cytometry analysis. Human GBM cell A172 and U87 were treated with 10 μM XL765 for 24 hrs. After treatment, cells were stained with Fluoro-3AM for 30 mins and collected for flow cytometry analysis.

Transfection of small-interfering RNA (siRNA) and plasmid

Transfection of siRNA and plasmid was conducted using Lipofectamine® 2000 (Invitrogen) as described by the manufacturer. Active Akt plasmid (myr Akt delta PH, #10,841) and S6K expressing plasmid (pDONR223-RPS6KB1, #23,686) were purchased from Addgene (Cambridge, MA, USA). siRNA duplexes were synthesized by GenePharma (Shanghai, People’s Republic of China) and included: DR5 (AAGACCCUUGUGCUUGUU GUC), CHOP (GCACAGCUAGCUAGAGAGA).
Western blot analysis
RIPA lysis buffer supplemented with protease inhibitor cocktail (Complete Mini; Roche, Basel, Switzerland) was used for Whole cell lysates preparation. After sonicated for 5 mins, the lysates were centrifuged at 12,000× g for 20 mins at 4°C, and the supernatants were collected. Western blot was performed as previously described. The following primary antibodies were used: Tubulin (Sigma), cleaved caspase-3, p-AKT, total AKT, p-S6K, total S6K, PERK (Cell Signaling, Danvers, MA, USA), CHOP (EMD Millipore, Burlington, MA, USA), DR5 (Santa Cruz, Shanghai, People’s Republic of China).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)
RNeasy Mini Kit (Qiagen, Hilden, Germany) was used to extract total RNA from the cultured cells in accordance with the manufacturer’s protocol. One microgram of total RNA was used to generate cDNA using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed on the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using SYBR Green qPCR Master Mix (Thermofisher Scientific, Shanghai, People’s Republic of China). Values were normalized to the expression of ACTIN mRNA. Primers used in this study were: DR5: 5′-AAGACCCCTGTGCTCGTTGT-3′ and 5′-AGG TGGACACAATCCCTCTG-3′; ACTIN: 5′-AGAGCTACG AGCTGCTGAC-3′ and 5′-AGCAGCTGTGGCTGCGTAC AG-3′.

Xenograft experiment
The animal studies were approved by the Institutional Animal Ethics Committee of Jilin University and experiments were performed in accordance with the Animal Ethics guidelines of Jilin University. For in vivo tumorigenicity assays, severely immuno-compromised female BALB/C nude mice were used. Briefly, 1×10⁶ of A172 cells in 0.1 mL ice-cold Hanks Balanced Salt Solution were implanted subcutaneously into the right flank of female nude mice (N=5). Seven days after cell injection, the mice were received intraperitoneal (IP) injection of TMZ (25 mg/kg) every day, and/or oral gavage of XL765 (100 mg/kg) every 2 days. PBS injection was used as a negative control (control). The drugs were administrated for total of 2 weeks. The tumor volume was measured each other day, and determined using the formula: 4/3π (√major axis/2× minor axis/2).

Statistical analysis
Data are presented as means±standard error of the mean. Two-tailed Student’s t test was used for statistical calculation. Differences were considered statistically significant at P<0.05.

Results
XL765 suppressed GBM cells viability
To investigate the effect of XL765 on the GBM cell lines, different human GBM cell lines A172, U87, and T98G were treated with different concentrations of XL765 (0, 1, 2.5, 5, 10, and 20 μM) for 24and 48 hrs. We found that 5, 10, and 20 μM XL765 could inhibit the growth of human GBM cell lines in a time- and dose-dependent manner (Figure 1A). The IC50 values of these GBM cells were shown in Figure (1B). A cell viability assay by plate crystal violet staining was performed to further confirm the effect of XL765 on GBM cell viability, where the survival of GBM cells was suppressed by XL765 treatment in a dose-dependent manner (Figure 1C). The effects of XL765 on the PI3K/mTOR signaling pathway were then examined by Western immunoblot of AKT and S6K. XL765 was found to induce a marked dose-dependent decrease in the phosphorylation of AKT and S6K (Figure 1D). Induction of apoptosis is the major mode of cytotoxicity for many anti-cancer drugs. A flow cytometry assay and Hoechst 33,258 nuclei staining were performed to investigate whether XL765 could induce GBM cell apoptosis in vitro. The percentage of apoptotic A172 cells was higher after XL765 treatment compared to the control groups (Figure 1E). The apoptosis rates increased after higher XL765 treatment concentrations (Figure 1E). Furthermore, Hoechst 33,258 staining also revealed that XL765 can induce nuclei fragmentation in multiple GBM cells, including A172, U87, and T98G cells (Figure 1F). Inhibition of apoptosis by pan-caspases inhibitor, z-VAD, compromised the nuclei fragmentation induced by XL765 in these 3 cell lines (Figure 1G).

