and is an inhibitor of PIM kinases. The aim of this study was to explore further its mechanism of action *in vitro*, and to determine its cytotoxicity both *in vitro* and *in vivo*.
Methods: *In vitro* cytotoxicity of CXR1002 was determined using an ATP depletion assay, alone and in combination with 8 other drugs in a panel of tumor cell lines. The mode of action was examined using microarray analysis (followed by analysis using the Ingenuity system) and western blots. Five CXR1002 xenograft studies (25 mg/kg *p.o*. 3x per week) were performed in nu/nu mice.
Results: CXR1002 was cytotoxic to a wide range of human tumor cells, including pancreatic and ovarian carcinoma and sarcoma. Cell lines derived from hematological malignancies were the most sensitive to CXR1002. The IC50 value was lower after 7 days vs 2 days exposure (range 100−590 μM vs 175−>1000 μM), suggesting potency was linked to duration of exposure. IC50 values for the most sensitive cell lines were substantially lower than the plasma concentrations achieved with a non-toxic dose in an on-going phase I trial. Drug combination studies with 8 anti-cancer drugs in 11 human cancer cell lines indicated that CXR1002 was synergistic with other anti-tumor drugs, particularly gemcitabine. Microarray studies in the pancreatic cell line PANC-1 treated with an IC15 dose of CXR1002 showed 4996 gene changes. Representation analysis of the 4996 signature list identified a number of pathways that were over-represented, in particular, genes in the ER stress pathway, including the ATF family of transcription factors. Western blot analysis of PANC-1 cells using PCNA, cleaved PARP and caspase antibodies showed that PCNA was reduced and cleaved PARP and cleaved caspases 3 & 7 were increased 24 hr after treatment with 300 μM CXR1002, suggesting a pro-apoptotic/anti-proliferative outcome. CXR1002 was active in all 5 human xenograft (*i.e*. pancreatic, liver, prostate, lung and colon) models examined with best absolute tumor volume as a % of control of 50.13%, 77.05%, 19.14%, 75.04% and 49.22% respectively.
Conclusions: CXR1002 is a unique potential anti-cancer therapy that exhibits unique pharmacokinetics and a wide spectrum of biological activities. CXR1002 appears to act in part by its ability to induce ER stress. A phase I human trial is on-going.

124 POSTER

Mechanisms of action of histone deacetylase inhibitors (HDACi)

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Background: Histone deacetylases (HDACs) are promising targets for anti-cancer drug development as evidenced by the rapid development of HDAC inhibitors (HDACi) as chemotherapeutics. While HDACi are now approved agents for the treatment of certain haematological malignancies, their mechanisms of action are not fully understood. New isoform-specific HDACi are being designed to target specific HDACs in the hope that a more tailored, less toxic approach to cancer therapy will be achieved. The purpose of this study is to determine whether an HDAC 1/2-specific HDACi will have more potent anti-cancer activities than an HDACi specific for HDACs 1/2/3 and 6.
Material and Methods: Two structurally different HDACi were used: vorinostat, an HDACi that inhibits class I and II HDACs (HDAC1/2/3 and 6) and MRLB223, a recently developed HDACi specific for HDAC1 and HDAC2 (class I). Eμmyc lymphomas were used to assess the biological activities of the two HDACi both in vitro and in vivo. Apoptosis readouts used were: propidium iodide staining to assess cell membrane permeabilisation; TMRE staining to assess mitochondrial function; histone acetylation and; TUNEL staining. C57BL/6 mice bearing Eμ-myc lymphomas were used for therapy studies. Anti-tumor efficacy was determined by assessing the tumor-free and overall survival of tumor-bearing, HDACi-treated mice.
Results: Both vorinostat and MRLB-223 killed Eμmyc lymphoma cells in vitro and engaged the same apoptotic pathways. Both HDACi induced histone hyperacetylation prior to cell death. The kinetics of apoptosis induced by MRLB-223 was slower than vorinostat and required significantly higher concentrations. In vivo, the survival of vorinostat- and MRLB-223-treated mice was significantly extended compared to vehicle-treated mice However, while MRLB-223 still provided a therapeutic benefit to the mice, the effect was not nearly as robust as that provided by vorinostat.
Conclusions: We have shown the HDAC1/2 inhibitor MRLB-223 can achieve similar anti-tumor activities as vorinostat in vitro and in vivo. However, MRLB223 was limited in its efficacy as higher concentrations were required to achieve the same effects as vorinostat. Interestingly, MRLB223 displayed increased toxicity in vivo compared to vorinostat. Therefore, while targeting the enzymes HDAC1 and HDAC2 may be sufficient to cause apoptosis in Eμmyc lymphomas, vorinostat was found to be the superior therapeutic agent.

