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

C. Haglund[1], M. Fryknäs[1], R. Larsson[1], S. Linder[2], L. Rickardson[1].
[1]Uppsala University, Medical Sciences, Uppsala, Sweden; [2]Karolinska Institute and Hospital, Oncology and Pathology, Stockholm, Sweden

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.

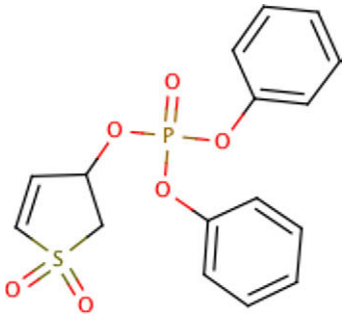

Figure 1. Structure formula of CB3

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

N. Buron[1], M. Porceddu[1], D. Disley[2], C. Torrance[2], A. Borgne-Sanchez[1].
[1]Mitologics SAS, Mitologics Research Laboratory, Paris, France; [2]Horizon Discovery Ltd, Horizon Discovery, Cambridge, United Kingdom

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.

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

P. McGowan[1], M. Mullooly[1], S. Sukor[1], N. O'Donovan[2], M.J. Duffy[1].
[1]University College Dublin, School of Medicine and Medical Science Conway Institute, Dublin, Ireland; [2]Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland

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

C. Mayne[1], A. Grabowska[1], S. Watson[1]. *[1]University of Nottingham, Division of Pre-Clinical Oncology, Nottingham, United Kingdom*

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

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

G. Kelter[1], T. Metz[1], J. Fehr[1], M. Hiss[1], H.H. Fiebig[1], T. Beckers[1].
[1]Oncotest GmbH, Institute for experimental Oncology, Freiburg, Germany

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

M. Belvin[1], L. Berry[1], J. Chan[1], D. den Otter[1], L. Friedman[1], K. Hoeflich[1], H. Koeppen[2], M. Merchant[1], C. Orr[1], K. Rice[3]. *[1]Genentech Inc., Cancer Signaling/Trans Onc, South San Francisco CA, USA; [2]Genentech Inc., Pathology, South San Francisco CA, USA; [3]Exelixis Pharmaceuticals Inc., Chemistry, South San Francisco CA, USA*

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

L. Salphati[1], L.B. Lee[2], J. Pang[1], E.G. Plise[1], X. Zhang[1], M.C. Nishimura[3], L. Friedman[4], D. Sampath[2], H.S. Phillips[3]. *[1]Genentech Inc, Drug Metabolism and Pharmacokinetics, South San Francisco, USA; [2]Genentech Inc, Translational Oncology, South San Francisco, USA; [3]Genentech Inc, Tumor Biology and Angiogenesis, South San Francisco, USA; [4]Genentech Inc, Cancer Signaling, South San Francisco, USA*

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