Thus, our data demonstrated that XL765 suppressed GBM cell viability by inducing apoptosis in a time- and dose-dependent manner.

XL765 induces endoplasmic reticulum (ER) stress-related apoptosis in GBM cells
It was reported that ER stress was negatively regulated by AKT/TSC/mTOR pathway, and is a major cellular stress to induce apoptosis. To investigate this phenomenon, calcium disorder, an important marker of the ER
stress, was analyzed by Fluo-3 AM cytoplasmic calcium staining followed by flow cytometry assay. We found that a significantly elevated cytoplasmic calcium level was detected in XL765 treatment GBM cell groups (Figure 2A). To further clarify this, the expression levels of ER stress marker PERK, p-Eif2α, and CHOP were assessed. We found that XL765 treatment could significantly increase the expression of PERK, p-Eif2α, and CHOP in a dose-dependent manner (Figure 2B). Inhibition of ER stress by Eif2α inhibitor, salubrinal, suppressed the phosphorylation of Eif2α and induction of CHOP by XL765 (Figure 2C). Suppression of ER stress by salubrinal also compromised the XL765-induced apoptosis in A172 cells, as showed by cleaved caspase-3 expression and fragmented nuclei staining (Figure 2C and D). Moreover, knockdown of CHOP,
The ER stress sensor that has a protective effect in ER stress-related apoptosis, which significantly suppressed XL765-induced GBM apoptosis (Figure 2E and F). These findings demonstrated that XL765 could induce ER stress-related apoptosis in human GBM cell lines.

**DR5 induction mediated the GBM apoptosis induced by XL765**

Death receptor 5 (DR5) plays an critical role in ER stress-induced apoptosis, we therefore investigated its regulation in response to XL765 treatment. We found that XL765 treatment induced DR5 expression in a dose-dependent manner (Figure 3A). Accordingly, the mRNA level of DR5 was also induced by XL765 in A172 cells with a dose-dependent manner (Figure 3B). Suppression of ER stress by salubrinal, depletion of CHOP by siRNA suppressed the induction of DR5 in protein and mRNA level, further confirmed that DR5 is the downstream effector of ER stress induced by XL765 (Figure 3Cand D). To further evaluate the role of DR5 in XL765-induced apoptosis, we applied the siRNA to depletion the expression of DR5. Our results showed that DR5 knockdown by siRNA compromised the cleavage of caspase-3 (Figure 3E), and abrogated the apoptosis induced by XL765 in A172 cells (Figure 3F). Collectively, our results suggested that DR5 is the downstream effector of XL765-induced ER stress to promote GBM cell apoptosis.
Inhibition of mTOR by XL765 contributes to ER stress-associated apoptosis

