options that may enhance this standard regimen. Enzastaurin, which has completed phase 2 clinical trials in combination with TMZ and radiation for newly diagnosed GBM, blocks signaling through the PKC and PI3K/AKT pathway sensitizing GBM cells and xenografts to the effects of TMZ.
Methods and Results: We sought to understand the mechanism by which enzastaurin enhances the effectiveness of TMZ-based therapy. We now show that TMZ treatment alone fails to induce apoptosis and triggers activation of the pro-survival transcription factor CREB, eliciting a profound increase in CREB-regulated transcription as assessed by promoter-reporter assays and transcriptional array analyses. Enzastaurin blocks TMZ-induced CREB activation, profoundly diminishing CREB transcriptional activity and inducing a robust apoptotic response in GBM cells regardless of p53, PTEN or MGMT status. To investigate further the importance of CREB function, we depleted CREB expression with siRNAs. As with enzastaurin co-treatment, CREB reduction was alone sufficient to induce a profound apoptotic response to TMZ treatment. In both subcutaneous and intracranial GBM xenografts, enzastaurin also synergized with TMZ to block tumor growth. In these xenograft studies, as in cell culture, enzastaurin blocks TMZ-induced CREB activation.
Conclusions: These data indicate that enzastaurin enhances the effectiveness of TMZ-based therapy by blocking CREB activation and strongly implicate pharmacologic inhibition of CREB as an attractive approach to enhancing the response of GBM to TMZ-based therapy.

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Characterization of novel series of selective PI3Kalpha and PI3Kalpha/mTOR-dual inhibitors

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Background: Activation of the PI3K signaling pathway occurs with high frequency in human cancers and promotes tumor growth and survival. Mechanisms for pathway dysregulation include loss of PTEN and mutation and/or amplification of the *PIK3CA* gene. Mutational activation of *PIK3CA* occurs in ~25% of human tumors and is predominantly associated with point mutations in either the helical (E545K) or kinase domains (H1047R). The effects of different PI3K pathway-activating genetic lesions are not equivalent. PTEN-null tumor cell lines demonstrate high basal pAKT levels that are primarily driven by PI3Kβ. PIK3CA mutant cell lines are either RAS-dependent with low basal levels of pAKT (E545K) or RAS-independent with moderate basal levels of pAKT (H1047R). We have developed novel series of inhibitors that selectively target either PI3Kα or PI3Kα and mTOR and are using these to explore the impact of tumor genetic background on sensitivity to PI3Kα inhibition.
Methods: Two distinct series of PI3Kα-selective inhibitors targeting either PI3Kα or PI3Kα and mTOR were identified via high-throughput screening and optimized by medicinal chemistry. Compound effects on PI3K pathway signaling were assessed in a panel of tumor cell lines using ELISA or western blot techniques.
Results: Compounds with low nM potency for PI3Kα or for PI3Kα and mTOR, with >100-fold selectivity over other PI3K isoforms and protein kinases were identified. These compounds inhibit PI3K pathway signaling in tumor cell lines harboring activating mutations in PIK3CA. Dual inhibitors of PI3Kα and mTOR show a differential pattern of activity when compared with selective inhibitors of PI3Kα. The impact of tumor genetic background on the activity of these inhibitors is being explored in vitro and in vivo.
Conclusions: Novel PI3Kα and PI3Kα/mTOR-selective small molecule inhibitors have been identified and are being used to explore the role of PI3Kα and mTOR in PI3K pathway signaling in the context of different activating genetic lesions. Selective PI3Kα and dual selective PI3Kα/mTOR compounds had distinct profiles of activity with respect to tumor cell genetic background, providing a rationale for advancing both classes of compound.

