62.5 mg/kg for 3 consecutive days (days 0, 1, 2). PET imaging was carried out on days −1 (pretreatment baseline), 1 (24 h after dose 1) and 3 (24 h after dose 3).
Results: During the first treatment cycle, mean tumor volume in the placebo group increased by approximately 49% vs a 6% decrease and 7% increase in the groups treated with GSK923295A at 125 mg/kg and 62.5 mg/kg treated groups, respectively. Subsequently (day 7 onwards), tumor regression (>50% decrease in tumor volume relative to pre-treatment volume) was observed in 11/13 mice dosed at 125 mg/kg, but only 4/14 dosed at 62.5 mg/kg. FDG-PET imaging showed significant decreases in mean FDG standardized uptake values (SUV) relative to placebo at both dose levels. On day 3, the mean SUV's in GSK923295A-treated tumors decreased by approximately 25% and 30% at 62.5 and 125 mg/kg respectively. The day 3 SUV's for both treatment groups were significantly lower than either day −1 (pre-treatment) ($p < 0.05$) or placebo ($p < 0.001$).
Conclusion: The results demonstrate that in a preclinical setting, GSK923295A treatment significantly affected FDG uptake early after onset of therapy and prior to tumor regression. FDG-PET may provide a means of evaluating pharmacodynamic activity in patients treated with GSK923295A.

343 POSTER

Preclinical validation of the TrpM8 ion channel as a cancer target

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Background: TrpM8, a transmembrane cation channel protein, is expressed in cancers including prostate, breast, lung, and colon. In normal tissues, its expression is primarily confined to prostate and a subset of sensory neurons. We have previously reported that small molecule agonists of TrpM8 can selectively kill cells that express TrpM8.
Methods: Activity and selectivity of agonists were measured in vitro in cell killing assays using CHO cells and CHO cells stably transfected to express TrpM8 (CHO/TrpM8). Cell viability was used to determine EC_{50} values. Plasma pharmacokinetics (PK) was determined in mice. Compounds were assessed in vivo in murine xenograft models using CHO and CHO/TrpM8 cells. Tumor growth inhibition (%TGI) was defined as the difference between the control and treated tumor volumes as a % of control. The plasma PK of the lead compound was evaluated in rats and dogs. In vivo activity was evaluated in human prostate cancer (LuCap) xenograft models and in a rat model of androgen-induced, benign prostate hyperplasia (BPH).
Results: Several compounds were identified that were potent and selective in vitro and efficacious in vivo via oral administration. Compound D-3263 proved to be the most potent and preliminary PK studies conducted in mice, rats and dogs suggest the plasma half-life ranges from 10 hrs (mice and rats) to 27 hrs (dogs). The compound appears to inhibit LuCap growth in Nude mice ($p = 0.004$, 123.7 mm^3 for treated vs 207.6 mm^3 for vehicle on Day 4) and inhibited androgen-induced hyperplasia of normal rat prostates (mean prostate weights for treated 620.1 g vs 1009.9 g for untreated; $p = 0.004$).

Table 1

Compound	MW	*In vitro* EC50 (uM)		*In vivo* %TGI
		CHO/TrpM8	CHO	
D-3263	373	0.003	>10	70%
D-3457	368	0.01	>10	62%
D-3517	346	0.01	>10	48%

Conclusions: These results establish TrpM8 as a tractable therapeutic target and endorse the likelihood of an agonist demonstrating clinical activity against cancers that express TrpM8. The orally bioavailable small molecule agonist D-3263 has been selected for clinical development.