We next addressed the mechanism of ER stress raised by XL765 treatment in GBM cells. As we found XL765 treatment inhibited S6K and AKT activation, we further investigated whether inhibition of PI3K or mTOR can induce ER stress in GBM. Transfection of constitutively active AKT in A172 cells suppressed XL765 inhibition of phosphorylation of AKT (Figure 4A). However, restore of AKT activity has mild effect on suppressing the XL765-induced ER stress, as indicated by expression CHOP (Figure 4A). Accordingly, enhanced AKT activity did not significantly suppress XL765-induced apoptosis, caspase-3 activation, and DR5 induction in mRNA level (Figure 4A–C), suggesting that inhibition of PI3K by XL765 did not contribute to ER stress-induced apoptosis. It was reported that PI3K is the upstream effector of mTOR,22 however, we found restore AKT activity only slightly reduced the inhibition of mTOR by XL765, also suggesting that XL765 inhibits PI3K-independent mTOR signaling.16 Inhibition of mTOR is also an important source of ER stress,23 we therefore tested whether XL765-induced ER stress by mTOR inhibition. Transfection of constitutively active S6K in A172 cells suppressed XL765 inhibition of phosphorylation of S6K, but did not affect the inhibition of phosphorylation of
AKT (Figure 4D). As predicted, enhanced S6K expression in A172 cells suppressed XL765-induced CHOP expression, cleavage of caspase-3, cell apoptosis, as well as DR5 expression in mRNA level (Figure 4D–F). Therefore, these data collectively indicated that XL765-induced ER stress-related apoptosis in GBM by suppression of mTOR activity.

**XL765 enhancing the killing effect of TMZ in vitro and in vivo**

We found that XL765 treatment suppressed cell viability by apoptosis in different GBM cell lines (Figure 1A–C), which has different responses to TMZ treatment, a major chemotherapy drug in treating GBM. We therefore tested whether XL765 can benefit the TMZ-based chemotherapy of GBM. We found that TMZ treatment induced higher apoptosis and caspase-3 activation in TMZ sensitive cells, U87 and A172 cells (Figure 5A and B). In contrast, TMZ treatment did not obviously induce apoptosis in TMZ resistant cells, T89G cells (Figure 5C). However, the combination of XL765 and TMZ can maximize the killing effect of TMZ in different GBM cells (Figure 5A–C), suggesting that XL765 combination can overcome the TMZ resistance, and achieve better therapy results. To further confirm the therapy effect of XL765 in GBM, we tested the effect of combination treatment of TMZ and XL765 on the growth of A172 xenograft in vivo. We found that XL765 single treatment suppressed the tumor growth, which was comparable with the effect of TMZ single treatment (Figure 5D and E). And combination of TMZ with XL765 treatment dramatically had more suppression effects on tumor growth, compared with the TMZ or XL765 single treatment (Figure 5D and E). Therefore, our data verified that XL765 has antitumor activity in vivo, and combination with TMZ treatment could enhance its killing effect in vitro and in vivo.

**Discussion**

The PI3K/mTOR pathway, as one of the most important signaling pathways in human GBM, has multiple amplification, mutation, or deletion in its key pathway regulatory components. Multiple evidence have shown that aberrant PI3K/mTOR pathway signaling has a critical role in many aggressive malignancy tumors, supporting the development of drugs targeting this axis. Studies here demonstrated...
that dual PI3K/mTOR blockade by XL765 markedly suppressed the growth of human GBM in vitro and in vivo. In this study, we found that inhibition of PI3K/mTOR by XL765-induced apoptosis in GBM cells, which was dependent on the ER stress-mediated DR5 activation. The ER stress induced by XL765 was more related to mTOR inhibition rather than PI3K inhibition. Furthermore, our studies investigated the effect of XL765 by using oral administration of clinically relevant dosing regimens (100 mg/kg every 2 days), and combination treatment with the standard care of TMZ in GBM xenograft mice model. Our in vivo data suggested that combination of XL765 with chemotherapy has a better anti-cancer effect, which provided a novel therapy combination strategy for GBM patients.