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Induction of endoplasmic reticulum stress by the novel anti-cancer compound KP46 and synergism with proteasome inhibition

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Introduction: Tris(8-quinolinolato)gallium(III) (KP46) is a novel oral compound with promising anticancer activity in vitro and in vivo. The underlying mechanism of KP46's anticancer activity are still widely unknown. This study investigates whether the unfolded protein response (UPR) and NF-kB signaling are involved in the cytotoxic activity of KP46 against lung cancer cells.
Methods: Cytotoxic/antiproliferative effects were tested against diverse lung cancer cell lines (A549, A427, VL-8) by MTT assay. Furthermore, alterations in expression of proteins related to endoplasmic reticulum (ER) stress and the NF-kB pathway were determined by western blot.
Results: The IC50 values for KP46 after 72h exposure were in the low μM range (0.4−3.9 mM). In this study, it is shown that KP46 activates UPR and ER stress in a time- and dose-dependent manner. Up-regulation of major chaperones and signaling molecules was found after 1 to 3 hours. Additionally, phosphorylation of SAP/JNK and expression of Bim were increased. Both have been linked to ER stress and induction of apoptosis. Long-term incubation with KP46 led to a decline of UPR signals with sustained pSAP/JNK and Bim activation in the lung cancer cell models. Interestingly, the NF-kB pathway was stimulated in the same time frame as ER stress. In general, A427 cells showed a stronger response against KP46 than A549, leading to more distinct changes in protein expression. These data are consistent with the higher sensitivity of the A427 cell line against KP46. UPR and ER stress can lead to an enhanced ubiquitination and degradation of proteins. Therefore, it was not surprising that treatment of A549 cells with KP46 led to higher levels of ubiquitinated proteins. These data were corroborated by the additive to synergistic effects of KP46 in combination with proteasome inhibitors.
Conclusion: Taken together, these data suggest that stimulation of UPR and NF-kB pathways contribute to the anticancer activity of KP46. These findings are in agreement with the observed synergistic effects with proteasome inhibitors. Respective combination approaches will be further investigated in preclinical studies.

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Allosteric MEK inhibitor BAY 86-9766 (RDEA119) shows anti-tumor efficacy in mono-and combination therapy in preclinical models of hepatocellular carcinoma and pancreatic cancer

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Introduction: The RAS-RAF-MEK-ERK pathway has been identified as one of the main pathways activated in cancers. BAY 86-9766 (RDEA119) is an orally available, potent, non-ATP competitive inhibitor targeting MEK1/2, the central switch in the RAS signal transduction cascade. We have characterized BAY 86-9766 in preclinical tumor models of hepatocellular carcinoma (HCC) and pancreatic cancer.
Method/Results: Tumor cell proliferation assays were performed with BAY 86-9766 in more than 15 cell lines. Strong antiproliferative activity was observed in cell lines carrying mutations that activate the MAPK pathway. BAY 86–9766 retained its antiproliferative activity in PGP overexpressing cells, indicating that it is not a MDR transporter substrate. In vitro combination studies with BAY 86-9766 showed strong synergy when combined with sorafenib in several HCC cell lines and for combination with gemcitabine in pancreatic cancer cell lines. *In vivo*, significant tumor growth inhibition was observed in the subcutaneous PLC/PRF/5 HCC model in monotherapy. In the syngeneic orthotopic Hepa129 HCC model, survival time was more than doubled after BAY 86-9766 treatment. In pancreatic cancer models, BAY 86-9766 was tested in MiaPaCa xenografts, and showed both, dose-dependent tumor growth inhibition and evidence of tumor shrinkage (PRs). In two additional pancreatic *in vivo* cancer models (Capan-1, DanG) synergistic effects of BAY 86-9766 were observed in combination with gemcitabine.
Conclusion: BAY 86-9766 demonstrates robust inhibition of tumor cell growth and has potent *in vivo* preclinical anti-tumor activity in a variety of human xenograft models in the indications HCC and pancreatic cancer. Activation of the RAS-RAF-MEK-ERK pathway increases the sensitivity of tumor cells to the allosteric MEK inhibitor BAY 86-9766. Strong synergy of BAY 86-9766 (RDEA119) was observed in combination with sorafenib and gemcitabine in preclinical tumor models. BAY 86-9766 is currently in phase I clinical trials in combination with sorafenib.

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The novel highly selective and efficacious MET inhibitor BAY853474: mode of action, basic in vitro characteristics and preclinical pharmacology

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BAY853474 is a potent, highly selective and orally available inhibitor of MET, a receptor tyrosine kinase implicated in tumor growth, angiogenesis