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A phase Ib dose escalation study to evaluate safety and tolerability of the combination of the aminopeptidase inhibitor CHR-2797 and paclitaxel in patients with advanced or treatment refractory tumors

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Background: CHR-2797 is an orally bioavailable aminopeptidase inhibitor. In a single agent phase I study in solid tumors the recommended daily dose was 240 mg. The active metabolite, CHR-79888 accumulates intracellularly. In vitro and in vivo experiments confirmed good synergy between CHR-2797 and paclitaxel.
Methods: Patients with histologically confirmed advanced solid tumors refractory to standard therapy and performance score $\leqslant 2$ were treated every 21 days with paclitaxel given i.v. over 3 hrs (escalating from 135 mg/m^2 in the first cohort to 175 mg/m^2 in subsequent cohorts) and escalating doses of CHR-2797 (90–240 mg). The first 21 day cycle (Cy) constituted the dose finding phase. Patients received up to 6 Cy of paclitaxel and could remain on CHR-2797 therapy until evidence of PD or unacceptable toxicity.
Results: 22 patients (median age 59 years [range 34−72], 18M/4F) were treated. At the 1st dose level (DL) 3 patients were given paclitaxel 135 mg/m^2 and 90 mg CHR-2797. DLs 2−5 received paclitaxel 175 mg/m^2 and CHR-2797 at 90, 130, 180, and 240 mg (4, 3, 9, and 3 patients respectively). One DLT (dyspnea G3) was seen at DL 4. Common Grade 1−3 toxicities during treatment included alopecia and fatigue (each in 95% of patients), sensory neuropathy (59%), myalgia (50%), anorexia and dizziness (each in 45% of patients), rash (32%). Infusion reactions developed in 13 (59%) patients. At DL 4 and 5 CHR-2797 was withheld for 5 days, from d18 of each Cy, in an attempt to decrease the risk of infusion reactions. However, this had no clear effect on the number of infusion related reactions. Six patients continued CHR-2797 after discontinuation of paclitaxel. Neither agent influenced the pharmacokinetics of the other. PR was achieved in 3 patients (melanoma, non small cell lung cancer, esophageal squamous cell).
Conclusions: Except for an unexpected high number of infusion reactions to paclitaxel the combination of paclitaxel-CHR-2797 was otherwise well tolerated. Further investigation into the potential immunological mechanisms is warranted. Formal MTD was not reached. Anti-tumor activity was observed in several patients.

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Myofibrillogenesis regulator 1 as a potential target for cancer therapy

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Background: The phosphorylation of myosin light chain-2 (MLC2) is pivotal in the regulated assembly and disassembly of focal adhesions and adherens junctions contributed to cell motility and tumor invasion. MLC kinase inhibitor abrogates MLC2 phosphorylation, cell polarization and migration, and MLC2 dephosphorylation induces apoptosis. Our previous data show that MLC2 interacts with human myofibrillogenesis regulator 1 (MR-1). Thus, we investigate whether MR-1 is associated with the proliferation and migration of cancer cells.
Material and Methods: The transcription and expression of MR-1 were detected by RT-PCR and Western blot analysis. Functional analysis of MR-1 small hairpin RNA (shRNA) was conducted in HepG2 cells using Lipofectamine-mediated gene transfer. The changes of cell spreading, adherence and migration in response to the treatments were evaluated by immunofluorescent staining, immunohistochemistry and Boyden chamber invasion assay. The tumorigenicity of HepG2 cells stably transfected with MR-1-shRNA was assessed by transplantation into nude mice.
Results: RT-PCR and Western blot analysis showed that MR-1 was overexpressed in human cancer cells and especially in hepatoma HepG2 cells. Transient treatment of cells with shRNA against MR-1 or stable transfection of cells with plasmid expressing MR-1-shRNA led to impairment of cell proliferation, adhesion and migration. Following inhibition of MR-1 by MR-1-shRNA, the phosphorylations of MLC2, focal adhesion kinase (FAK) and Akt were decreased dramatically and formation of stress fiber was destroyed. In the same condition, MLC kinase inhibitor could block exogenous MR-1-induced phosphorylations of MLC2, FAK and Akt and F-actin polymerization inhibitor also decreased phosphorylations of FAK and Akt, indicating that activation of MLC2 and intact actin cytoskeleton was upstream of FAK and Akt in MR-1 modulating pathway. *In vivo* data showed that knockdown of MR-1 markedly inhibited the tumorigenicity of human

liver cancer HepG2 cells, and that the percentage of growth inhibition was 91.1%.
Conclusions: MR-1 is overexpressed in human liver cancer cells and plays an important role in the tumorigenicity. Knockdown of MR-1 blocks the proliferation, adhesion and migration of cancer cells. The data provide evidence for a functional role of MR-1 in the control of MLC2 dependent signaling events as a potential cancer therapeutic target.

