cells as a positive control, were grown either as monolayers, or, to provide a 3D *in vitro* tumour model, as colospheres. Linear-after-the-Exponential (LATE)-PCR was used to quantify CCK-2R gene expression and its sensitivity compared with a Taqman assay. Flow cytometry (FACS) was used to investigate receptor protein expression. Activity of CCK-2R promoter reporters constructed in pGL4, using 250 to 2000 bp of DNA upstream of the CCK-2R start codon, was quantified using luciferase assays.
Results: LATE-PCR for CCK-2R gene expression is 1000-fold more sensitive than the Taqman-based assay. Cell-lines from the panel, including HCT116 (colorectal) and AGS (gastric), in which CCK-2R mRNA was not detectable by the Taqman assay, were positive using the LATE-PCR, confirming the results of previous inhibitor studies. CCK-2R siRNAs resulted in up to 86% ($p < 0.005$) knockdown of the receptor in CCK-2R-transfected AGS cells, confirming the LATE-PCR's specificity. FACS analysis suggests the presence of a small population of cells within HCT116 and AGS cell-lines that express CCK-2R very highly. CCK-2R expression was enriched when cells were grown as colospheres. The CCK-2R promoter constructs were active in cancer cell-lines; however, transcriptional activity did not always correlate with gene expression.
Conclusions: LATE-PCR provides a highly sensitive method for detection of genes such as CCK-2R which have important biological functions but low expression. An element within the 250bp proximal to the CCK-2R transcriptional start site controls transcription of the CCK-2R gene, demonstrating a potential drug target. CCK-2R protein expression is elevated in a subset of cells, and may play a role in promoting survival of cancer stem cells, thereby encouraging drug resistance and cancer recurrence in patients. Thus, CCK2R provides a potential target for therapeutic intervention in GI cancer.

131 POSTER

Systematic drug combination studies with new targeted agents using 30 cell lines established from patient-derived tumor xenografts

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In recent years, the focus of anticancer drug development has changed from conventional cytotoxic drugs to targeted agents interfering with cell proliferation, migration or survival. In most cases, signaling pathways, key regulatory complexes or biological processes of pathophysiological relevance are selected and proteins with key functions targeted. These include signaling cascades originating from receptor tyrosine kinases (e.g. EGFR, HER2 or c-Met,), the mitotic machinery (eg5, Aurora/ARK or polo-like kinases/Plk) and the nucleosome (e.g. HDACs, HATs). However, as a single agent new targeted drugs often demonstrate weak antitumor activity in preclinical testing and clinical trials. Combinations of new targeted agents with each other or with standard cytotoxic drugs are a suitable strategy resulting in potent anti-cancer therapies. As the number of possible drug combinations is essentially limitless and a scientific rationale is only available in few cases, a screening strategy to identify the most promising drug combinations *in vitro* is crucial for success in further development.
At Oncotest a panel of 30 proprietary solid tumor cell lines established from patient-derived xenografts is routinely used. All major histologies are represented such as NSCLC, colon and breast cancer, as well as niche tumors like pleuramesothelioma, bladder and liver cancer. Chemosensitivity information for most standard-of-care drugs and experimental compounds as well as genomic and proteomic characterization data are available. By using this cell line panel, systematic combination studies were performed with the propidium iodide standard cytotoxicity/proliferation assay. Synergistic activity was assessed (i) according to the method of Chou-Talalay (combination at fixed ratio) by calculating "Combination Index" (CI) values using the CalcuSyn software or (ii) by shift of IC50 values (combination at fixed concentration). Activity profiles were established for well described experimental and registered agents targeting, for example, the EGFR and HER2 receptor tyrosine kinases (Lapatinib, Erlotinib), Eg5 (Ispinesib, HR22C16, S-tritylcysteine/STC), HDAC (Entinostat, Vorinostat, LBH-589, SBHA) in combination with each other as well as with standard chemotherapeutic agents like Cisplatin, Paclitaxel or 5-Fluorouracil. The most promising combinations were found to be Ispinesib with Lapatinib and Ispenisib with Erlotinib. A lower level of synergy was evident for combinations of Erlotinib with MS275, Erlotinib with STC, Erlotinib with SAHA or SBHA and Lapatinib with MS275.
In conclusion, the Oncotest solid tumor cell line panel is suitable for a broad, systematic evaluation of drug combinations including cytotoxic and new targeted anticancer agents with the purpose of identifying potential beneficial combinations for further preclinical and clinical studies.

