without significantly affecting the maximum plasma concentration (C_{max}) or time to maximum plasma concentration (t_{max}) of CS-7017. There were no deaths, serious adverse events (SAEs) or discontinuations due to AEs in this study. All treatment emergent AEs(TEAEs) were mild in severity. Only two TEAEs (abdominal distension and abdominal discomfort) were considered to be related to study treatment (ketoconazole).
Conclusions: Co-administration of ketoconazole significantly increased the bioavailability of CS-7017. Administration of a single dose oral dose of 0.25 mg CS-7017 either alone or concomitantly with 400 mg ketoconazole was well tolerated in healthy male subjects.

327 POSTER

Activating transcription factor 3 as a novel regulator and predictor of cisplatin response in human cancers

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Background: The development of resistance to platinum-based chemotherapy is a major obstacle in the treatment of cancer. Activating transcription factor 3 (ATF3) is a stress-inducible gene that we recently demonstrated to be a regulator of cisplatin induced cytotoxicity. Further characterization of ATF3's role in cisplatin cytotoxicity will lead to novel therapeutic approaches that could improve the efficacy of this important class of chemotherapies.
Methods: A panel of human cancer cell lines was treated with a range of cytotoxic and non-cytotoxic doses of cisplatin. Cell viability was assessed by the MTT assay and a time course of ATF3 protein expression was evaluated by Western blot. The expression of potential ATF3 targets in response to cisplatin treatment was determined by Western blot and confirmed by the chromatin immunoprecipitation assay. ATF3 levels were evaluated in a clinical setting where total RNA was extracted from 51 resected ovarian tumors and basal ATF3 mRNA was evaluated by QPCR. Immunohistochemistry using an anti-ATF3 antibody was performed on ovarian and lung cancer tissue microarrays.
Results: ATF3 protein expression was induced upon cytotoxic doses of cisplatin treatment in all cell lines examined. Two potential targets of ATF3 had altered expression in response to cisplatin treatment: cyclin D1, a cell cycle regulator, showed reduced protein expression and CHOP/gadd153, a pro-apoptotic transcription factor, showed increased protein expression. ATF3 was also found to be bound to the CHOP/gadd153 promoter in response to cytotoxic doses of cisplatin. In a clinical setting, basal ATF3 mRNA levels were variable among ovarian tumors and there was a trend toward higher ATF3 expression correlating with better outcomes. ATF3 protein expression on tissue microarrays containing ovarian and non-small cell lung cancer cases was found to be differentially expressed among tumor subtypes. A higher proportion of ATF3 expression was seen in ovarian mucinous adenocarcinomas and lung squamous cell carcinomas suggestion that stratifying treatment based on tumor subtype might yield better patient outcomes.
Conclusions: ATF3 is a novel regulator of cisplatin cytotoxicity acting through the suppression of cyclin D1 and activation of CHOP/gadd153 protein expression. The basal expression of ATF3 might be predictive of cisplatin treatment response and may present itself as a novel biomarker.

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P-glycoprotein antagonists as opposed to inhibitors of glucosylceramide synthase enhance short-chain ceramide cytotoxicity in human ovarian cancer cells