125 POSTER

Targeting different conformations of BRAF kinase: efficacy of Omni-Raf inhibitors in NRAS and BRAF mutant tumors

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Background: The RAS-RAF-ERK cascade is aberrantly activated in many types of cancer. RAF inhibitors such as PLX4032 have demonstrated clinical efficacy in melanoma patients carrying the oncogenic mutant form of BRAF. Tumors driven by RAS or other elevated upstream signaling can bypass mutant BRAF-targeted compounds by mechanisms that are currently under investigation. In particular, the induced or autonomous conformational changes of the RAF kinases potentially can dislodge a conformation-specific inhibitor and even turn an inhibitor-bound RAF molecule into an activator of other RAF molecules. By applying the scaffold-based discovery method, we have identified a new generation of RAF inhibitors that show potent inhibition of all RAF isoforms, including mutant BRAF. As revealed by X-ray co-crystallography, these compounds have the unique ability to bind in both the active and inactive states of the RAF kinases, thereby avoiding the conformational restraints of the activation loop. We call this class of compounds the Omni-Raf inhibitors (ORIs).
Material and Methods: This study used $BRAF^{V600E}$ cell lines and NRAS mutant cell lines from commercial sources. The sensitivities of these cell lines to ORIs and $BRAF^{V600E}$-specific inhibitor PLX4720 were determined by both growth and MTT assays. Balb/C nude, female mice were used for the xenograft studies. The treatment was started when mean tumor size reaches approximately 100 mm^3. On the last day of the efficacy studies, the blood samples were collected at different time points after dosing to determine the plasma exposures of the compounds.
Results: In cell culture, the ORIs not only showed improved activity against BRAF mutant cell lines, including some previously known to be resistant to $BRAF^{V600E}$-specific inhibitors, but also potently inhibit melanoma cell lines driven by mutated NRAS. In xenograft models using $BRAF^{V600E}$ and NRAS-driven cells, the ORIs demonstrated over 90% tumor growth inhibition, including significant tumor regression, whereas the $BRAF^{V600E}$-specific inhibitors showed no effect on NRAS-driven tumors.
Conclusion: These results show that RAF inhibitors structurally designed to target multiple conformations of the enzymes can prevent upstream signals from bypassing RAF inhibition.

126 POSTER

Impairment of S-nitrosothiol homeostasis and nitrosative stress modulate proliferation of breast cancer cells

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Background: Augmented nitric oxide (NO) levels in tumors have been usually detected compared to surrounding healthy tissue, and protein modifications induced by NO may constitute a significant regulating factor affecting both tumor progression and antitumoral treatment. S-nitrosothiol (SNO) formation is a cysteine modification, also referred to as S-nitrosation or S-nitrosylation, that controls the function of proteins in a manner similar to phosphorylation. One of the specific mechanisms governing protein de-nitrosylation is the system thioredoxin/thioredoxin reductase (Trx/TrxR). Manipulation or alteration of this enzymatic system may alter SNO homeostasis in tumor cells, providing new insights into the role of NO in cancer and its therapeutic significance.
Materials and Methods: Human breast cancer cells (MCF-7, MDA-MB-231 and BT-474) were pretreated or not with the specific TrxR inhibitor auranofin and exposed to different doses of S-nitroso-L-Cysteine (CSNO). Cell proliferation was measured using the XTT assay, and phosphorylation of Akt and Erk1/2 and cyclin D1 levels were determined by westernblot using the corresponding specific antibodies.
Results: Treatment with auranofin and 100 nM CSNO enhanced cell proliferation of MCF-7 (ER+), but not of MDA-MB-231 (ER-, mut p53), or BT-474 (ER+, mut p53) cells. The augmented rate of cell growth was associated with Akt and Erk1/2 phosphorylation and higher expression of cyclin D1. Significantly, this pro-proliferative effect was abolished by the estrogen receptor (ER) antagonist fulvestrant or the p53 specific inhibitor pythiphrin-alpha. In contrast, in all the three cell lines, a high CSNO dose (500 μM) reduced cell proliferation and this effect was potentiated by pretreatment with auranofin.
Conclusions: Impairment of SNO homeostasis modulated tumour cell growth depending on the grade of the subsequent nitrosative stress. A