132 POSTER

Intermittent dosing of the MEK inhibitor, GDC-0973, and the PI3K inhibitor, GDC-0941, results in prolonged accumulation of Bim and causes strong tumor growth inhibition in vivo

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Background: Combinations of MEK and PI3K inhibitors have shown promise in pre-clinical cancer models, which has led to combination clinical trials co-targeting these two key cancer signaling pathways. Here we show that continuous exposure of the two drugs in combination is not required for efficacy in cancer models, and that sustained biomarker effects can result from intermittent dosing.
Methods: GDC-0973, a potent and selective MEK1/2 kinase inhibitor, and GDC-0941, a potent and selective Class 1 PI3K inhibitor, were tested alone and in combination with or without drug wash-out followed by assessment of cell viability, apoptosis, and downstream signaling. Pharmacodynamic (PD) response and anti-tumor efficacy were evaluated in mouse xenograft models dosed with GDC-0973 and/or GDC-0941 at varying doses and schedules. Modulation of glucose uptake in xenograft tumors was evaluated in vivo using FDG-PET.
Results: GDC-0973 shows strong cellular potency in a broad panel of tumor types, particularly in BRAF or KRAS mutant cancer cell lines. In vitro, the combination of GDC-0973 and GDC-0941 in BRAF and KRAS mutant cell lines results in synergistic cell growth inhibition and leads to a combinatorial decrease of phosphorylated S6, and increases in cleaved PARP and BimEL that lead to apoptosis. Inhibition of Bim by RNAi attenuates the cell death induced by MEK and PI3K blockade. In vivo, GDC-0973 displays dose-dependent anti-tumor activity in BRAF mutant and KRAS mutant xenograft models, and causes knockdown of pERK that persists up to 8 hours at efficacious doses. In vivo combination efficacy greater than either single agent is observed when GDC-0973 and GDC-0941 are administered in combination, either daily or intermittently. Intermittent dosing results in transient pathway knockdown as measured by levels of pERK, pAkt, pS6 and cyclin D1, but sustained accumulation of Bim. The sustained accumulation of Bim in response to transient MEKi/PI3Ki treatment is also observed in vitro. Combination of GDC-0973 on a high, intermittent schedule dosed with GDC-0941 on a daily schedule resulted in greater combination efficacy with a corresponding decrease in FDG-PET uptake.
Conclusions: These findings suggest that intermittent dosing regimens may be efficacious for combinations of MEK and PI3K inhibitors, and that sustained exposure to inhibitors may not be required for maximal combination efficacy.

133 POSTER

Role of Abcb1 (P-glycoprotein) and Abcg2 (Bcrp1) in the brain penetration of the novel PI3K Inhibitor GDC-0941 and efficacy in orthotopic xenograft models of glioblastoma and metastasis

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Background: The PI3K pathway is a major regulator of cell proliferation, survival and migration, and is aberrantly regulated in multiple cancers. In recent years, this pathway has emerged as a major target for the investigation of anticancer drugs. GDC-0941 is a novel small molecule inhibitor of PI3K currently being evaluated in the clinic as an anticancer agent. The objectives of these studies were to determine *in vitro* whether GDC-0941 was a substrate of P-glycoprotein and Bcrp1 and to investigate the impact of Pgp and Bcrp1 on the absorption, disposition and brain penetration of GDC-0941 in FVBn mice (wild type), Mdr1a/b(−/−), Bcrp1(−/−) and Mdr1a/b(−/−)/Bcrp1(−/−) knockout mice. In addition, efficacy of GDC-0941 against orthotopic xenograft models of glioblastoma and brain metastasis was evaluated *in vivo*.
Results: In vitro studies with MDCK cells transfected with Pgp or Bcrp1 established that this compound was a substrate of both transporters. Following intravenous (IV) and oral (PO) administrations, GDC-0941 brain-to-plasma ratios ranged from 0.02 to 0.06 in the wild type mice, were unchanged in the Bcrp1(−/−) and were 3- to 4-fold higher in the Mdr1a/b(−/−) knockout mice. In contrast, the brain-to-plasma ratio of GDC-0941 in Mdr1a/b(−/−)/Bcrp1(−/−) was 30-fold higher than in the wild type