It has been reported that XL765 exhibits a wide range of anti-tumor activity in multiple cancers. There is a trend that enhanced sensitivity to XL765 in the cells exhibiting mutations in PI3K pathway, which is consistent with previous reports about PI3K inhibitors GDC-0941 and CH5132799. However, it is still unclear whether PI3K mutations or PTEN deficiency can be the general predictive marker for clinical responsiveness to PI3K pathway inhibitors. Large-scale molecular profiling of tumors in the ongoing XL765 clinical studies is being performed to further address this question. It was reported that oral administration of XL765 resulted in substantial tumor growth suppression in multiple xenograft tumor models, suggesting that XL765 may have broad utility in tumors. In contrast, PTEN-absent cell lines had multiple sensitivities to XL765, since some are very sensitive but some are intractable. In our study, the three human GBM cells, A172, U87-MG, and T98G, are PTEN deficient or mutant, and showed high sensitivity to XL765-induced apoptosis, which suggested that XL765 might have favorable therapy outcomes in PTEN mutated tumors.

It has been reported that XL765 could promote apoptosis through genetic and pharmacologic autophagy blockade in malignant peripheral nerve sheath tumors. Meanwhile, Radoul et al described the phenomenon that XL765 treatment either alone or in combination with TMZ significantly reduced the tumor burden in xenografted mice, but without further mechanism discovered. Here in this study, we first discovered that XL765 could block GBM cell survival by inducing apoptosis in a time- and dose-dependent manner. Further research on these XL765-treated GBM cell lines detected an increasing expression of ER stress markers after XL765 treatment, such

Figure 5 XL765 enhanced the killing effect of TMZ in vitro and in vivo. (A–C) A172 (A) U87MG (B) and T98G (C) cells treated with 10 μM XL765 and/or 15 μM TMZ for 24 hrs. Upper, the apoptosis was analyzed by Hochest-33,258 staining, lower, the expression of cleaved caspase-3. (D) Tumor growth of A172 cells xenografted nude mice (n=5) received TMZ (25 mg/kg) and/or XL765 (100 mg/kg) treatment. *, P<0.05; **, P<0.01. (E) The representative tumors in each treatment group.
as PERK, EIF2α, and CHOP. PERK is pivotal for cell adaptation to ER stress, promoting either survival or apoptosis.28 The activation of PERK/EIF2α axis leads to the expression of CHOP, a pro-apoptotic protein in the downstream of PERK/EIF2α axis.29 Our results showed that pharmacological inhibition of ER stress by EIF2α inhibitor resulted into abandon of cell apoptosis, suggesting the activation apoptotic pathway by of PERK/EIF2α pathway.

As to the induction of ER stress by XL765, we found that the ER stress is more dependent on the mTOR inhibition. mTOR and unfolded protein response have been shown to act coordinately in a number of biological processes.30 mTOR inhibitors were able to suppress the tumor growth by trigger ER stress-dependent apoptosis.31 Up to date, several pre-clinical studies using rapamycin or its derivatives have yielded favorable outcomes. However, accumulating data from other solid malignancies suggest that the clinical effects of mTOR inhibitors only have transient tumor stabilization, and there is tumor re-growth during and/or after treatment discontinuation.-

XL765, as a novel developed small molecule, inhibits both class I PI3Ks and mTOR. Although XL765 seems to be more potent against PI3K, it also inhibits mTOR-dependent phosphorylation events and PI3K-independent in tumor cells.16 Different from previous described mTOR inhibitor, XL765 not only suppressed the GBM cell growth, but also demonstrated obvious killing effect on GBM cells. However, the clinical usage of XL765 still needs to be further investigated.

XL765 is currently in Phase I and II clinical studies, and used as a single agent or in combination with other chemotherapy agents in various cancer patients.34,35 Consistent with previous observation.36 XL765 has demonstrated significant efficacy in a GBM xenograft model, both as a single treatment or combination with TMZ. Taken together, these data support the ongoing clinical function of XL765 for the treatment of GBM cancer.

Disclosure
The authors declare no conflicts of interest in this work.

