growth kinetics and 48−72 hours for functional assays, allows analysis of several parameters simultaneously. U87MG and MDA MB 231 show normal growth kinetics which are inhibited in a dose-dependent manner using 17-AAG. Both models also showed significant migration and invasion mirroring their in-vivo behaviour. Furthermore, MDA MB 231 MTS migration and invasion was inhibited with sub GI50 doses of 17-AAG. Direct comparison of cell viability (CellTiterGlo assay) following drug treatment in 2D and 3D showed that both tumour models are more resistant to 17-AAG in 3D. A method to analyse protein expression was optimised using the MDA MB 231 MTS and characteristic client depletion following HSP90 inhibition was demonstrated.
Conclusions: We provide evidence that the MTS model and derived functional assays are modifiable for a relatively high-throughput format. The reproducibility and simplicity of the assays make them attractive options for drug discovery projects potentially increasing the strength of prediction of in vivo activity.

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Novel Mcl-1 inhibitors for pancreatic cancer therapy

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The anti-apoptotic myeloid cell leukemia protein Mcl-1, a member of the Bcl-2 family proteins, has emerged as a promising therapeutic target. It was demonstrated that Mcl-1 is an important survival factor for pancreatic cancer cells; its down-regulation with siRNA for example, enhances the induction of apoptosis, chemosensitivity and radiosensitivity of pancreatic cancer cells. Therefore targeting Mcl-1 to overcome apoptosis resistance is an important strategy for the development of new drugs to treat pancreatic cancer.
Through high throughput screening approach we have identified several promising lead compounds which bind to the BH3 binding site in Mcl-1 selectively over Bcl-2 and Bcl-xL, and disrupt interactions between Mcl-1/Bid BH3 peptide and Mcl-1/Bax protein. We have synthesized several analogues and established initial structure–activity relationships. The novel synthetic analogue E288 is the most potent compound with K_i = 400 nM, 10 times more potent than the identified hits. NMR spectroscopy demonstrates that E288 binds to the same BH3 domain of Mcl-1 as the Bim BH3 peptide and antagonizes Mcl-1, inhibiting cell growth and inducing apoptosis in pancreatic cancer cells with high Mcl-1 levels (BxPC-3 and Panc-1) in a time and dose-dependent manner. By using murine embryonic fibroblasts (MEFs), wild type and deficient in both Bax and Bak (double knock out), it was demonstrated that the cytotoxic activity and induction of apoptosis by several analogues, depend on Bax and/or Bak, suggesting that they function as BH3 mimetics. Furthermore, the observed induction of apoptosis was Mcl-1 dependent demonstrated through applying siRNA approach, where the transient suppression of Mcl-1 abrogated E288 mediated apoptosis in both BxPC-3 and Panc-1 cell lines.
Collectively, these findings provide good promise for further chemical modifications of this compound and further optimization toward developing a new class of anticancer drugs, Mcl-1 inhibitors.

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Combination drug screening at the NCI

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A hurdle in selection of combinations to test in clinical trials is the complexity of signaling networks, feedback loops, and incomplete knowledge about how these are affected by the myriad genetic and epigenetic variations present in cancer. As a pragmatic approach to overcoming the challenges in choosing drug combinations, we have recently initiated an *in vitro* combination drug screen that accomodates testing of rationally designed choices, but also allows for serendipity. The screen utilizes 3 cell lines, chosen from the NCI-60 panel for diversity of their molecular characteristics. The non-small cell lung cancer cell line A549 is near triploid, with mutations in CDKN2A, KRAS and STK11. HCT-116, a colon cancer cell line has a nearly normal 2N karyotype, with microsatellite instability, and mutations in BRCA2, CDKN2A, CTNNB1, FGFR2, KRAS, MLH1 and PIK3CA. The final line in the screen is the prostate cancer cell line PC-3, which is a near tetraploid with many chromosomal rearrangements, and is mutant for TP53 and PTEN. Agents being considered for combination trials (test agents) are assayed in each of these 3 cell lines against a panel of well characterized agents (modifier agents), including recently approved kinase inhibitors and conventional cytotoxic agents. Cells are exposed to drugs for 3 days at 3 concentrations of both the “test” agent and each modifier agent, yielding a 3×3 concentration matrix. A Wilcoxon statistic is used to test the hypothesis that the growth inhibitory activity of the combination is better than that expected if the 2 single agents are independent and additive. The screen has identified a number of promising combinations, including some that would not have been predicted.

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Role of the epithelial phenotype in the sensitivity of pancreatic and breast cancer cell lines to Irvalec; in vitro synergism of the combination with gemcitabine

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Background: Irvalec is a novel marine-derived cyclic peptide belonging to the Kahalalide family of compounds, currently in phase II clinical trials. Epithelial–mesenchymal transition (EMT) is a biological process defining progression from polarised epithelial phenotype to a mesenchymal phenotype, which is distinguished by fibroblast-like features. We have studied the role of EMT markers in the sensitivity to Irvalec in a panel of pancreatic and breast cancer cell lines. Furthermore, we analyzed the combination of Irvalec with gemcitabine, the most widely used chemotherapeutic drug in pancreatic cancer, in the pancreatic cancer cell lines.
Material and Methods: Six pancreatic (BxPC-3, HPAC, AsPC-1, CFPAC-1, PANC-1, MIAPaCa-2) and five breast cancer cell lines (SK-BR-3, BT-474, MDA-MB-468, MCF7, MDA-MB-231) were obtained from the ATCC. Cell viability was measured by a cristal violet assay after treatment for 72 h. Protein expression levels of different EMT markers (E-cadherin, β-catenin, snail, twist-1, slug and vimentin) were analyzed by immunohistochemistry, immunocytochemistry and western blot. The combination of Irvalec and gemcitabine was analyzed using the median effect method of Chou and Thalalay using Calcusyn software program.
Results: All cell lines were tested with Irvalec. IC50 ranges were 0.06−8.7 uM and 0.1−6.5 uM for the pancreatic and breast cancer cell lines, respectively. The most sensitive Irvalec cell lines exhibited an epithelial phenotype (high E-cadherin, low vimentin and high twist-1 expression), whereas the mesenchymal phenotype was observed in the least sensitive cell lines. The potential synergism of the combination of Irvalec with gemcitabine was also evaluated in the panel of six pancreatic cancer cell lines, after treatment for 72 h with the different drugs, as single agents or in combination. The combination of Irvalec and gemcitabine had a synergistic effect at high doses (IC90 concentrations) in all pancreatic cancer cell lines tested, whereas at IC70 concentrations synergism was observed in the three most sensitive cell lines (CI values = 0.79, 081 and 0.85 for BxPC-3, CFPAC-1 and AsPC-1, respectively).
Conclusions: Sensitivity of pancreatic and breast cancer cell lines to Irvalec positively correlates with an epithelial phenotype. The *in vitro* synergism of the combination of Irvalec and gemcitabine provide a rationale for further development of this combination.

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Lithocholic acid competitively inhibits EphA2–ephrinA1 binding: pharmacological and structural considerations

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Background: Eph–ephrin system plays a central role during multiple morphogenetic processes and recent data suggest that, in a large variety of human cancers, up-regulated expression and/or de-regulated function of Eph–ephrin system may promote tumorigenesis and the development of a more aggressive and metastatic tumour phenotype.
In particular EphA2 upregulation is correlated with tumour stage and progression and expression of EphA2 in non-transformed cells induces malignant transformation and confers tumorigenic potential. Based on these evidences our aim is to develop small molecules able to modulate EphA2–ephrinA1 activity.