mild nitrosative stress caused by TrxR inhibition promoted breast cancer cell growth in a ER positive and intact p53 setting. On the contrary, the severe nitrosative stress caused by exposure to higher doses of CSNO and TrxR inhibition promoted growth arrest in breast cancer cells. Our results suggest that drugs modulating SNO homeostasis are potential therapeutic agents in breast cancer treatment.
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127 POSTER

Identification of CB3, a novel inhibitor of the ubiquitin-proteasome system

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Background/aim: As the ubiquitin–proteasome system (UPS) represents a promising therapeutical target we set out to identify novel UPS inhibitors with tolerable toxicity.
Material and Methods: A chemical library consisting of 10 000 compounds was screened for cytotoxicity in the colon carcinoma HCT 116 cell line using a non-clonogenic 72 hour cytotoxicity assay. Cytotoxic compounds were further investigated for UPS activity in the HEK 293 ZsGreen Proteasome Sensor cell line using an image-based screening assay. Inhibition of the proteasome led to accumulation of the fluorescent protein ZsGreen which was measured using automatic fluorescence microscopy. To validate UPS as the target and to obtain kinetic information of hit compounds, live cell monitoring was performed in the IncuCyte FLR using the ubiquitin sensor cell line MelJuSoUb^{G76V}-YFP which fluoresce when the UPS is inhibited. Microarray-based gene expression analysis was performed on hit compounds to characterize global effects after compound-treatment. To study the effect of hit compounds on normal cells, in vitro toxicity profiling was performed using a bone marrow, epithelial, liver, lymphocyte and renal toxicity assay.
Results: When screening 10 000 substances, 382 showed cytotoxic activity at 25 μM in HCT 116. Of these 382 compounds, one (CB3, Phosphoric acid, 2,3-dihydro-1,1-dioxido-3-thienyl diphenyl ester, figure 1) was identified as an inhibitor of proteasomal activity in the HEK 293 ZsGreen cells. The effect on the UPS was subsequently studied in live cell monitoring where the MelJuSoUb^{G76V}-YFP cells showed a dramatic increase in fluorescence when treated with CB3 compared to control. The global gene expression profile evoked by CB3 was similar to that of the known proteasome inhibitors MG132 and MG262. The toxicity of CB3 in the normal cell systems was relatively low compared to conventional cytotoxic compounds and was favourable compared to the approved proteasome inhibitor bortezomib. Since there are several possible targets in the UPS, detailed mechanistic studies of CB3 are ongoing.
Conclusion: CB3 is a novel inhibitor of the UPS with a promising toxicity profile and will therefore be investigated further.

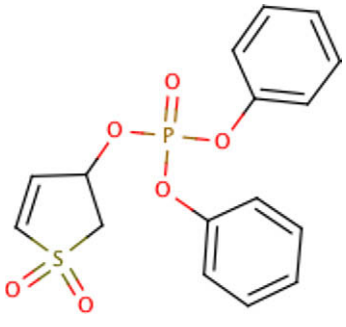

Figure 1. Structure formula of CB3

128 POSTER

GDC-0941 and ABT-737 cooperate to sensitize isolated mitochondria from PI3K mutant cells