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Myofibroblasts and TGF-beta1 induce upregulation of tumoral L1CAM thereby promoting malignant transformation of pancreatic ductal epithelial cells

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Background: Pancreatic ductal adenocarcinoma (PDAC) originating from ductal epithelial cells is characterized by its high malignancy. Since PDAC exhibits strong desmoplastic reaction with stromal pancreatic myofibroblasts (PMFs), PMFs are supposed to drive PDAC tumorigenesis. Previously, we observed high expression of the adhesion molecule L1CAM (CD171) in PDAC cells accounting for chemoresistance. Thus, this study aimed to investigate whether PMFs are involved in the induction of L1CAM in ductal epithelial cells thereby pomoting malignant transformation of these precursor cells and to identify the mechanisms underlying L1CAM induction.
Material and Methods: An in vitro transwell coculture model was employed lasting up to 6 weeks including the immortalized human pancreatic ductal epithelial cell line H6c7 and freshly isolated pancreatic fibroblasts – representative for the main compartment of the tumor stroma. As parameters for apoptosis induction, AnnexinV binding and caspase-3/-7 activity were measured. Cell migration was determined by transmigrationassays in a modified Boyden chamber. Expression of L1CAM and signaling molecules involved in L1CAM induction were detected by western blotting. DNA binding activity of AP-1 subunits was detected by Gelshift- and ChIP assays. Knock down of L1CAM expression for apoptosis and migration assays was performed by siRNA transfection.
Results: When cocultured together with PMFs, L1CAM expression was upregulated in the human pancreatic duct cell line H6c7 in a TGF-b1-dependent fashion accounting for a migratory and chemoresistant phenotype. Accordingly, TGF-b1 treatment of monocultured H6c7 cells increased L1CAM expression thereby enhancing migratory and chemoresistant abilities. Thus, knock down of L1CAM expression reversed the chemoresistant phenotype and diminished cell migration of cocultured and TGF-b1 stimulated H6c7 cells. The TGF-b1- and PMF-induced L1CAM expression was dependent on the activation of JNKs, but not of Smad2/3, a mechanism that was verified in the L1CAM expressing and TGF-b1 responsive PDAC cell lines Colo357 and Panc1. Accordingly, DNA binding activity of the AP-1 subunits c-Fos and c-Jun was detected within the L1CAM promotor of TGF-b1 stimulated H6c7 cells.
Conclusion: These data provide the first explanation how tumoral L1CAM expression is induced and provide new insights into the mechanisms by which PMFs contribute to malignant transformation of pancreatic ductal epithelial cells quite early, thereby promoting tumorigenesis of PDAC. Since L1CAM mediates both chemorestistance and cell migration of PDAC cells, it represents an interesting therapeutic target to overcome chemoresistance and to concomitantly interfere with the process of metastasis.

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Anti-cancer activity of human ribonuclease conjugates with enhanced pharmacokinetic profiles

