tumors transplanted into syngeneic FVB mice, perturbation of HER2/HER3 signaling pathway with EZN-3920 resulted in tumor growth inhibition, reduced expression of HER3, and induction of apoptosis as assessed by immunohistochemical methods. It is concluded that EZN-3920 provides a novel strategy to effectively down modulate HER3-mediated addiction to growth factors. Furthermore, down regulation of HER3 may provide a novel strategy to overcome resistance to HER1 and HER2 targeted therapies.

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Utility of microRNA analysis for understanding treatment mechanism of action: Necitumumab +/− gemcitabine/cisplatin in NSCLC models

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The contribution of mRNA regulation through effects on microRNA (miRNA), to the anti-tumor effects of therapy needs further study. Here, we utilized SABiosciences PCR arrays to identify oppositely regulated miRNA and predicted mRNA targets in A549 and NCI-H1650 non-small cell lung cancer (NSCLC) xenograft tumors growing in nu/nu athymic mice. Necitumumab, a recombinant human IgG1 targeting EGFR, alone or in combination with cisplatin+gemcitabine (cis/gem), inhibited the growth of these tumors. In the A549 model, 34% of 125 human mRNAs that were >2-fold up or down-regulated by necitumumab, cis/gem, or necitumumab+cis/gem ($p < 0.05$ by t-test versus saline control, $n = 3$), were predicted to be targets of at least one of the 16 tumor miRNA affected oppositely by the same respective treatment, indicating potential regulation of mRNA by miRNA in these tumors. In the NCI-H1650 model, cis/gem treatment did not affect the expression levels of the miRNAs evaluated, however 40% of 52 human mRNAs that were >2-fold up or down-regulated by necitumumab or necitumumab+cis/gem treatment were predicted to be targets of at least one of the 5 miRNA affected oppositely by the same respective treatment, again supporting the potential involvement of miRNA in the regulation of mRNA in tumors. The potential value of miRNA analysis for understanding mechanism of action *in vivo* was further highlighted by the observed necitumumab induced increase in mir-15b (18.3 fold, $p = 0.037$), let-7g (6.5 fold, $p = 0.03$) and miR-150 (2.3 fold, $p = 0.026$), that may impact tumor cell apoptosis and cell proliferation through modulation of BCL2 (-46.5 fold, $p = 0.002$), Cyclin D1 (−11.1 fold, $p = 0.0009$) and TP53 (-133.8 fold, $p = 0.0003$) mRNA in NCI-H1650 tumors. In the A549 model necitumumab increased the level of miR-148a (3.44 fold, $p = 0.0034$), miR-148b (2.54 fold $p = 0.006$), and miR-29 (2.1 fold, $p = 0.02$), that may target mRNA for the methyltransferase DNMT3b (−4.5 fold, $p = 0.02$) to restore normal patterns of methylation-silenced tumor suppressor genes such as CDKN2A (66.7 fold, $p = 0.004$), RARA (8.7 fold, $p = 0.006$), RARB (9 fold, $p = 0.007$) and RARG (6.67 fold, $p = 0.001$), contributing towards the treatment induced reduction in tumor growth. To summarize, utilizing necitumumab +/− gem/cis efficacy in NSCLC subcutaneous xenograft tumor models, we demonstrate that miRNA analysis can be an important tool in understanding the mechanism of action underlying antitumor effects of therapy.

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Specific MET inhibition using SU11274 impairs cholangiocarcinoma cells proliferation, motility and invasion

