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Chemical-genetic Screenings for Synthetic-lethal Interactions in Breast Cancer

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Background: Breast cancer (BC) has a huge epidemiological impact in developed countries and is a leading cause of cancer-related mortality among women. Despite progresses made in diagnosis and treatment, especially in its advanced or metastatic presentation, it is hardly curable. This is mostly due to the paucity of therapeutics that specifically target BC's genetic and phenotypic peculiarities. Synthetic-lethal therapeutics exploit cancer-associated mutations as Achilles' heels by taking advantage of the weaknesses that such mutations expose in malignant cells (i.e, PARP inhibitors for BRCA1/2-mutated cancers). Here, we report on the execution of chemical-genetic interaction screens aimed to identify compounds that specifically kill mammary epithelial cells engineered to express common BC-associated mutations.
Material and Methods: MCF10A, a spontaneously immortalized but non-transformed human mammary epithelial cell line, was engineered to express HRas, Her2/neu, PI3K H1047R or to stably silence the tumour suppressors PTEN, p53 and Rb. Cells engineered with empty retroviral vectors were used as controls. The introduced genetic modifications were verified by western blotting and by flow cytometry. The so-generated isogenic cells lines were probed against different collections of chemical compounds (including the Diversity and Mechanistic set from NIH's NCI Developmental Therapeutics Program) for a total of more than 10.000 genotype-compound combinations. Viability was assessed by colorimetric assay after a 48h-incubation. The experiments were performed in a 384-well format by means of a robotic liquid handling workstation.
Results: We developed a system to perform chemical-genetic screens in genetically-modified epithelial breast cells. Our approach is able to detect susceptibility phenotypes, since MCF10A cell engineered to overexpress HER2 do show a markedly increase susceptibility to Lapatinib, a dual EGFR/HER2 tirosine kinase inhibitor which is known to preferentially kill HER2-over-expressing cells. Moreover, previously reported resistance phenotypes due to aberrant activation of the PI3K-Akt and MAPK pathway were also readily detected in preliminary experiments.
Conclusions: We aim to identify synthetic-lethal compounds that may serve as leads for subsequent development of clinically-effective treatments for BC. The use of this type of chemical-genetic approach appears to be feasible and able to detect mutation-associated susceptibility phenotypes.

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Chemical Screening for Potentiators of Lapatinib Activity in Human Breast Cancer

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Background: 10−30% of breast cancers overexpress the oncogenic receptor tyrosine kinase HER2 as a consequence of gene amplification. In these tumours, HER2 acts as a driving oncogene which cancer cells are addicted to. Monoclonal antibodies against HER2 and small-molecule inhibitors of its tyrosine kinase activity proved effective in the treatment of HER2+ breast cancer. However, the benefit of these treatments is limited by primary and acquired resistance. Lapatinib is an inhibitor of the tyrosine kinase activity of both HER2 and EGFR and it is highly active against HER2-overexpressing breast cancers. Resistance to lapatinib in breast cancer is due to estrogen-dependent pro-survival mechanisms, and to activating mutations in the PI3K and ras pathways. We chose lapatinib as a drug model and performed chemical screens in order to identify compounds which synergistically enhance lapatinib efficacy in breast cancer cells.
Materials and Methods: The HER2+ overexpressing breast cancer cell line SKBR3 was used in our experiments. Cells were incubated in the presence or absence of lapatinib, and subsequently probed against the over 2000 compounds of the Mechanistic Set and the Diversity Set from NCI/NHI Developmental Therapeutic Program. Cells were plated in 384 well plates and 4 different concentration of each compound were tested. After 72h of incubation, cell viability was determined using a colorimetric assay and then the cooperative index (CI) was calculated as the sum of the specific cell deaths induced by the single agents divided by the specific cell death in response to their combination. CI values <1, indicate a synergistic effect between compounds.
Results: We identified 13 different compounds which showed a synergistic activity with lapatinib in SKBR3 cells. Most of these show antiproliferative activity in vitro in NCI-60 cell lines, but their molecular target is unknown. These compound were retested and 9 of them confirmed to synergistically potentiate the activity of the tyrosine kinase inhibitor. Follow-up experiments are now ongoing with the aim to define their mode of action and their usefulness in combination regimens.
Conclusions: Our preliminary results show that lapatinib activity can be potentiated in combination with compounds identified through chemical screens. Such drug combinations shall be further investigated and may lead to new treatment strategies for HER2-overexpressing malignancies.

