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.

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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.

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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.

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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