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Background: Cholangiocarcinomas (CCA) are highly malignant tumors of unmet medical needs often displaying aberrant MET signaling. MET activation either by HGF stimulation, MET over-expression or mutations induces cell proliferation, invasion, and angiogenesis, offering opportunities for investigating the potential of novel MET inhibitors such as SU11274.
Materials and Methods: SU11274, a pyrrole indolinone, specifically inhibits overexpressed and oncogenic MET activation at nanomolar concentrations. Antiproliferative effects of SU11274 were evaluated in human CCA cell lines (Mz-chA1, Mz-chA2 and SK-ch) using MTT assay. Baseline and phosphorylated (p-) protein levels were assessed by Western blot analysis. mRNA expressions used qRT-PCR. Mobility was investigated by wound-healing and matrigel invasion assays. Cell cycle distribution was studied by FACS analysis.
Results: MET-protein and -mRNA expression was detectable in our three CCA cell lines and cells were found responding to HGF stimulation as detected by activation of p-MET$^{Tyr1234/35}$ in all cell lines. MET activation by HGF was associated with increase of p-GAB1, p-ERK1/2, and p-AKT^{ser473} in Mz-chA2 cells. SU11274 displayed antiproliferative effects at concentrations ranging 2−5 μM after 48−72h exposures in our three cell lines without HGF stimulation. Cell cycle analysis of CCA cells exposed to 5μM SU11274 for 72h demonstrated accumulation of cells in G2/M phase. In HGF-stimulated Mz-chA2 and SK-ch cells, SU11274 blocked p-MET and p-GAB1 at MET-specific concentrations ranging 0.5−2 μM that were also shown yielding antiproliferative effects. At these concentrations, SU11274 inhibited HGF-induced downstream MET signaling by reducing p-AKT^{473} and p-ERK1/2. No significant effect of SU11274 on E-cadherin and vimentin expression was observed. SU11274 (5μM) decreased spontaneous HGF-independent cell motility of Mz-chA2 cells and their invasion in matrigel.
Conclusion: Inhibition of p-MET by SU11274 inhibited HGF-dependent MET signaling that resulted in inhibition of cell proliferation, motility and invasion in CCA cells. CCA may be an interesting tumor type to evaluate novel MET inhibitors either as single agents and/or in combination with other targeted therapies.

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Pharmacological characterization of NMS-P506, a novel second generation HSP90 inhibitor

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Background: the molecular chaperone heat shock protein 90 (HSP90) is essential for the conformational maturation and stability of a variety of key proteins, including kinases, implicated in cancer development and progression.
Prototype geldanamycin derivatives are the most advanced compounds, but their liabilities may ultimately limit clinical applications and justify the development of new non ansamycin drugs. In this perspective, the identification of inhibitors capable of long lasting pharmacodynamic modulation of client proteins in tumour tissues might prove important in order to improve the therapeutic window in the clinics.
Results: here we describe the *in vitro* and *in vivo* characterisation of NMS-P506, representative of a novel class of fully synthetic non-ansamycin HSP90 inhibitors. NMS-P506 binds HSP90α with an affinity of 65 pM, and has no significant activity against a broad panel of kinases, as well as other relevant ATPases.
When tested against a panel of tumor cell lines of various tissue origins, NMS-P506 showed widespread antiproliferative activity, with an average IC_{50} of 95 nM. When characterized by Biacore analysis the compound showed a very slow rate of dissociation from HSP90, with a K_{off} of $8.09E^{-5}$ 1/sec, which translates in cells in a long lasting degradation of HSP90-dependent oncoproteins and up regulation of HSP70.
In mice, NMS-P506 had a favourable pharmacokinetic profile with a $t_{1/2}$ of 6 hours in plasma. Selective retention was observed in tumours, with an extended half-life of 139 hours, and relevant concentrations of the compound were found in the brain.
In vivo, NMS-P506 showed an excellent anti tumor efficacy in the B-RAF V600E driven A375 melanoma model, as well as in the A2780 ovarian cancer model, resulting in tumor shrinkage after weekly intravenous administrations with a good tolerability profile. This *in vivo* activity was associated with apoptosis induction and prolonged degradation of HSP90 client proteins.
In conclusion, we report the characterization of NMS-P506, a new second generation Hsp90 inhibitor, capable of long lasting pharmacodynamic modulation of client proteins in cells and in xenograft tumors. NMS-P506 has a very potent *in vitro* and *in vivo* activity, with a good PK profile and selective retention in tumors, which makes it a candidate for further development. Moreover, the brain penetration makes this compound potentially attractive also for brain tumors or metastases.

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Therapeutic targeting of the pro-survival transcription factor CREB sensitizes glioblastomas to temozolomide-based therapy

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Background: Glioblastoma multiforme (GBM) is the most common, lethal primary brain tumor in adults. Following debulking surgery, adding temozolomide (TMZ) with, and after, radiation therapy has become the standard of care for newly diagnosed GBM, yielding 15 months median overall survival versus 12 months with radiation alone. Despite this advance, more effective therapeutic options are needed, particularly

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