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

P. Foster[1], K. Rice[2], J. Bussenius[2], C. Jaeger[3], L. Nguyen[1], A. Virone-Oddos[4], D. Markby[5]. *[1]Exelixis, Biochemistry, South San Francisco CA, USA; [2]Exelixis, Medicinal Chemistry, South San Francisco CA, USA; [3]Exelixis, New Lead Discovery, South San Francisco CA, USA; [4]Sanofi-Aventis, Oncology − Drug Discovery, Vitry/Seine, France; [5]Exelixis, Molecular and Cellular Pharmacology, South San Francisco CA, USA*

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

J. Gojo[1], U. Jungwirth[1], P. Heffeter[1], B. Atil[1], M. Micksche[1], B.K. Keppler[2]. *[1]Medical University of Vienna, Department of Medicine I Institute of Cancer Research, Vienna, Austria; [2]University of Vienna, Institute of Inorganic Chemistry, Vienna, Austria*

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

F. Puehler[1], A. Scholz[1], M. Kissel[1], A. Adjei[2], J.N. Miner[3], M. Hitchcock[1], R. Schmieder[1], D. Mumberg[1], K. Ziegelbauer[1]. *[1]Bayer Schering Pharma AG, TRG Oncology, Berlin, Germany; [2]Roswell Park Cancer Center, Buffalo NY, USA; [3]Ardea Biosciences Inc., San Diego CA, USA*

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

D. Zopf[1], M. Kissel[1], M. Mamounas[1], K. Schuck[1], G. Fachinger[1], U. Bömer[1], H. Hess-Stumpp[1], P. Lienau[1], S. Ince[1]. *[1]Global Drug Discovery Bayer Schering Pharma, GDD, Wuppertal Berlin, Germany*

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

and metastasis. In vitro BAY853474 inhibited MET auto-phosphorylation and MET driven tumor cell proliferation in vitro, which translated into strong inhibition of tumor growth in MET-dependent tumor xenograft models *in vivo*. In preclinical studies the compound demonstrated besides the high selectivity for the MET receptor a favourable physical-chemical, DM/PK and tolerability profile in support of oral administration route for further clinical development. We present representative data supporting pharmacological mode-of-action and efficacy in tumor xenograft models *in vivo*.

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Novel pyrazolopyrimidine derivatives as potent mTOR kinase inhibitors with anticancer activities

S. Chen[1], L. Meng[1], C. Liang[2], J. Ding[1]. [1]*Shanghai Institute of Materia Medica, Division of Anti-Tumor Pharmacology, Shanghai, China;* [2]*Xcovery LLC, Drug Discovery, West Palm Beach, USA*

Background: The mammalian target of rapamycin (mTOR),which is deregulated in about 50% of all human malignancies,sits in the center of signaling network regulating cell growth, metabolism, and angiogenesis. mTOR exists in two complexes: the mTOR-raptor complex and the mTOR-rictor complex. Rapamycin and its analogues partially inhibit mTOR through allosteric binding to TORC1 without inhibiting TORC2 and their efficacy is moderate as anticancer agents in the clinic. A few mTOR kinase inhibitors that inhibit both TORC1 and TORC2 have been reported to possess more potent anticancer activities. Herein, we designed and synthesized a series of pyrazolopyrimidine derivatives as novel specific ATP-competitive mTOR kinase inhibitors which inhibit both TORC1 and TORC2 and display potent antitumor activities.
Materials and Methods: mTOR kinase activity was measured by an ELISA assay employed purified truncated mTOR protein. Protein levels were detected by Western blot analysis. Cell cycle distribution was assessed by FACS analysis and cell proliferation was evaluated via SRB assay.
Result: Compounds specifically inhibited mTOR while sparing a panel of over 400 kinases tested. Further study indicated that the compounds acted in an ATP-competitive manner, blocked mTOR signaling pathway in cell lines with different characteristics such as A549 cells with Kras and LKB1 mutations, MCF-7 cells with PIK3CA mutation and p70S6K1 amplification, and rapamycin resistant HCT116 cells. The compounds inhibited the phosphorylation of TORC1 substrate p70S6K1 and 4EBP1 as well as phosphorylation of the TORC2 substrate AKT and downstream protein GSK3β, which happened within 15 minutes of compound treatment. As a consequence, cells were arrested in G1 phase upon treatment with compound for 24 h or 48 h. Consistent with the reported role of mTOR in regulating autophagy in mammalian cells, we found that the compounds induced autophagy in A549 cells after 24 h of treatment. We further evaluated the anticancer activity of the compounds in a broad panel of tumor cells originating from different tissue types and they displayed potent inhibition of the tumor cell proliferation with IC_{50}s ranging from 0.27 mM to 1.17 mM across all cell lines tested.
Conclusion: Pyrazolopyrimidine derivatives are a novel class of specific mTOR kinase inhibitors targeting both TORC1 and TORC2. They displayed broad anticancer activities and deserve further evaluation and optimization as new anticancer compounds.