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Background: A major focus in the field of drug resistance has been on design and testing of antagonists that limit drug effluxing properties of P-glycoprotein (P-gp). This direction however has shown little clinical benefit. The purpose of this study was to assess the utility of P-gp antagonists as enhancers of ceramide-induced cell death in ovarian cancer, a malignancy in which P-gp occurs with high frequency and correlates with disease progression.
Materials and Methods: A2780 wild-type and the multidrug resistant (MDR) counterpart 2780AD, and MDR NCI/ADR-RES human ovarian cancer cell lines were employed. Cultures were exposed to the ceramide (cer) analog C6-cer (5−10 μM) in the absence and presence of P-gp antagonists (2.5−5 μM) tamoxifen, cyclosporin A, and VX-710 (Biricodar®), or the glucosylceramide (GC) synthase (GCS) inhibitor, ethylenedioxy-P4 (P4). Cell viability was measured by Cell Titer 96 Aqueous®. C6-cer metabolism was followed using N-hexanoyl[1−^{14}C]-D-erythro-sphingosine, thin-layer chromatography, and liquid scintillation counting. Cell-free GCS assays employed [^{3}H]UDP-glucose and C6-cer.
Results: Compared to wild-type A2780 cells, P-gp rich 2780AD cells converted 4-fold more C6-cer to nontoxic C6-GC (C6-GC), whereas cell-free GCS activities were equal. In 24−72 hr viability assays, 2780AD cells demonstrated resistance to C6-cer and A2780 cells were sensitive. Tamoxifen and cyclosporin A inhibited conversion of C6-cer to C6-GC by 70−90% in 2780AD cells. C6-cer resistance in 2780AD cells was reversed by tamoxifen but not by addition of the GCS inhibitor P4, even though P4 inhibited conversion of C6-cer to C6-GC by 90%. For example, cell viabilities measured 78, 84, and 83% of control in C6-cer-, tamoxifen-, and P4-treated cells, respectively, and 15 and 68% in cells exposed to C6-cer/tamoxifen and C6-cer/P4, respectively. Co-administration of C6-cer and P-gp antagonists was also effective in reducing NCI/ADR-RES cell viability; whereas C6-cer, VX-710, and cyclosporin A exposure resulted in viabilities of ~90%, C6-cer/VX-710 and C6-cer/cyclosporin A exposure resulted in viabilities of 22 and 17%, respectively.
Conclusions: Many studies have focused on inhibiting GCS to enhance ceramide-related cytotoxicity, as GCS catalyzes GC synthesis. Here we have taken advantage of the function of P-gp in GC trafficking and shown that targeting P-gp is more effective than inhibiting GCS for increasing ceramide cytotoxicity.

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Insights into the cellular responses against different DNA-binding agents

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The cell response to anti-cancer agents is largely determined by the expression of the genetic materials into proteins in space and time. Here, we used the cDNA microarray technology to follow the biological responses to different classes of anti-cancer DNA binding agents. Although all chosen agents are a DNA minor groove binding agents, they exert differ mechanisms of killing towards the cancer cells. The examined agents include doxorubicin (topoisomerase poison), echinomycin (transcription inhibitor), alkamin (minor groove alkylating agent).The cultured human CEM cells were the model and the 5 × IC_{50} for 24 h were the concentration and the time of exposure. The expression of 6000 human genes printed cDNA was the target. Genes that have been up-regulated more than 3 folds were selected and used as a representative for the cell responses against these agents. Applying ontological and pathway analysis results three distinguish profiles. The first profile that representing topoisomerase poisons mainly characterized by the up-regulation of those functions that related to protein metabolic process, regulation of macromolecule metabolic process, regulation of apoptosis, regulation of signal transduction. Importantly the ubiquitination pathway was largely up-regulated suggesting an attempt to remove the trapped cleavable complex by an ubiquitin-dependent mechanism, marking it a substrate for proteolysis by the 26S proteasome. The second resulted profile, which represent the transcription inhibitors, distinguished from the first by the abundance of those functions that related to RNA metabolic process, regulation of signal transduction, protein kinase cascade, mRNA metabolic process. One significant point to be mention here is that echinomycin profile is characterized by many negative impacts on the important cellular functions, It is as if the cells are down regulating many of its essential functions to cope with DNA assault. The third biological profile, the alkamin profile, is dominated by those functions related to transcription activation and RNA biosynthesis and cell cycle. Interestingly, in the alkamin profile the basic transcription factor pathway and the RNA elongation ontological category were largely up-regulated alarming the idea that alkamin mechanism of action may involve transcription inhibition. Finally we concluded that the muted response of the drugs suggests a degree of biological resistance amongst functions that might be expected to be most damaged by drug action.