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Enhanced Cellular Delivery of Idarubicin by Surface Modification of Propyl Starch Nanoparticles Employing Pteroic Acid Conjugated Polyvinyl Alcohol

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Background: Improved efficacy of anti-cancer agents with simultaneous reduction of their systemic exposure and non-specific toxicity is possible through enhanced internalization of nanoparticles encapsulating these. The routinely used hydrophilic stabilizers used in formulating nanoparticles may however hinder their interaction with the hydrophobic cell membranes. The present investigation attempts to overcome this problem by employing pteroic acid modified polyvinyl alcohol (ptPVA) as a novel surfactant to formulate idarbicin (IDA) encapsulated propyl starch nanoparticles [1]. This modification was hypothesized to enhance their uptake through improved adsorption of various proteins, the receptors of the latter being dominant on numerous cancer cells.
Materials and Methods: IDA-loaded ptPVA nanoparticles were formulated using solvent emulsification-diffusion technique and optimized for relative amounts of drug to polymer and stabilizer. The optimum formulation was characterized with regards to particle size, surface charge, morphology, drug encapsulation and loading and *in vitro* release pattern. Enhanced protein adsorption of ptPVA modified nanoparticles was confirmed employing BCA assay with BSA as the standard. The safety and efficacy of the nanoparticles was confirmed by MTT and ATPase cytotoxicity assays in Caco-2 cell line. Enhancement of cellular uptake was confirmed using Confocal laser scanning microscopy.
Results: A homogenous distribution of spherical IDA nanoparticles (245 nm) were formulated with IDA encapsulation of about 85%. These nanoparticles which exhibited a controlled drug release were found to be safe for cellular evaluations in both the cytotoxicity assays. Moreover an enhanced efficacy of the IDA nanoparticles was observed as compared to the free drug control thus indicating their efficacy. Higher protein binding was exhibited by ptPVA nanoparticles compared to non-modified ones indicating the possible influence of protein adsorption on improved internalization and hence efficacy. Furthermore, a higher cellular internalization was observed for ptPVA nanoparticles in HT-29 cells and A-549 cells, thus confirming the proposed hypothesis of higher protein adsorption being responsible for this effect.
Conclusion: Higher uptake and efficacy along with cellular safety indicate the potential of the IDA nanoparticles for *in vivo* evaluations to validate these results. Such selective internalization and efficacy in cancer cells is significant for toxic anti-cancer drugs like IDA.
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[1] Santander-Ortega, M. J.; Stauner, T.; Loretz, B.; Ortega-Vinuesa, J. L.; Bastos-González, D.; Wenz, G.; Schaefer, U. F.; Lehr, C. M. Nanoparticles made from novel starch derivatives for transdermal drug delivery. *J. Control Release* 2010, 141, 85−92.

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Docetaxel Delivery Mediated by Nanoparticles of Novel Hydrophobic Starch

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Background: Polymeric nanoparticles can overcome challenges in delivery of anti-cancer agents due to their enhanced internalization, retention and

controlled release of the encapsulated drug. This investigation focuses on preparation and characterization of nanoparticles of propyl starch, a novel hydrophobic polymer [1], for encapsulating and modulating the release of docetaxel. Docetaxel was selected due to its evident efficacy in numerous cancers limited by its low aqueous solubility and severe toxicity.
Material and Methods: Docetaxel nanoparticles were formulated using solvent emulsification-diffusion technique and optimized with respect to relative amounts of docetaxel and propyl starch, influence of various stabilizers and their quantity. Optimum nanoparticles were characterized with regards to particle size, morphology, surface charge, docetaxel encapsulation and it's *in vitro* release profile. Cytotoxicity assays in cancer cells (Caco-2) were conducted to determine the safety and efficacy of nanoparticles. Cellular internalization of nanoparticles was observed by Confocal laser scanning microscopy. Results in Caco-2 cells were compared with those in non-cancer cells (NHDF-p) to confirm their benignity towards the latter.
Results: Nanometric, homogenous and spherical nanoparticles were formulated with a mean particle size of ~250 nm and a negative surface charge of −23 mV. Encapsulation efficiency of docetaxel was greater than 80% with a controlled release being observed from the selected polymer indicating probability of increased concentration and duration at the affected area. Cytoxicity tests of un-loaded particles in Caco-2 and NHDF-p cells exhibited their safety for cellular evaluations. Cytotoxicity of encapsulated drug was higher than free drug control indicating nanoparticle efficacy attributable to their enhanced internalization. Further, a superior action was observed in cancer versus non-cancer cells. Internalization studies confirmed these results by exhibiting a better uptake of nanoparticles into the cancer cells with a distinct evidence of their peri-nuclear localization.
Conclusions: Docetaxel nanoparticles may be regarded as a safe yet efficacious therapeutic with probability of enhanced drug bioavailability as a direct consequence of the 'nano' dimensions of its carrier.
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1225 POSTER

A Randomised, Double-blind, Placebo Controlled, Multi-site Study of Subcutaneous Ketamine in the Management of Cancer Pain

