maintain exponential growth. All experiments were conducted with cells in exponential growth phase. Cells were free of mycoplasma contamination.
Results: 17-AAG was toxic to A549 cells and synergistic toxicity was observed during simultaneous exposures with each of the four TKIs. Flow cytometry analysis of cell surface expression of EGFR using monoclonal anti-EGFR antibodies showed 50% decrease upon pre-treatment with 17-AAG for 24 h. Uridine transport in A549 cells mediated by human equilibrative nucleoside transporter 1 (hENT1) was inhibited by 17-AAG with an IC_{50} value of 15 μM.
Conclusions: Combination therapy with 17-AAG and several clinically used TKIs looks promising and should lead to the design of future trials based on these combinations. The inhibition by 17-AAG of hENT-1 mediated uridine transport suggests an explanation for the observed antagonism between cytarabine with 17-AAG in leukemic cells since cellular uptake of cytarabine is mediated primarily by hENT1. These results caution against combination of nucleoside analogs with 17-AAG in patients.

174 POSTER

Investigating the role of Hedgehog signaling in tumor models

R.J. Austin[1], W. Aaron[1], A. Chong[1], M.G. Johnson[2], B.S. Lucas[2], D.L. McMinn[2], J. Orf[1], M. Rong[1], Q. Ye[3]. *[1]Amgen Inc, Oncology Research, South San Francisco, USA; [2]Amgen Inc, Medicinal Chemistry, South San Francisco, USA; [3]Amgen Inc, Pharmacokinetics & Drug Metabolism, South San Francisco, USA*

Background: Hedgehog (Hh) signaling is activated in medulloblastoma (MB) and basal cell carcinoma, either through loss of the inhibitory protein, Patched (Ptch), or through genetic activation of Smoothened (SMO), a protein that transduces Hh signals. In some colon and pancreatic tumor xenograft models, antagonism of Hh signaling has been reported to attenuate tumor growth, and inhibition of growth correlated with reduced stromal expression of Gli1, a marker of Hh signaling. Here we describe the effects of antagonizing Hh signaling a Ptch-deficient model of MB. We also describe the effects of antagonizing Hh signaling in models of pancreatic, prostate, lung, and bile duct cancers, including models with expression of Hh in the tumor and Gli1 in the stroma.
Materials and Methods: Tumor bearing nude mice were treated with an antagonistic anti-Hh antibody or with specific SMO small molecule antagonists. Effects of these treatments on Gli1 RNA expression and tumor growth rate were assessed.
Results: Mice bearing tumor allografts derived from a Ptch+/− p53−/− mouse model of MB were treated with SMO antagonists. These treatments caused regression of the allografts and robust reduction in tumor Gli1 expression. Mice bearing tumors of ten different xenograft models were also treated with Smoothened antagonists or with anti-Hh antibody. The xenograft models were patient-derived pancreatic or lung tumors or were cell line models of pancreatic, prostate, or bile duct cancers. In these models, antibody and compound treatment significantly reduced Gli1 expression in the stroma, but neither treatment affected tumor growth. All treatments were well tolerated, and no significant weight loss was observed.
Conclusions: The SMO antagonists used in these studies are efficacious in a preclinical model of MB and are ineffective in other tumor models. Although antagonism of Hh signaling in the stroma has previously been correlated with attenuated growth of some tumor models, our data indicate antagonism of Hh signaling in the stroma is insufficient to inhibit growth of all tumor models.

175 POSTER

The treatment of breast cancer tumor growth and metastasis with an anti-MMP9 deoxyribozyme

M.A. Hallett[1], B. Teng[1], T. Seagroves[2], T. Sweatman[3], T. Pourmotabbed[1]. *[1]University of Tennessee Health Science Center, Molecular Sciences, Memphis TN, USA; [2]University of Tennessee Health Science Center, Pathology, Memphis TN, USA; [3]University of Tennessee Health Science Center, Pharmacology, Memphis TN, USA*

Background: Despite continued improvements in diagnosis, surgical techniques, and chemotherapy, breast cancer patients are still overcome by cancer metastasis. Tumor cell proliferation, invasion and metastasis are known to be mediated, at least in part, through degradation of basement membrane by neutral MMPs produced by tumor and stromal cells. Evidence suggests that MMP-9 plays a significant role in breast tumor cell proliferation, invasion and metastasis. Our novel catalytic DNA molecule (DNAzyme) based strategy is capable of specifically down regulating MMP9 expression without affecting other MMPs.
Materials & methods: DNAzymes are catalytic enzymes that bind to and cleave specific mRNA, resulting in a decreased protein expression. The application of anti-MMP-9 DNAzyme (AM9D) for the treatment of metastatic breast cancer was evaluated *in vitro* and *in vivo* using MDA-MB-231 human breast cancer cells and a MMTV-PyMT transgenic breast cancer mouse model, respectively. AM9D was intratumorally injected into the mammary tumors of the transgenic mice, once a week for 4 weeks. Tumor sizes were monitored bi-weekly and final tumor size was measured by weighing tumors. The role of AM9D on MMP-9 protein production and blood vessel formation were determined by immunohistochemistry. To determine safety and efficacy of AM9D systemically delivered to animals, AM9D was labeled with ^{35}S and injected intravenously into the tail vein of mice. Distribution and clearance rates were determined by excising tissues and quantizing the amount of radioactivity in each tissue.
Results: The DNAzyme studied in these experiments represent a novel application of this nucleic acid. Treatment with AM9D in vitro lead to reduced expression of MMP-9 mRNA and in vivo resulted in delayed rate of tumor growth, retarded final tumor volume by up to 70%, and a reduction in lung macrometastases. This decrease in tumor growth and lung metastasis was correlated with decreased MMP-9 protein production within the treated tumor tissues. Tumors treated with AM9D were also less vascular compared to control and untreated tumors. Furthermore, DNAzyme is distributed to major organs including lung through intravenous injection, thus, breaking ground for a clinical treatment strategy.
Conclusion: These results show that targeting and down regulation of MMP-9 by a novel DNAzyme molecule could prove useful as a therapy against breast carcinoma tumor growth and invasion.