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P-glycoprotein in doxorubicin resistant MCF-7 breast cancer cells: DPAGT1 approach to N-glycosylation regulation

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Background: Dysregulation of DPAGT1 (Dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase) causes disturbances in P-glycoprotein (Pgp) expression in multidrug resistance. The resent results are in favour of the idea that N-glycosylation in cancer cells is limited by Dolichyl Phosphate (DolP). The aim of the present study is to investigate the effect of polyprenol (PP)

which provides a Dolichol Phosphate (DolP) substitute on regulation of Pgp expression in Doxorubicin resistant MCF-7 breast cancer cells.
Methods: Breast cancer cell lines, MCF-7 and MCF-7/ADR were used. Pol concentration in the culture medium made up 10^{-2}–10^{-6}. Immunohistochemical and Western blotting methods were used to detect the changes in the expression levels of MDR1 and DPAGT1 expression. Intermediates of DPC fractions were analysed by HPLC method.
Results: Overexpression of DPAGT1 was detected MCF-7/ADR cells, but not in MCF-7 cells. It is confirmed that plasmatic membranes of MCF-7 cells contain 5.6–6.4% of Pgp (the total protein amount) as a resistance marker. Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in Pgp content by 10−12 times. The study showed 8.5-fold DolP decrease in MCF-7/ADR cells. The investigations demonstrate that the situation can be changed by treatment with DolP and PP. The DolP concentration in MCF-7/ADR cells was returned to the normal level. It is established that DolP in the concentration 10^{-6} M aid 7−9-fold reducing Pgp in membranes of MCF-7/ADR cells. The MCF-7/ADR cells cultivation in medium with polyprenol proceeded to give lowered Pgp content in membranes no over 0.4−0.6%, which amount was consistent with the level of Pgp in MCF-7 cells. Overexpression of DPAGT1 was detected in MCF-7 and in MCF-7/ADR cells. It is established that Pol in the concentration 10^{-4} M aid 7−9-fold could overcome DPAGT1 overexpression which leads to regulation of Pgp N-glycosylation. Pol in concentration 10^{-2}−10^{-3} M induced apoptosis in MCF-7/ADR cells within 3−4 hours.
Conclusions: These results indicate that noncontrollable accumulation of Pgp, after MDR1 expression in MCF-7/ADR cells can be overcome using stimulation with dolichyl phosphate substitution. DPAGT1 overexpression in MCF-7/ADR can be overcome with Pol, which provides a DolP substitute for DPAGT1 normal expression.

Drug targets

331 POSTER DISCUSSION

Atrimers as a novel class of potent trimeric therapeutics inducing cancer cell death

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TRAIL death receptors DR4 and DR5 are attractive therapeutic targets in oncology as they are expressed in a wide variety of tumors, and DR4/DR5 agonists can induce p53-independent apoptosis. Agonistic monoclonal antibodies against DR4 or DR5, and recombinant TRAIL are currently being evaluated in clinical trials in combination with chemotherapy. However, these monoclonal antibodies target a single receptor type and, due to their bivalent nature, are not ideal to mimic the receptor-trimerizing effect of the potent natural trimeric ligand. The efficacy of recombinant trimeric TRAIL is hampered by its short half-life and its binding to decoy receptors that do not mediate apoptosis. We aimed to overcome these shortcomings of current therapeutics by developing stable trimeric death receptor agonists that do not cross-react with the decoy receptors and with half-lives expected to significantly exceed that of TRAIL. Potent DR4 and DR5 agonists were engineered using human tetranectin, a trimeric human serum protein of 60 kDa size, as a scaffold to generate Atrimers™. A panel of DR4 binders was selected from phage libraries displaying the C-type lectin domain (CTLD) of tetranectin with randomized loop sequences. Current lead DR4 Atrimers have sub-nanomolar affinity to DR4-Fc and show no detectable binding to DR5 or the decoy receptors. *In vitro*, they efficiently kill DR4-positive cancer cell lines with sub-nanomolar ED50, but do not kill DR4-negative cell lines. DR5 agonists were engineered by fusing phage-display-selected DR5 binding peptides to the N-terminus of tetranectin. Such DR5 agonists were equipotent to TRAIL in cell death assays with Colo-205 cells. Bi-specifics are currently being engineered by genetically fusing our most potent DR5 agonist peptide with our DR4 agonist Atrimers. Such a bi-specific Atrimer will target both DR4 and DR5 as stable trimer offering i) greater coverage due to differential expression with some patients expressing both DR4 and DR5 and ii) greater potency mediated in part by super-clustering via tumor cell specific binding on both ends of the molecule. In addition, improved tumor penetration (vs. antibodies) is expected due to smaller size (~70 kD). DR4/DR5 Atrimers therefore represent a novel class of cancer therapeutics selectively targeting the TRAIL receptors to efficiently induce apoptosis and provide many promises for the treatment of a broad range of cancer types.