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Establishment and evaluation of patient-derived tumor models of adenoid cystic carcinoma: Effects of chemotherapeutics and targeted therapies on human ACC xenografts

F. Nieves[1], M. Moore[1], M. Mann[1], C. Moskaluk[2], A. Tolcher[3], K. Papadopoulos[3], A. Patnaik[3], J. Kaufman[4], M. Wick[1]. [1]*South Texas Accelerated Research Therapeutics (START), Preclinical Research, San Antonio TX, USA;* [2]*University of Virginia Charlottesville, Pathology/Biochemistry and Molecular Genetics, Charlottesville VA, USA;* [3]*South Texas Accelerated Research Therapeutics (START), Clinical Research, San Antonio TX, USA;* [4]*Adenoid Cystic Carcinoma Research Foundation, Needham MA, USA*

Adenoid cystic carcinoma (ACC) is a rare form of malignant neoplasm defined by a distinct histologic appearance which arises within secretory glands, most commonly the salivary glands of the head and neck. Standard treatment options for this malignancy include resection and local radiation therapy; however, currently no standard of care exists for this cancer type. Several novel therapies have demonstrated single agent or combination activity across a range of in vivo models of human cancer; however, lack of validated ACC models has limited evaluation of these agents in treating this disease.
Previously we and others in collaboration with the Adenoid Cystic Carcinoma Research Foundation (ACCRF) established and evaluated two preclinical models of ACC (ACCx6 and ACCx9) using tumor explants from donor patients implanted into immunocompromised mice. Three additional tumor models (ACCx5M1, ACCX14 and ACCx16) have been established and screened against a panel of 35−40 agents representing each class of approved anticancer agent as well as candidate compounds obtained from academic and pharmaceutical collaborators; follow up studies were also performed evaluating previously untested agents in the ACCx6 and ACCx9 models. Designated endpoints for these studies were a mean control tumor volume of approximately 1−2 cm^3 or sixty days following treatment initiation. Treatment with standard chemotherapeutics was ineffective in most screens except for docetaxel which demonstrated statistically significant ($p < 0.05$) tumor growth inhibition in all evaluated models including partial and complete tumor responses towards ACCx16. Activity was reported in ACCx16 and ACC×9 with the tyrosine kinase inhibitor (TKI) sunitinib and sorafenib was active towards ACC×16 but inactive in ACC×5M1. Irinotecan and temsirolimus were active towards ACC×16 but inactive in remaining models. ACC×14 was most insensitive to evaluated chemotherapeutics with only docetaxel reporting activity in this model while ACC×16 was most sensitive in these studies.
Data from these studies demonstrate these low passage models as excellent screening tools to identify potentially useful approved and candidate agents for treatment of ACC. Antitumor activity of docetaxel in these models suggests it as an appropriate combination agent for future studies and activity of TKI agents over several models suggest this class of agents as potentially useful in the treatment of ACC. Additional studies evaluating single agent and combination treatments including docetaxel plus TKI are warranted.

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EML4-ALK signaling is required for the maintenance of neoplastic phenotype of non-small cell lung cancer cells: novel strategy for lung cancer tailored therapies

C. Voena[1], E. Panizza[1], E. Pellegrino[1], L. D'Amico[1], F.E. Boccalatte[1], B. Ruggeri[2], R.D. Polakiewicz[3], E. Medico[4], R. Chiarle[1], G. Inghirami[1]. [1]*University of Torino, Department of Biomedical Science and Human Oncology, Torino, Italy;* [2]*Cephalon Inc, Discovery Research, West Chester PA, USA;* [3]*Cell Signaling Technology Inc., Cell Signaling Technology Inc., Danvers MA, USA;* [4]*Institute for Cancer Research and Treatment (IRCC) University of Torino, The Oncogenomics Center, Torino, Italy*

Background: Lung cancer is the most common cancer in the world, and is lethal in 90% of the cases. In non-small cell lung cancer (NSCLC), deregulated receptor tyrosine kinases (RTKs) are among the causal dominant oncogenes. In a subset of NSCLC, the Anaplastic Lymphoma Kinase (ALK) gene has been described to be translocated and fused to EML4. Here, we investigated the ALK oncogenic addiction of human NSCLC and studied the putative co-operative role of other kinases.
Materials and Methods: Human lung cancer cell lines H2228, H3122 (EML4-ALK+ cells) and H1395 (negative control) were treated with small molecule ALK (Cephalon) or EGFR inhibitors. Cells were stably transduced with doxycycline inducible shRNA against ALK (A5). Phosphoproteomic and GEP analysis were performed after treatment with ALK inhibitors. Apoptosis was measured by TMRM staining and cell proliferation by iodidum propide staining followed by FACS analysis. Immunocompromised mice were inoculated s.c. and treated with an ALK inhibitor. Tumor growth measuring, bioluminescence imaging and immunohistochemistry were performed.
Results: The ectopic expression of EML4-ALK in ALK positive NSCLC cell lines (H2228 and H3122) resulted in the activation of multiple signaling pathways as described for other known ALK fusions. Although EML4-ALK can induce transformation in lung *in vitro* and *in vivo*, ALK inhibition via shRNA or small molecule inhibitors induced only the apoptosis of H3122 cells, whereas in H2228 it caused cell growth arrest. Moreover, the treatment with ALK inhibitors led to tumor regression *in vivo.* Based on Phosphoproteomic analyses we demonstrated that the phosphorylation status of several TKs (EGFR, Met, FGFR, Jak1 or IGFR) was affected by ALK inhibition only in H2228 cell line. Notably, the combined treatment with anti-ALK and EGFR inhibitors resulted in an increased cell death of H2228 cells to values similar to those observed for ALK treated H3122 cells. Finally, GEP analyses showed that known EGFR substrates were specifically down regulated upon the combined treatment in H2228 cells.
Conclusions: ALK signaling is required for the maintenance of the neoplastic phenotype of some ALK positive NSCLC cells and its abrogation could represent a novel strategy for the treatment of a well-defined subset of human lung cancer (complete ALK addiction). More importantly, the tumor survival and maintenance of ALK positive neoplastic cells might relay on the concomitant activation of multiple RTKs.