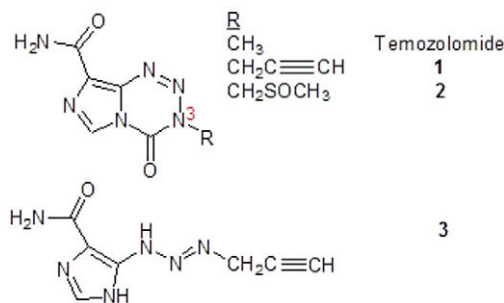


treatment for MGMT positive GBM tumors and possess broader spectrum anticancer activity.



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POSTER

#### Amino-carbonyl 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 PI3Kα 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 exploration and biological characterization of C-2 amino-carbonyl fused imidazoles series, reporting its SAR/SPR (ADME). We identified lead compounds with a potency in the low nanomolar range vs. p110α, b and d and selectivity against other related PIKK family members such as mTOR, DNA-PK or ATR. In general, this series show high selectivity versus a panel of 24 protein kinases. 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 ETP-992.

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POSTER

#### Preclinical characterization of 4SC-202, a novel isotype specific HDAC inhibitor

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Alterations of protein acetylation regulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC) are associated with various types of cancer and thus HDACs have emerged as attractive drug targets for neoplastic disease. The family of HDACs is divided into four classes, of which three consist of Zn<sup>2+</sup>-dependent enzymes. Several pan-HDAC inhibitors are currently under clinical investigation in a broad range of tumour indications. Based on their chemical structure they can be categorized into hydroxamates, benzamides, cyclic peptides, fatty acid analogs and ketons. While promising, these compounds have exhibited side effects that might limit their clinical potential. It might be possible to reduce some of the toxicity associated with HDAC inhibition by specifically targeting only selected HDAC isoforms.

4SC-202 is a novel orally available class I specific HDAC inhibitor of the benzamide type compound family that harbors additional strong anti-mitotic potential associated with cell cycle arrest and pronounced induction of apoptosis. This HDAC inhibitor shows strong anti-tumoural activity *in vitro* against a broad range of human cancer cell lines with submicromolar activity on tumour cell growth and also *in vivo* after oral application in relevant xenograft animal models. It has been well tolerated in a number of toxicological studies in rodent and non-rodent species. A clinical phase I First-in-Man trial with 4SC-202 in hematological malignancies is currently under preparation and is planned to commence in H2 2010. A comprehensive overview of the preclinical characterization of this novel

class I selective HDAC inhibitor and an outlook on the planned first clinical application to man will be presented.

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POSTER

#### Allelic loss on p16, BRCA1, BRCA2, PTEN and p53 genes in sporadic invasive ductal carcinomas

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**Background:** Breast cancers show various molecular and genetic alterations in its development and progression. The major tumor suppressor genes (TSGs) such as p16, PTEN and p53 may play important roles in cell cycle regulation, apoptosis and the regulation of the expression of other genes as well as tumor suppression. BRCA1 and BRCA2 genes are TSGs involved in familial breast cancer. Loss of heterozygosity (LOH), novel mechanisms of carcinogenesis, has been known to be a useful prognostic factor in many kinds of malignant tumors. LOH is related to the allelic loss of various TSGs. This study was planned not only to evaluate LOH of 5 TSGs in sporadic invasive ductal carcinomas (IDCs) and correlate these results with the clinicopathological factors, but also to investigate the role of BRCA1 and BRCA2 TSCs.

**Material and Methods:** LOH analysis was carried out using a polymerase chain reaction with 20 polymorphic microsatellite markers (including D9S162, TP53, D13S290, D17S1323, D10S541, etc) of 5 TSGs in 50 surgically resected tumors and their non-tumorous counterparts. IDC case having three or more LOH was grouped as LOH-H.

**Results:** There was no detectable LOH in normal tissue. At least one LOH was detected in 88% of 50 cases of IDCs. LOH results detected on all chromosomes showed statistical discrimination between benign tumor and malignant tumor. LOH rates of p16, BRCA1, BRCA2, p53 and PTEN TSGs were detected in 38%, 32%, 42%, 56 and 48%, respectively. Especially, LOH rates on D13S290, D17S1323 markers were 25.0% and 30.2%, respectively. LOH of p16, BRCA1 and PTEN TSGs inversely correlated with tumor grade 1. Low LOH detection rate on BRCA2 gene was measured in T1 tumor and stage I. LOH of p53 and PTEN TSGs correlated well with the lymph node metastasis and stage. The LOH-High results correlated well with the tumor size, lymph node metastasis and stage.