mice, ranging from 0.5 to 1. The plasma clearance of GDC-0941 was similar in the four strains of mice. Exposure following PO dosing was also comparable in the wild type and all knockout mice. Following administration of GDC-0941 to Mdr1a/b(−/−)/Bcrp1(−/−) mice, the PI3K pathway was markedly inhibited in the brain for up to 6 hours post-dose, with a 60% suppression of the pAkt signal, while no effect on the PI3K pathway was detected in the brain of wild type mice. GDC-0941 was efficacious in the U87 glioblastoma orthotopic model that contained compromised blood–brain barriers (BBB) but inactive in a neurosphere-derived model with non-compromised BBB. Additionally, in the Fo1282 breast cancer brain metastasis model, GDC-0941 treatment increased survival benefit and decreased phospho-S6 ribosomal protein levels indicative of PI3K pathway suppression *in vivo*.
Conclusions: These findings show concerted effects of Pgp and Bcrp1 in restricting GDC-0941 access and pathway modulation in the brain of non-tumor bearing mice. Anti-tumor activity of GDC-0941 is observed in orthotopic brain tumor and metastasis models that contain compromised blood–brain barriers.

134 POSTER

Biological characterization of ETP-46321 a potent and selective phosphoinositide-3-kinase inhibitor with antitumor activity

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The phosphoinositide-3-kinase (PI3K) signaling 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, p110α or inactivating mutations or deletions of the tumor suppressor PTEN. In addition, the PI3K pathway is activated by mutations in certain receptor tyrosine kinases as well as by mutation of the oncogene KRAS. These data provide a strong rationale for the discovery of PI3K inhibitors for treatment for cancer. Following a rational design strategy, we identified the fused imidazole derivative ETP-46321 as a potent inhibitor of PI3K (e.g., K_i = 2.4 nM *vs.* p110α, K_i = 549 nM *vs*. p110β, K_i = 14 nM *vs.* p110δ, and K_i = 153 nM *vs*. p110γ, respectively). ETP-45321 also inhibits three oncogenc mutants of p110α: p110α E542K K_i = 1.9 nM, p110α E545K K_i = 1.8 nM and p110α H1047R K_i = 2.4 nM. The compound does not significantly inhibit the related PIKK family members such as mTOR, DNA PK or ATR (K_i's >10 μM), or an additional 280 protein kinases that were screened. The compound blocks PI3K signaling, induces cell cycle arrest and inhibits VEGF-dependent sprouting of HUVEC cells. ETP-46321 has a pharmacokinetic profile suitable for oral dosing in mice (%F = 95%, Cl = 0.56 L/hr/kg; Vds = 0.016 L). Analysis of xenograft tumor tissue after acute dosing reveals a reduction in P-Akt levels. Once a day treatment with ETP-46321 of mice with human tumor xenografts with ETP-46321 results in tumor growth delay and is well tolerated. In a mouse model of lung cancer induced by expression of an oncogenic mutant KRAS, treatment with ETP-46321 results in tumor growth delay and a significant PET response. These and combination data will be presented.

135 POSTER

Pediatric Preclinical Testing Program (PPTP) stage 1 evaluation of JNJ-26481585, a second generation histone deacetylase inhibitor