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The phosphatidylinositol 3-kinase subunit PIK3 is frequently mutated in human cancers and therefore represents an interesting therapeutic target. Cancer resistance to therapies is often related to expression of anti-apoptotic Bcl-2 family members which negatively regulate mitochondrial cell death. We investigated the influence of PI3K mutations on Bcl-2 family proteins both at the cellular and mitochondrial level. We used cell lines with "knock-in" PI3K mutation (ex. human breast epithelial cells HME-1 PI3K H1047R/+) from which we purified the mitochondria to homogeneity (95%). We first characterized these mitochondria for their sensitivity to reference compounds (Calcium, t-Bid ...) on 3 parameters: swelling, $\Delta\psi$m loss and cytochrome c release. Mitochondrial preparations from wild-type and mutated HME-1 were analysed for their protein pattern in Bcl-2 family members. The PI3K mutation gives a tumoral profile to mitochondria (accumulation of anti-apoptotic Bcl-xL, Bcl-2 and decrease of pro-apoptotic Bim, Bax and Bad) and induces sensitivity to t-Bid. Cell treatment with the PI3K inhibitor GDC-0941 counteracts PI3K mutation by increasing mitochondria-associated pro-apoptotic proteins. Furthermore, these isolated mitochondria become sensitive to the Bcl-2 family inhibitor, ABT-737 suggesting an interesting cooperative effect between PI3K and Bcl-2 inhibitors. Investigations with HCT-116 PI3K +/− cells are currently under investigation to confirm the impact of PI3K mutations in cancer cells.

129 POSTER

ADAM17: A new therapeutic target for triple negative breast cancer?

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Background: Triple negative breast cancer (TNBC) can be defined as tumors lacking expression of ER, PR and HER2. Unlike other subgroups of patients with breast cancer, targeted therapy is currently unavailable for these patients. It has been shown that these cancers possess high levels of EGFR and thus may be dependent on EGFR signaling. Due to the fact that ADAM (a disintegrin and metalloprotease)10 and ADAM17 are involved in the proteolytic release of all EGFR ligands, inhibiting these ADAMs may be a potential therapeutic option for TNBC, either alone or in combination with EGFR-targeted agents.
Materials and Methods: We used the EGFR inhibitor, gefitinib, and an ADAM17 specific inhibitor (Wyeth), to determine their effects on TN cell lines. IC_{50} values were determined (using SoftmaxPro software) by treating cells for 5 days (1×10^3 cells/well) and measuring cell growth using acid phosphatase assay. MDA-MB-435 cells were stably transfected with ADAM17 shRNA (2 clones used) using Nucleofection technology (Lonza).
Results: In MDA-MB-435 cells, ADAM-17 silencing resulted in a decrease in the IC_{50} of gefitinib from 8.34 μM to 6.96 μM and 5.98 μM for clone 1 and clone 2, respectively (Student's paired t-test: $p = 0.004$ and $p = 0.002$), when compared with the IC_{50} for parental MDA-MB-435 cells. These findings suggest that EGFR may be involved in mediating the effects of ADAM17 on proliferation in MDA-MB-435 cells. SUM159PT cells were chosen for further investigation of the involvement of EGFR in mediating the effects of ADAM17 on *in vitro* cellular invasion and proliferation, as they express the EGFR at relatively high levels (gefitinib IC_{50}: 1.01 ± 0.35 μM). IC_{50} of the ADAM inhibitor (AI) in these cells was 6.97 ± 0.26 μM. SUM159PT cells were treated with a combination of gefitinib and AI at a ratio of 1:5, for 5 days. Selective inhibition of ADAM17 showed similar growth inhibitory effects on SUM159PT breast cancer cell lines as the EGFR inhibitor, geftinib. No synergism however, was observed using a combination of the AI and gefitinib. However adding gefitinib 72 hr following AI treatment was more effective than adding both inhibitors simultaneously, though this did not reach statistical significance.
Conclusion: ADAM17 inhibition resulted in similar growth inhibitory response to EGFR inhibition in SUM159PT TNBC cells. Due to the current issues with resistance to gefitinib, ADAM17 inhibition could be pursued as a second-line treatment.
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130 POSTER

A role for the cholecystokinin 2 receptor (CCK-2R) in promoting cancer progression

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Background and aims: The gastrointestinal (GI) hormone, gastrin, promotes cancer progression and its down-regulation has been linked to reduced cancer stem cell numbers. Gastrin acts through the cholecystokinin 2 receptor (CCK-2R) and its biological effects are blocked by CCK-2R inhibitors. We investigated a potential role for CCK-2R in promoting survival of cancer stem cells using RNAi combined with a sensitive method to detect CCK-2R mRNA.
Materials and Methods: A panel of cancer cell-lines, including GI, glioblastoma and small cell lung cancer (SCLC), with CCK-2R-transfected