**Conclusions:** These results suggest that LOH of BRCA1, BRCA2 as well as 3 major TSGs may contribute to the development and invasion of IDCs. Also combined use of various LOH markers and application of LOH-H concept may help in deciding prognosis of IDCs.

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POSTER

#### Is sorafenib effective in colorectal cancer?

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**Background:** We initiated this preclinical study in order to analyze if tyrosine kinase inhibitor sorafenib might be an effective therapeutic option in human colorectal cancer.

**Material and Methods:** The expression status of VEGFR1-3, PDGFRα/β and EGFR1 was analysed in 100 colorectal cancer samples and in 4 different CRC cell lines. Expression was correlated with clinico-pathologic parameters. The K-ras, Raf, PI3K and PTEN mutation status of cell lines was obtained by PCR-RFLP or sequencing, respectively. The effect of increasing sorafenib doses on proliferation, apoptosis, migration and invasion was analysed *in vitro*. In addition, we analysed the efficacy of sorafenib monotherapy and different combination therapies (sorafenib + 5-FU, Irinotecan or oxaliplatin) *in vitro* and *in vivo* in a xenograft tumor mouse model.

**Results:** The majority of colorectal carcinoma samples revealed a VEGFR1 (92%), PDGFRα (83%), EGFR1 (88%) and PDGFRβ (62%) expression, whereas VEGFR2 (51%), VEGFR3 (50%) were expressed only in half of all samples. Expression of VEGFR3 and PDGFRα significantly correlated with lymphatic metastatic disease (P = 0.01 and P = 0.05, respectively), whereas VEGFR3 did correlate with distant metastases (P = 0.05). Human colorectal cancer cell lines revealed varying expression levels of TKs. *In vitro*, sorafenib did impact on growth of SW480 and HT29 cells but not on SW620 or Caco2 cells. Migration was decreased in all cell lines analysed, while sorafenib did not modulate invasion in any cell line. Combination of sorafenib and 5-FU, irinotecan or oxaliplatin was not superior to a sorafenib mono-therapy and even seemed to stimulate proliferation in some cell lines. Similarly, the combination of 5-FU and sorafenib was inferior to a 5-FU or sorafenib monotherapy, in an *in vivo* mouse model. Response did not correlate with the mutation status of K-ras, Raf, PTEN or PI3K. Nor did we observe any evident association of response with the expression of pPI3K/PI3K, pAKT/AKT, mTOR/pmTOR or pMEK/Mek. However, responsive cell lines (SW480 and HT29) decreased AKT expression upon sorafenib exposition, which was not observed in resistant cell lines (Caco2, SW620).

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

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POSTER

# **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  $0.08 \mu M$ . 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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POSTER

# **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 (Erbix<sup>®</sup>), 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)  $\leq 50\%$ , 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 ( $\geq 25\%$  decrease in tumor volume in  $\geq 10\%$  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%  $\leq 50\%$  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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POSTER

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

J. Pastor<sup>1</sup>, S. Martínez<sup>1</sup>, R.M. Álvarez<sup>1</sup>, C. Varela<sup>1</sup>, A.I. Hernández<sup>1</sup>, S. Rodríguez<sup>1</sup>, J. Oyarzábal<sup>1</sup>, M. Albarrán<sup>1</sup>, W. Link<sup>1</sup>, J.R. Bischoff<sup>1</sup>. <sup>1</sup>Spanish National Cancer Research Centre, Experimental Therapeutics, Madrid, Spain

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 PI3K $\alpha$ , p110 $\alpha$ ; 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 p110 $\alpha$  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 PI3K $\alpha$ . 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. p110 $\alpha$  and d, selective versus the other isoforms and versus other related PI3K 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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POSTER

# **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 G2-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<sub>L</sub>. 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/bid) 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 ( $>1.0 \text{ mM}$ ) and in chronic treatment (from 10–14 days) with low doses of drug ( $< 0.1 \text{ mM}$ ). 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