Conclusion: Sorafenib inhibits proliferation and migration but not invasion in colorectal cancer. Combination therapies with 5-FU, oxaliplatin or irinotecan deem not feasible.

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Inhibition of erlotinib on bone metastasis of human non-small-cell lung cancer cell line NCI-H292

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Background: Bone metastases occur in 30−40% of non-small-cell lung cancer (NSCLC) patients, and are often associated with significant morbidity. Previous pre-clinical and clinical findings suggested a potential role of epidermal growth factor receptor (EGFR) in osteoclast differentiation and the pathogenesis of bone metastasis. In the present study, we investigated the effect of erlotinib, an orally active EGFR tyrosine kinase inhibitor, on the bone metastases of human non-small-cell lung cancer (NSCLC) cell line NCI-H292.
Material and Methods: To establish a novel bone metastasis model, the NCI-H292 cells were injected into both tibiae and the mice were randomly divided into two groups of 10 mice on day 1. The mice were daily administered either oral erlotinib (75 mg/kg/day) or vehicle (Captisol) for 27 days from day 1. For the in vitro cell proliferation assay, NCI-H292 cells were seeded into 96-well plates. On the following day, cells were treated with erlotinib for 4 days and then MTT assays were done. The concentration of osteolytic factors produced by NCI-H292 cells in the culture media was determined by immunoassays. To investigate the inhibitory effect of erlotinib on the osteoclast differentiation, the standard osteoclast differentiation culture system using mouse bone marrow cells was used.
Results: We established a novel bone metastasis model of NCI-H292 cells. In this model, erlotinib significantly inhibited osteolytic bone destruction of NCI-H292 cells ($P < 0.05$). Erlotinib showed a marked inhibition of NCI-H292 cell proliferation *in vitro*, and the IC_{50} value was 0.08 μM. Furthermore, erlotinib suppressed the production of osteolytic factors, such as parathyroid hormone-related protein (PTHrP), IL-8, IL-11 and vascular endothelial growth factor (VEGF) in NCI-H292 cells. In addition to the effects for NCI-H292 cells, erlotinib also inhibited osteoclast differentiation from mouse bone marrow cells.
Conclusion: Erlotinib inhibits tumor-induced osteolytic metastases by the effect of suppressing tumor growth as well as suppressing osteoclast development, by blocking osteolytic factor production in tumor cells, and osteoclast differentiation from bone marrow.

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Defining efficacy thresholds in preclinical models of cancer: a comparative analysis of cetuximab efficacy and biomarkers in colorectal cancer models

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Clinical studies have demonstrated a dramatic difference in clinical benefit with epidermal growth factor receptor (EGFR) antibodies in metastatic colorectal cancer (mCRC) patients with wild type (WT) versus mutant (MT) KRAS gene harboring tumors. These findings provide a unique opportunity to address a frequent issue arising in preclinical cancer drug development, i.e. the criteria for efficacy in cancer models predictive of clinical benefits.
The chimeric antibody to EGFR, cetuximab (Erbitux®), was tested alone or in combination with irinotecan+oxaliplatin [IROX] in 13 subcutaneous xenograft tumor models established in mice with human CRC cell lines. Cetuximab has previously been shown to increase the effects of these chemotherapies. KRAS mutation status was evaluated by sequencing and EGFR gene copy number was evaluated by RT-PCR for each CRC cell line utilized.
With a threshold for efficacy of T/C% (Treatment/Control relative tumor volume ratio) $\leqslant$ 50%, cetuximab alone was efficacious in 50% of KRAS MT models and 57% of KRAS WT models tested. Cetuximab+IROX was efficacious in 100% of KRAS MT models and 71% of KRAS WT models. Utilizing a threshold for efficacy of tumor regression ($\geqslant$25% decrease in tumor volume in $\geqslant$10% of mice), cetuximab alone was efficacious in 8% of KRAS WT models (DiFi model). A very high EGFR gene copy number in DiFi (535 copies) stands out as a potential biomarker for this significant level of activity. Cetuximab + IROX was efficacious in 50% of KRAS MT models and 43% of KRAS WT models, while IROX alone was not efficacious. The benefits of adding IROX to cetuximab were mostly related to the effects of irinotecan, although the percentage of mice achieving tumor regression was generally increased by including oxaliplatin in the combination.
Subcutaneous xenograft models of colorectal cancer do not predict the clinically demonstrated KRAS dependence for the benefits of an EGFR antibody when T/C% $\leqslant$ 50% is utilized to claim the treatment "works". Results may speak against the validity of these models, or may support shifting the threshold for response away from tumor growth inhibition towards partial or complete tumor regressions for advancement of therapeutic strategies into clinical testing. In this regard, significant preclinical benefits of cetuximab in combination with IROX demonstrated in KRAS MT models may indicate the potential for clinically demonstrable benefits of anti-EGFR therapy in novel combination strategies.