References
1. Cloughesy TF, Cavenee WK, Mischel PS. Glioblastoma: from molecular pathology to targeted treatment. Annu Rev Pathol. 2014;9:1–25. doi:10.1146/annurev-pathol-011110-130324
2. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352:987–996. doi:10.1056/NEJMoa043330
3. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRα, IDH1, EGFR, and NF1. Cancer Cell. 2010;17:98–110. doi:10.1016/j.ccr.2009.12.020
4. Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. Cell. 2013;155:462–477. doi:10.1016/j.cell.2013.09.034
5. Patel AP, Tirosch I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science. 2014;344:1396–1401. doi:10.1126/science.1254257
6. Touat M, Idbaih A, Sanson M, Ligon KL. Glioblastoma targeted therapy: updated approaches from recent biological insights. Ann Oncol. 2017;28:1457–1472. doi:10.1093/annonc/mdx106
7. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase Akt pathway in human cancer. Nat Rev Cancer. 2002;2:499–501. doi:10.1038/nrc839
8. N. Cancer Genome Atlas Research. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;455:1061–1068. doi:10.1038/nature07385
9. Kong D, Yamori T. Advances in development of phosphatidylinositol 3-kinase inhibitors. Curr Med Chem. 2009;16:2839–2854.
10. Rodon J, Dienstmann R, Serra V, Tabernerio J. Development of PI3K inhibitors: lessons learned from early clinical trials. Nat Rev Clin Oncol. 2013;10:143–153. doi:10.1038/nrclinonc.2013.10
11. Ma DJ, Galanis E, Anderson SK, et al. A phase II trial of everolimus, temozolomide, and radiotherapy in patients with newly diagnosed glioblastoma: NCCTG N057K. Neuro Oncol. 2015;17:1261–1269. doi:10.1093/neuonc/nou328
12. Kuger S, Graus D, Brendtke R, et al. Radiosensitization of glioblastoma cell lines by the dual PI3K and mTOR inhibitor NVP-BEZ235 depends on drug-irradiation schedule. Transl Oncol. 2013;6:169–179. doi:10.1016/j.tranon.2012.04.002
13. Fan QW, Knight ZA, Goldenberg DD, et al. A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. Cancer Cell. 2006;9:341–349. doi:10.1016/j.ccr.2006.03.029
14. Papadopoulos KP, Tabernerio J, Markman B, et al. Phase I safety, pharmacokinetic, and pharmacodynamic study of SAR245409 (XL765), a novel, orally administered PI3K/mTOR inhibitor in patients with advanced solid tumors. Clin Cancer Res. 2014;20:2445–2456. doi:10.1158/1078-0432.CCR-13-2403
15. Salphati L, Heffron TP, Alickie B, et al. Targeting the PI3K pathway in the brain—efficacy of a PI3K inhibitor optimized to cross the blood-brain barrier. Clin Cancer Res. 2012;18:6239–6248. doi:10.1158/1078-0432.CCR-12-0720
16. Yu P, Laird AD, Du X, et al. Characterization of the activity of the PI3K/mTOR inhibitor XL765 (SAR245409) in tumor models with diverse genetic alterations affecting the PI3K pathway. JCI Insight. 2017;2:c97952. doi:10.1172/jci.insight.97952
17. Radoul M, Chaimel MM, Eriksson P, Wang AS, Phillips JJ, Ronen SM. MR studies of glioblastoma models treated with dual PI3K/mTOR inhibitor and temozolomide: metabolic changes are associated with enhanced survival. Mol Cancer Ther. 2016;15:1113–1122. doi:10.1158/1535-7163.MCT-15-0769
18. He K, Chen D, Ruan H, et al. BRAFV600E-dependent Mcl-1 stabilization leads to everolimus resistance in colon cancer cells. Oncotarget. 2016;7:47699–47710. doi:10.18632/oncotarget.10277
19. Hickman JA. Apoptosis induced by anticancer drugs. Cancer Metastasis Rev. 1992;11:121–139.
20. Qin L, Wang Z, Tao L, Wang Y. ER stress negatively regulates AKT/TSC/mTOR pathway to enhance autophagy. Autophagy. 2010;6:239–247. doi:10.4161/auto.6.2.11062