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Background: Ketamine is used commonly as an adjunct to opioids in the management of pain. The evidence to support this practice is limited. The aim of this study was to evaluate the role of subcutaneous ketamine in cancer pain.
Materials and Methods: Patients with pain related to malignant disease or its treatment, rated as $\geqslant$3/10 despite adequate co-analgesia, were eligible if there has been no change in baseline opioid dose within the previous 48 hours. Participants were randomised to either ketamine or placebo, delivered subcutaneously at a dose titrated from 100 to 500 mg/24hours, according to response and toxicity. Response was defined as a $\geqslant$2 point reduction in average Brief Pain Inventory (BPI) pain score from baseline with $\leqslant$4 breakthrough doses of analgesia. The primary endpoint was average pain score at start day 6. Secondary endpoints included adverse events, response at days 2−5 and quality of life. Ketamine would be considered superior to placebo if the response rate at start day 6 was 25% greater than that of placebo (assuming a placebo response rate of 30%).
Results: One hundred and eighty five participants were randomised from March 2008 to February 2011 to complete the planned sample size of 150. Primary analysis has confirmed the high placebo response rate (26/92 = 28%) with no difference between active and placebo arms ($p = 0.78$).
Conclusion: This adequately powered, randomized controlled trial demonstrates the power of placebo and does not support the role of subcutaneous ketamine in the treatment of cancer pain in advanced cancer.

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An ErbB-3 Antibody, MP-RM-1, Inhibits Tumour Growth by Blocking Ligand-dependent and Independent Activation of ErbB-3/Akt Signaling

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Background: The ErbB receptors, such as ErbB-1 and ErbB-2, have been intensely pursued as targets for cancer therapeutics. Although initially efficacious in a subset of patients, drugs targeting these receptors led invariably to resistance which is often associated with reactivation of the ErbB-3-PI3K-Akt signaling. This may be overcome by an ErbB-3 ligand binding molecule that abrogates ErbB-3 mediated signaling.
Materials: Toward this end, we have generated a mouse monoclonal antibody, MP-RM-1, against the extracellular domain (ECD) of ErbB-3 receptor. The ability of the antibody to suppress NRG-1b-dependent and independent ErbB-3 signaling was evaluated in vitro by western blotting using a panel of human tumour cell lines (breast, melanoma, stomach and prostate) as well as early passage tumour cells obtained from patients. The effect on tumour growth in vitro was evaluated with clonogenic assay and in vivo using human tumour xenograft nude mouse models.
Results: Assessment of human tumour cell lines as well as early passage tumour cells collected from patients revealed that MP-RM-1 effectively inhibited both NRG-1β stimulated and basal ErbB-3 activation. MP-RM-1 treatment led, in most instances, to decreased ErbB-3 expression. In addition, MP-RM-1 was able to inhibit the colony formation ability of tumour cells and tumour growth in two human tumour xenograft nude mouse models. Treatment with the antibody was associated with a decreased ErbB-3 and Akt phosphorylation and ErbB-3 expression in the excised tumour tissue.
Conclusions: Collectively these results indicate that MP-RM-1 has the potential to interfere with signaling by ErbB-3 and reinforce the notion that ErbB-3 could be a key target in cancer drug design.

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Molecular Portrait of Breast Cancer Cell Lines and Response to Artemisinin

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Background: Artemisinin (ART) is a sesquiterpene lactone originally used as an antimalarial drug. Broad anti-cancer activities of ART have been documented in the last decade, including activity against breast cancer (BC). The molecular mechanism of ART against BC, especially its selectivity for different BC subtypes, is largely unknown. In this study we therefore aimed to identify potential target genes associated with ART sensitivity using gene expression microarray, and to determine the selectivity of ART against a panel of BC cell lines representing the various known intrinsic subtypes.
Material and Methods: Cell growth inhibition of ART was measured using sulforhodamine B protein staining to determine sensitivity to ART of 31 different BC cell lines with known molecular subtypes. The half maximal inhibitory concentration (IC50) values of these cell lines were associated with their morphological properties and intrinsic subtypes. Also, differentially expressed genes (Affymetrix U133A array) between the 7 most sensitive versus the 10 most resistant cell lines were identified. Finally, to identify possibly involved networks and pathways, a pathway analysis involving the most significant differentially expressed genes (with $p < 0.01$) was carried out using Ingenuity.
Results: IC50 values of ART showed that this molecule had activity against all the 31 cell lines but had a significant preference to target spindle-shaped ($p = 0.044$), and also triple negative BC (TNBC) cell lines ($p = 0.025$) which lack expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2. We identified 119 differentially expressed probes, associated with 105 unique genes, between the 7 ART-sensitive and the 10 ART-resistant cell lines. Pathway analysis revealed that the top interaction network (25 out of 35 involved genes were differentially expressed) was associated with functions related to "cell-mediated immune response, cellular development, cellular function and maintenance", while the most significantly involved canonical pathway was "role of IL-17F in allergic inflammatory airway diseases" ($p = 0.002$).
Conclusions: Our studies showed the selectivity of ART against TNBC cell lines and identified a putative gene profile related to sensitivity to ART. Further studies will focus on confirming the differential expressed genes at protein level and functionally verifying the discovered pathway in differential ART sensitivity of BC cell lines.