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Amino-alkyl substituted fused imidazoles: potent, selective and orally bioavailable inhibitors of PI3K

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The phosphatidylinositol 3-kinase (PI3K) signaling pathway plays a crucial role in cell growth, proliferation and survival. This pathway is activated in a variety of solid and non-solid tumors. In many instances this is due to either activating mutations in the catalytic subunit of PI3Ka, p110α; or inactivating mutations or deletions of the tumor suppressor PTEN.
In addition, persistent signaling through the PI3K/Akt pathway has been shown to be a major mechanism of resistance to therapy. Hence, PI3K, and in particular the p110α subunit of PI3K, is a highly promising candidate for cancer therapy.
Using a rational drug design strategy, we identified a novel fused imidazoles series, with potent activity against PI3Kα Depending on the C-2 substitution fragment we have observed different isoforms profiles. Here, we describe the design, synthesis and biological characterization of C-2 amino alkyl fused imidazoles subseries, reporting its SAR/SPR (ADME).
We identified lead compounds with potency in the low nanomolar range *vs.* p110α and d, selective versus the other isoforms and versus other related PIKK family members such as mTOR, DNA-PK or ATR. In general, this series show high selectivity versus a 24 kinase panel. The compounds display cellular activity by blocking PI3K signaling, S473 P-Akt in U2OS cells, in the low nanomolar range.
Finally, we will show in vivo PK data for selected compounds.

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Histone deacetylase inhibitor Belinostat (PXD-101) represses androgen receptor expression and acts synergistically with castration and bicalutamide treatment to inhibit prostate cancer growth hormone refractory models

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Background: Growth of prostate cancer cells is initially androgen dependent. However, resistance to hormone therapy inevitably occurs. Histone deacetylase (HDAC) inhibitors are currently undergoing clinical trials in cancer patients on the basis of their effect. One of them is the hydroxamic acid belinostat (PXD101) that has demonstrated therapeutic efficacy for several clinical indications.
Materials and Methods: We investigated the in vitro effects of PXD-101 in a panel of prostate cancer cells and the in vivo effects of this drug using two aggressive hormone refractory prostate cancer cell lines expressing (22rv1) or not (PC3) the AR.
Results: PXD101 rapidly induced histone H3 and H4 acetylation and upregulated p21 expression. PXD101 significantly decreased also the expression of the cell cycle regulatory proteins (p27, cyclin D1/cyclin dependent kinase (CDK) 4, CDK6, and cyclin E/CDK2) with reduced mitotic rate and accumulation of cells both in G0/G1 and in G_2-M cell cycle phase. Apoptosis was associated with up-regulation of the pro-death Bak and Bim, as well as with attenuation of the levels of Akt, XIAP, survivin, Bcl-2, and Bcl-x_L. The effects were higher in less differentiated cells when compared to more differentiated androgen sensitive cells. In mice bearing 22rv1 tumors, PXD101 (20 mg/kg/biday) caused significant suppression of tumor growth compared with mice receiving vehicle alone and treatment with 40 mg/kg/day resulted in a 60% reduction in the mean final tumor volume compared with controls. In PC3 bearing mice, 40 mg/Kg, bid, i.p PXD101 reduced PC3\ tumor proliferation of about 47%. The apoptotic program was triggered both in acute treatment with high PXD101 dose (>1.0 mM) and in chronic treatment (from 10−14 days) with low doses of drug (< 0.1 mM). The significant effects of chronic treatments suggest the possibility to use low doses of this drug to reduce the side effects. Culture of 22rv1 cells in steroid-free medium sensitized these cells to