21. Szegedzi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep. 2006;7:880–885. doi:10.1038/sj.embor.7400779
22. Ghadimi MP, Lopez G, Torres KE, et al. Targeting the PI3K/mTOR axis, alone and in combination with autophagy blockade, for the treatment of malignant peripheral nerve sheath tumors. Mol Cancer Ther. 2012;11:1758–1769. doi:10.1158/1535-7163.MCT-12-0015
23. Sanchez-Alvarez M, Del Pozo MA, Bakal C. AKT-mTOR signaling modulates the dynamics of IRE1 RNAse activity by regulating ER-mitochondria contacts. Sci Rep. 2017;7:16497. doi:10.1038/s41598-017-16662-1
24. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. Front Oncol. 2014;4:64. doi:10.3389/fonc.2014.00064
25. O’Brien C, Wallin JJ, Sampath D, et al. Predictive biomarkers of sensitivity to the phosphatidylinositol 3’ kinase inhibitor GDC-0941 in breast cancer preclinical models. Clin Cancer Res. 2010;16:3670–3683. doi:10.1158/1078-0432.CCR-09-2828
26. Tanaka H, Yoshida M, Tanimura H, et al. The selective class I PI3K inhibitor CH5132799 targets human cancers harboring oncogenic PIK3CA mutations. Clin Cancer Res. 2011;17:3272–3281. doi:10.1158/1078-0432.CCR-10-2882
27. Janku F, Wheler JJ, Naing A, et al. PIK3CA mutation H1047R is associated with response to PI3K/AKT/mTOR signaling pathway inhibitors in early-phase clinical trials. Cancer Res. 2013;73:276–284. doi:10.1158/0008-5472.CAN-12-1726
28. Maas NL, Diehl JA. Molecular pathways: the PERKs and pitfalls of targeting the unfolded protein response in cancer. Clin Cancer Res. 2015;21:675–679. doi:10.1158/1078-0432.CCR-13-3239
29. Yang SY, Wei FL, Hu LH, Wang CL. PERK-eIF2alpha-ATF4 pathway mediated by endoplasmic reticulum stress response is involved in osteodifferentiation of human periodontal ligament cells under cyclic mechanical force. Cell Signal. 2016;28:880–886. doi:10.1016/j.cellsig.2016.04.003
30. Appenzeller-Herzog C, Hall MN. Bidirectional crosstalk between endoplasmic reticulum stress and mTOR signaling. Trends Cell Biol. 2012;22:274–282. doi:10.1016/j.tcb.2012.02.006
31. He K, Zheng X, Li M, Zhang L, Yu J. mTOR inhibitors induce apoptosis in colon cancer cells via CHOP-dependent DR5 induction on 4E-BP1 dephosphorylation. Oncogene. 2016;35:148–157. doi:10.1038/onc.2015.79
32. Johannessen CM, Reczek EE, James MF, Brems H, Legius E, Cichowski K. The NF1 tumor suppressor critically regulates TSC2 and mTOR. Proc Natl Acad Sci U S A. 2005;102:8573–8578. doi:10.1073/pnas.0503224102
33. Johannson G, Mahller Y, Collins MH, et al. Effective in vivo targeting of the mammalian target of rapamycin pathway in malignant peripheral nerve sheath tumors. Mol Cancer Ther. 2008;7:1237–1245. doi:10.1158/1535-7163.MCT-07-2335
34. Brown JR, Hamadani M, Hayslip J, et al. Voxtalisib (XL765) in patients with relapsed or refractory non-Hodgkin lymphoma or chronic lymphocytic leukemia: an open-label, phase 2 trial. Lancet Haematol. 2018;5:e170–e180. doi:10.1016/S2352-3026(18)30030-9
35. Mehnert JM, Edelman G, Stein M, et al. A phase I dose-escalation study of the safety and pharmacokinetics of a tablet formulation of voxtalisib, a phosphoinositide 3-kinase inhibitor, in patients with solid tumors. Invest New Drugs. 2018;36:36–44. doi:10.1007/s10637-017-0467-7
36. Prasad G, Sottero T, Yang X, et al. Inhibition of PI3K/mTOR pathways in glioblastoma and implications for combination therapy with temozolomide. Neuro Oncol. 2011;13:384–392. doi:10.1093/neuonc/noq193