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***POLQ* (DNA polyermase theta) as a novel therapeutic target: preclinical and clinical data**

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Background: We have previously shown that *POLQ* is upregulated in multiple different tumour cell lines and that *POLQ* depletion causes radiosensitisation. We have therefore conducted further studies to assess the suitability of POLQ as a therapeutic target.
Methods: Tumour cell lines were transfected with either non-targeting or *POLQ* siRNA. At 48h after transfection, cells were treated with cytotoxic agents prior to being assayed for clonogenic survival. Homologous recombination (HR) was assessed by quantifying RAD51 foci formation 4h after exposure to cytotoxic drugs. The I-Sce-I assay was also used to assess whether POLQ is involved in HR.
To assess the prognostic importance of *POLQ* we analysed tumour samples from two retrospective series of breast cancer patients (n = 279 in total) treated in Oxford, UK. *POLQ* mRNA expression was assessed by Affymetrix U133 array and compared with clinical outcomes. Published clinical series containing details of a further 537 breast cancer patients were accessed to confirm the findings seen in the Oxford cohorts.
Results: Tumour cells depleted of POLQ are sensitized to DNA damaging agents such as cisplatin, etoposide, doxorubicin, and mitomycin C. They are not sensitized to mitotic spindle poisons such as docetaxel. Cells depleted of POLQ have fewer RAD51 foci after exposure to cytotoxic drugs. POLQ knockdown also resulted in decreased HR efficiency as assessed by the I-Sce-I assay. All of these findings suggest that POLQ plays a role in HR.
The prognostic implications of *POLQ* overexpression were assessed in retrospective series of patients with early breast cancer. *POLQ* overexpression was associated with clinical features known to confer an adverse prognosis such as ER negative disease ($p = 0.047$) and high tumour grade ($p = 0.004$). Multivariate analysis showed that *POLQ* expression was associated with markedly worse relapse free survival rates independently of these other clinical features (HR 8.086; 95% CI 2.340 to 27.948; $p = 0.001$). Analysis of other published series supported these findings.
Conclusion: *POLQ* has limited normal tissue expression, but is overexpressed in a wide variety of different tumours. POLQ appears to be involved in HR with POLQ depletion rendering tumour cells sensitive to radiotherapy and multiple different DNA damaging agents. *POLQ* overexpression confers an extremely bad prognosis in breast cancer patients. This fact, combined with the above data make POLQ a highly appealing target for clinical exploitation.

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Identification of drug-associated proteins in NSCLC xenograft models by reverse phase protein arrays

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The treatment of non-small cell lung cancer (NSCLC) is currently limited by the fact that most therapies are not adapted to the individual response of a patient. The stratification of patients for the most efficient response to conventional chemotherapeutics and targeted therapies will improve established therapy schemes. Our project aims at unravelling the influence of specific signalling molecules on the response rate to common NSCLC drugs.
We quantified protein expression levels in patient derived NSCLC xenograft models. The tumor models are characterized by different response rates upon treatment with established chemotherapeutics (e.g. Gemcitabine, Paclitaxel, Carboplatin) and EGFR-targeted therapies (Cetuximab, Erlotinib). Protein expression was analyzed using the reverse phase protein array technology (RPPA). Protein lysates of 53 tumor samples were spotted in dilution series and replicates on nitrocellulose coated glass slides. Signalling proteins of cancer-relevant pathways (e.g. MAPK/Erk, JAK/STAT, PI3K/AKT) were detected by specific primary antibodies followed by IR-