PXD101. Moreover, a combination of low, sub-effective doses of PXD101 and the AR antagonist, bicalutamide, resulted in a synergistic reduction in cell proliferation and increase in caspase-dependent cell death. In similar conditions but in acute treatment, this combination was not effective in AR negative PC3 cells. However, chronic administration of PXD101 at subeffective doses restored AR expression and sensitized to the activity of bicalutamide. In vivo, PXD101 showed higher effects when administered in castrated mice bearing both 22rv1 and PC3 cells and this seem to be associated also to the reduction of Her2 expression and Her2-mediated AR transactivation.
Conclusions: Taken together, our results demonstrate that HDAC inhibition can increase the sensitivity of antagonists resulting attractive in the treatment of hormone refractory prostate cancers. Further information, we need to develop clinical treatment strategies for prostate cancer.

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Anti-tumor activity of CU-201, an inhibitor of HDAC, SFK and Abl kinases

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Recent evidence has established Src Family Kinases (SFKs) as a critical component of multiple signaling pathways that regulate proliferation, survival, angiogenesis and metastasis. Increased levels of SFK protein and activity have been widely reported in human cancers. Dasatinib and bosutinib, multi-kinase inhibitors of Bcr-Abl and SFKs, are undergoing multiple clinical trails for the treatment of hematological and solid tumors associated with Src kinase. On the other hand, HDAC inhibitors downregulate oncoproteins and disrupt signaling pathways of tumor growth and metastasis via epigenetic modification. Synergy between HDAC and Src inhibition has previously been demonstrated in cancer cell lines. We have designed CU-201, a single small molecule that displays potent inhibition of HDAC (IC50 6.8 nM), Src (IC50 2.4 nM) and Brc-Abl (25.8 nM). In cell based assays CU-201 exhibits potent anti-proliferation and apoptosis-inducing effects in hematological as well as solid tumor cell lines. CU-201 displays preferential exposure to tumor tissues compared with plasma in tumor-bearing mice with a prolonged half life following intravenous administration. CU-201 displays antitumor efficacy in various xenograft models of hematological and solid tumors. MOA (mechanism-of-action) studies demonstrate that CU-201 is able to inhibit Src family kinases and upregulate acetylated histones. Importantly CU-201 is able to downregulate essential molecular mediators of the Src signaling pathways through epigenetic regulation, which may partially account for its synergistic effects. In summary, as a single agent with potent non-kinase and kinase inhibitory activities, CU-201 exhibits potent antitumor activity in preclinical models of cancer and deserves further investigation.

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Dasatinib blocks cetuximab- and radiation-induced nuclear translocation of the epidermal growth factor receptor in head and neck squamous cell carcinoma

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The aberrant expression of epidermal growth factor receptor (EGFR) has been linked to the etiology of head and neck squamous cell carcinoma (HNSCC). The first major phase III trial combining cetuximab with radiation confirmed a strong survival advantage. However, both cetuximab and radiation can promote EGFR translocation to the nucleus where it enhances resistance to both of these modalities. In this study we sought to determine how to block cetuximab- and radiation-induced translocation of EGFR to the nucleus in HNSCC cell lines. We utilized three established HNSCC cell lines, SCC1, SCC6 and SCC1483 and measured nuclear translocation of EGFR after treatment with cetuximab or radiation. We then utilized dasatinib (BMS-354825, sprycel®), a potent, orally bioavailable inhibitor of several tyrosine kinases, including the Src Family Kinases, to determine if SFKs blockade could abrogate cetuximab- and radiation-induced nuclear EGFR translocation. The results of these experiments showed that cetuximab and radiation treatment of all three HNSCC lines lead to translocation of the EGFR to the nucleus. Further, blockade of SFKs abrogated cetuximab and radiation-induced EGFR translocation to the nucleus. The data presented in this study suggests that both cetuximab and radiation can promote EGFR translocation to the nucleus and dasatinib can inhibit this process. Collectively these findings may suggest that dasatinib can limit EGFR translocation to the nucleus and may enhance radiotherapy plus cetuximab in HNSCC.