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Background: JNJ-26481585 is a 'second-generation' HDAC inhibitor with prolonged pharmacodynamic response *in vivo*. The agent has demonstrated superior efficacy compared to both standard of care agents and 'first generation' HDAC inhibitors in adult cancer preclinical models. The activity of JNJ-26481585 was evaluated against the *in vitro* and *in vivo* panels of the Pediatric Preclinical testing Program (PPTP).
Methods: JNJ-26481585, which was provided by Johnson & Johnson Pharmaceutical Research and Development, was tested against the PPTP *in vitro* panel (n = 23) at concentrations ranging from 1.0 nM to 10 mM and was tested against the PPTP *in vivo* panel using a dose of 5 mg/kg (solid tumor) or 2.5 mg/kg [acute lymphoblastic leukemia (ALL)] administered by the intraperitoneal route daily for 21 days. Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: JNJ-26481585 demonstrated potent cytotoxic activity, with T/C% values approaching 0% for all of the cell lines at the highest concentration tested. The median EC_{50} value for the PPTP cell lines was 2.2 nM, with a range from <1 nM (MOLT-4) to 19 nM (NB-EBc1). JNJ-26481585 was well tolerated and induced significant differences in EFS distribution compared to control in 20 of 31 (65%) of the evaluable solid tumor xenografts and in 5 of 8 (63%) of the evaluable ALL xenografts. JNJ-26481585 induced tumor growth inhibition meeting criteria for intermediate EFS T/C activity (EFS T/C > 2) in 5 of 30 (17%) evaluable solid tumor xenografts. Intermediate activity for the EFS T/C metric occurred most frequently in the glioblastoma panel (2 of 4) and was also observed for one xenograft in the rhabdoid, Ewing, and rhabdomyosarcoma panels. An objective response was observed in 1 of 31 solid tumor xenografts, Rh28 in the rhabdomyosarcoma panel that achieved a maintained complete remission (MCR). For the ALL panel, two xenografts (both T-cell ALL xenografts) achieved CR and MCR, respectively, and a third xenograft achieved stable disease (SD).
Conclusions: The activity signals observed for JNJ-26481585 against the PPTP preclinical models warrant follow-up. *In vivo* activity signals for rhabdomyosarcoma, glioblastoma, and T-cell ALL are particularly noteworthy, with further exploration of the preclinical activity of JNJ26481585 for T-cell ALL being a high priority.

136 POSTER

Activity of the Cdc7 inhibitor NMS-1116354 as single agent and in combination in breast cancer models

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NMS-1116354 is a potent ATP competitive oral inhibitor of the Cdc7 kinase (IC_{50} <3 nM). Consistently with the inhibition of this enzyme, treated cells show inhibition of phosphorylation of serine 40 in the Mcm2 protein and an impairment of DNA replication. While this event leads to apoptotic tumor cell death, in normal cells it induces a reversible cell cycle arrest. In addition, NMS-1116354 induces the down regulation of the pro-survival proteins Mcl-1 and XIAP expression thus exacerbating the effects of the Cdc7 inhibition on tumor growth.
NMS-1116354 has potent antiproliferative activity against a wide panel of tumor cell lines with IC_{50} values ranging between 0.1 and 3 μM. Oral administration of NMS-1116354 demonstrated significant antitumor activity in various tumor animal models as well as in disseminated human leukemia models. Particularly, strong tumor regressions were obtained in breast cancer models. In combination studies, NMS-1116354 exhibited synergistic effects when administered with Irinotecan, Gemcitabine, Erlotinib, Bortezomib and with other approved antineoplastic drugs. The combination with Docetaxel in triple negative breast cancer animal models gave tumor free animals lasting for >4 months.
The phase I clinical trials to evaluate the safety of orally administered NMS-1116354 as single agent with different schedules in cancer patients are ongoing. The results of the combination studies open a possible path for its clinical development in combination with approved drugs.

137 POSTER

Preclinical characterization of ACTB-1010, an orally activity Aurora kinase inhibitor

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Introduction: Aurora Kinases A and B are dysregulated in a number of human cancers and are essential to the regulation and function of mitosis and cytokinesis. ACTB-1010 is an oral kinase inhibitor targeting both Aurora Kinase A and B. ACTB-1010 was selected for development based on the nanamolar potency against targeting Aurora Kinase A and B, without cross reactivity to other major kinases such as VEGF, FGF, KIT and FLT3. We investigated the preclinical efficacy and mechanism of action of ACTB-1010.
Results: ACTB-1010 inhibits both Aurora Kinase A (IC_{50} = 1.6 nM) and Aurora Kinase B (IC_{50} = 9 nM) and is highly active in cell-based mechanistic assays. Human tumor cell lines treated with ACTB-1010 demonstrate a phenotype consistent with Aurora B inhibition including enlarged nuclei