176 POSTER

N3-Substituted temozolomide analogs overcome methylguanine DNA methyltransferase and mismatch repair

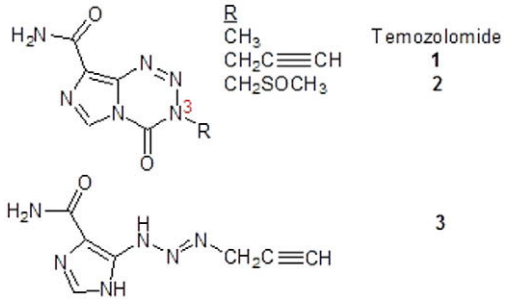
T.D. Bradshaw[1], M. Hummersone[2], J. Hartley[3], M.F.G. Stevens[4], J. Zhang[4]. *[1]University of Nottingham, School of Pharmacy University of Nottingham, Nottingham, United Kingdom; [2]Pharminox Ltd, Biocity, Nottingham, United Kingdom; [3]UCL, UCL Cancer Institute, London, United Kingdom; [4]University of Nottingham, School of Pharmacy, Nottingham, United Kingdom*

Glioblastoma multiforme (GBM) is the most prevalent and aggressive malignant adult CNS tumor. Treatment includes radiotherapy and temozolomide (TMZ) alkylating agent chemotherapy. TMZ methylates purine residues of DNA: *N7*-guanine, *N3*-adenine and *O6*-guanine. *O6*-methylguanine, the primary cytotoxic lesion, is a substrate for direct repair by methylguanine DNA methyltransferase (MGMT). Response to TMZ requires low MGMT levels and functional DNA mismatch repair (MMR). Resistance to TMZ (inherent or acquired), conferred by MGMT expression or MMR deficiency (a consequence of mutation(s) in MMR proteins), represents a huge barrier to successful treatment of GBM.
To address this problem, analogs of TMZ have been synthesized, substituting the N3 methyl moiety with substituents which may lead to DNA lesions able to evade MGMT and DNA MMR.
MTT assays were conducted to compare *in vitro* antitumor activity of TMZ and novel analogs in SNB19 and U373 isogenic glioma cell line pairs: (V = vector control; M = MGMT transfected). TMZ potency reduced 13- and 5.4-fold in SNB19 and U373 cells expressing MGMT; in contrast SNB19M and U373M cells were equi-sensitive as SNB19V and U373V cells to analogs **1** and **2** (Table).

N3-substituent	Analog	GI_{50} (μM)			
		SNB19V	SNB19M	U373V	U373M
CH_3	TMZ	35.7	469.9	68.0	368.7
$CH_2C{=}CH$	**1**	35.6	37.8	37.6	36.1
CH_2SOCH_3	**2**	28.9	14.3	8.2	7.3

In addition, analogs **1** and **2** inhibit growth of vector control glioma cells generated to possess acquired resistance to TMZ. GI_{50} values <50 μM were observed in SNB19VR (MMR deficient; hMSH6 loss) and U373VR (MGMT up-regulation) following analog **1** or **2** challenge.
Analogs **1** and **2** cause G2/M cell cycle arrest in glioma cells irrespective of MGMT status and MMR deficient HCT116 colorectal carcinoma cells. Comet assays demonstrate DNA single strand breaks following SNB19V cell treatment with TMZ and novel analogs, formation of γH2AX foci infer conversion to DNA double strand breaks preceding death by autophagy and apoptosis.
Taq polymerase stop assays reveal that N3 propargyl imidazotetrazine **1** and ring opened N3 propargyl imidazotriazene preferentially alkylate guanine rich DNA sequences. *N-7* guanine alkylation by analog **1** was detected by piperidine cleavage assay.
We conclude that novel imidazotetrazines **1** and **2** elicit *in vitro* antitumor activity irrespective of MGMT and MMR status. Such molecules may offer

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



177 POSTER

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

J. Pastor[1], S. Martínez[1], R.M. Álvarez[1], A.I. Hernández[1], C. Varela[1], A.B. García[1], O. Rabal[1], M.T. González-Granda[1], J. Fominaya[1], J.R. Bischoff[1]. [1]*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 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 PI3Ka. 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.

178 POSTER

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

S.W. Henning[1], R. Doblhofer[1], H. Kohlhof[1], R. Jankowsky[1], T. Maier[2], T. Beckers[2], M. Schmidt[2], B. Hentsch[1]. [1]*4SC AG, Martinsried, Germany;* [2]*Nycomed GmbH, Konstanz, Germany*

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^{2+}-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.

179 POSTER

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

C. Park[1], S. Choi[1], S. Cho[2]. [1]*Hallym University Kangdong Hospital, Surgery, Seoul, Korea;* [2]*Hallym University Kangdong Hospital, Pathology, Seoul, Korea*

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.

180 POSTER

Is sorafenib effective in colorectal cancer?

C. Schimanski[1], M.M. Markus Moehler[1]. [1]*University of Mainz, First Dept of Internal Medicine, Mainz, Germany*

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 oxalipaltin) *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 oxalipaltin 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).