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The EVade™ Ribonuclease (RNase) technology allows for the transformation of human pancreatic RNase 1 into proteins with anti-cancer activity by substitution of a handful of amino acid residues. The EVade™ RNases selectively cause apoptosis of cancer cells and have demonstrated efficacy in both xenograft and syngeneic models at doses with few to no side effects. An EVade™ RNase called QBI-139 has advanced to a Phase 1 clinical trial. The proteins are small (~15 kDa) and are cleared quickly from the circulation (half life <2 hours in mice, rats and dogs). While the EVade™ Ribonucleases are effective (~70% tumor growth inhibition), improving the pharmacokinetics of therapeutic proteins using polyethylene glycol (PEG) is a common strategy to increase potency. Additional benefits often seen for the PEG-protein conjugates include more convenient dosing and decreased immunogenicity.
The RNases presented were expressed in inclusion bodies in *E. coli* and purified by column chromatography. The RNases were then conjugated to PEGs of varying lengths (5, 12, 20 and 30 kDa). The EVade™ RNase PEG conjugates were tested for their ability to inhibit the growth of human tumors implanted in the flanks of Foxchase nude mice (xenograft models). In addition, the conjugates were tested against murine cancer cells implanted in the flanks of normal mice (syngeneic model). To assess the pharmacokinetics of the EVade™ RNase PEG conjugates, a fluorescence resonance energy transfer (FRET) assay was used to detect the presence of RNase activity in serum of treated animals. Among the EVade™ RNase PEG conjugates tested, QBI-206 has demonstrated up to 20% greater tumor growth inhibition than the first generation drug, QBI-139. This efficacy was achieved at doses using five-fold less RNase per dose with no toxicity. The enzymatic activity of the conjugates demonstrated that systemic exposure (area under the curve) is significantly increased relative to first generation EVade™ RNases, such as QBI-139. Additional studies are ongoing to select an EVade™ RNase PEG conjugate to advance to the clinic. The potency and safety profile of these conjugates provide substantial justification for continued development.

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Molecular pharmacology of benzamide riboside and sodium meta arsenite in chronic myeloid leukemia: a preclinical evaluation

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Background: Chronic myeloid leukemia (CML), a form of leukemia, has serious implications which call for innovative, cheaper but effective modes of treatment urgently.
The **aim** of this study was to evaluate Benzamide Riboside (BR), a new synthetic compound and Sodium meta arsenite (SMA) – previously used in the treatment of acute promyelocytic leukemia, as candidate drugs for CML. While a primary target or sole pathways for SMA induced cytotoxic response on CML has not been reported, BR is known to selectively target and inhibit inosine 5′monophosphate dehydrogenase (IMPDH II) – an isoform present only in highly proliferating cells and requires nicotinamide 5'-mononucleotide adenyl transferase (NMNAT) for its conversion into active form.
Materials: The foremost step was to look for presence of NMNAT, IMPDH in cells in our study. Leukemic cells from CML patients and K562 cells were screened and confirmed for expression of NMNAT and IMPDH by RT-PCR and imunocytochemistry. Subsequently, tumor cells were isolated from peripheral blood of 23 CML patients for exvivo studies. Effect of Glivec®, currently used active agent in the primary treatment of CML, was also studied in parallel.
Methods and Results: BR and SMA induced time dependent and dose dependent cytotoxicity in patient tumor cells as well as in leukemic cell line. Flow cytometric analysis showed that while BR provokes an 'S' phase arrest, SMA arrested the cells in 'G2′ phase. Both BR and SMA caused cell death through selective triggering of apoptosis as apparent from Hoechst, Acridine orange/Ethidium bromide staining (fluorescence microscopy), mitochondrial permeabilization, annexinV assay (flow cytometry), DNA ladder and TUNEL assay. Kinetic spectrofluorimetric studies confirmed involvement of caspases 9 and 3. Expression of apoptosis regulatory molecules – bcl-2, bax, survivin and X-IAP in BR and SMA treated tumor cells were found to be modulated at both RNA (RT-PCR) and protein level (Western blot). Whole genomic microarray analysis on BR and SMA treated K562 revealed up regulation of proapoptotic and cell growth inhibitor genes.
Conclusions: At any given time and dose, the study compounds were more effective than Glivec®, alone or in combination with BR/SMA on leukemic cells. The unifying feature of both BR and SMA seems to be induction of apoptosis. The results, involving primary tumor cells from CML patients, provide preclinical molecular pharmacology model supporting potential utilization of BR and SMA in the management of CML.