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The effect of KRAS mutations on the rectal cancer transcriptome: clinical implications

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Background: Mutations of the KRAS oncogene are predictive for resistance to treatment with antibodies against the epithelial growth factor receptor in patients with colorectal cancer. Overcoming this therapeutic dilemma could potentially be achieved by the introduction of drugs that inhibit signaling pathways that are activated by KRAS mutations.
Material: To comprehensively identify such signaling pathways we profiled pretreatment biopsies and normal mucosa from 65 patients with locally advanced rectal cancer − 30 of which carried mutated KRAS − using global gene expression microarrays.
Results: By comparing all tumor tissues exclusively to matched normal mucosa, we could improve assay sensitivity, and identified a total of 22,297 features that were differentially expressed (adjusted p-value $p < 0.05$) between normal mucosa and cancer, including several novel potential rectal cancer genes. We then used this comprehensive description of the rectal cancer transcriptome as the baseline for identifying KRAS-dependent alterations. The presence of activating KRAS mutations resulted in significant upregulation of 13 genes (adjusted p-value <0.05), among them DUSP4, a MAP-kinase phosphatase, and SMYD3, a histone methyltransferase. Inhibition of the expression of both genes has previously been shown using the MEK1-inhibitor PD98059 and the antibacterial compound Novobiocin, respectively.
Conclusion: These findings first have to be validated in rectal cancer, however, they suggest a potential approach to overcome resistance to treatment with antibodies against the epithelial growth factor receptor in patients with KRAS-mutant rectal carcinomas.

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Synergistic activity of the novel apoptosis-inducing compound KP1339 with the tyrosine kinase inhibitor sorafenib in cancer cell lines of diverse origin

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KP1339 is a promising novel apoptosis-inducing anti-cancer compound. The aim of this study was to test the in vitro and in vivo activity of KP1339 in combination with sorafenib. The combination of KP1339 and sorafenib was initially tested against a tumor cell line panel (n = 10) by MTT assay. The combination of the two drugs resulted in additive to synergistic effects in all cell lines tested. Particularly strong synergism with CI values between 0.1 and 0.5 were observed at higher sorafenib concentrations (10 μM). The synergistic activity of sorafenib and KP1339 was observed in sorafenib-resistant as well as sorafenib-sensitive cell lines. Recently, we have presented that KP1339 treatment led to cell cycle arrest in G2/M Phase and induced the phosphorylation of P38, JNK, and ERK. We therefore analyzed the effects of KP1339-sorafenib combination on the phosphorylation of these proteins and cell cycle arrest. Western blot analyses revealed that, compared to KP1339 monotherapy, KP1339-sorafenib combination exhibits significantly reduced phosphorylation of P38 and ERK. Moreover, addition of sorafenib shifted KP1339-induced G2/M arrest into a G0/G1 arrest. The activity of the KP1339-sorafenib combination was also evaluated in xenograft experiments (Hep3B cells). KP1339 monotherapy led to a 2.4-fold increase in life span (mean survival 80 days vs. 33 days in control) and thus was superior to sorafenib monotherapy, which induced a 1.9-fold survival increase compared to control (60 days vs. 33 days). Combination of KP1339 with sorafenib increased the mean survival by 3.9-fold to 96 days. Together, our data indicate that the combination of KP1339 with sorafenib displays promising activity in vitro and in vivo especially against human hepatoma cells.
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