colon, pancreas, lung and glioma tissue. A version of this virus expressing GM-CSF has shown promising results in Phase I and II clinical trials. The present study aims to test OncoVexGALV/CD as an intravesical therapy for superficial bladder cancer.
Material and Methods: In vitro tumour cell killing by OncoVexGALV/CD was assessed by Fusion/Prodrug MTS assays. In vivo efficacy of the treatment was studies through histology and IVIS imaging.
Results: Treatment of three human bladder carcinoma cell lines with the virus, resulted in higher tumour cell killing through oncolysis, pro-drug activation and glycoprotein fusion. To further test OncoVexGALV/CD we have developed a rat orthotopic bladder tumour model to assess intravesical tumour control. Using luciferase-expressing tumours, we will further delineate the effects of individual properties of the virus on tumour growth using the IVIS imaging system.
Conclusions: Preliminary results on human bladder carcinoma cell lines indicate, that OncoVexGALV/CD may improve local tumour control within the bladder, and potentially alter its natural history.

305 POSTER

Structure–activity relationships for lipophilic dinitrobenzamide mustards as prodrugs for Escherichia coli NfsB nitroreductase

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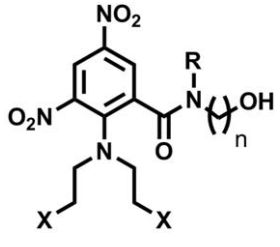
Background: A major weakness of cancer gene therapy protocols is the limited distribution of gene-delivery vectors within the tumour mass. Enzyme-prodrug activating systems (GDEPT) can potentially compensate by generating cytotoxic metabolites that diffuse locally to kill neighbouring vector-naive cells, creating what is known as a 'bystander effect'. E. coli nitroreductase *nfsB* (NTR) in combination with the prodrug CB1954 has been evaluated clinically but efficacy was constrained, at least in part, through inadequate bystander effects (Patterson, Can Res 2002;62:1425). New analogues of the dinitrobenzamide mustard (DNBM) class have demonstrably superior bystander efficiencies in vivo following *nfsB* activation (Singleton, Can Gen Ther 2007;14:953). We sought to maximise metabolite redistribution properties in the DNBM class by modifying the lipophilic nature of the prodrugs through placing additional alkyl groups at three available positions (R, n, X), as well as modification of mustard leaving groups (X).
Methods: A series of 14 novel lipophilic DNBM prodrugs were synthesised and characterised by HPLC, MS, NMR and combustion. Potency (24 h exp) was determined in vitro against $HCT116^{NTR}$ cells (relative to parental cells) in a 5 day proliferation assay. Nine candidates were advanced to mixed 3D tissue culture "bystander efficiency" testing in vitro containing only 1% NTR +ve cells with local toxicity transfer to cocultured WT cells being quantified by clonogenic survival.
Results: Prodrug Log P values spanned >4 orders of magnitude (0.14–4.4). All DNBM prodrugs were more dose-potent (2−60 fold) than CB1954 with $HCT116^{NTR}$ cell growth inhibition (IC_{50}) ranging from 0.05–1.4 μM (28-fold) in a manner that correlated with Log P ($r^2 = -0.82$). Increasing lipophilicity was paralleled with loss of NTR-dependent sensitisation of 2D monolayer cultures. However, only a weak correlation between 2D-IC_{50} and 3D-C_{10} values for $HCT116^{NTR}$ cells was seen ($r^2 = +0.58$), with no apparent relationship with WT-NTR potency ratios in the two systems ($r^2 = +0.24$). As expected, improvements in 3D bystander efficiency (% toxicity transfer from 1% NTR+ve cells) tracked with increasing lipophilicity ($r^2 = +0.69$), ranging from 14% for CB 1954 to 69% for the lead DNBM analogue.
Conclusion: This study establishes the importance of ranking prodrugs using in vitro models with appropriate tissue-like cell densities and identifies several promising leads for further development.



306 POSTER

Targeted suicide gene therapy for small cell lung cancer

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In suicide gene therapy, the introduced therapeutic gene encodes an enzyme capable of transforming a non-toxic prodrug into a cytotoxic drug. Utilizing cancer-specific promoters suicide gene expression can be selectively targeted to the cancer cells of interest. For that purpose we have identified several promotor regions, which are highly promising candidates for transcriptionally targeted gene therapy for small cell lung cancer (SCLC). The suicide gene yeast cytosine deaminase (YCD) converts the pro-drug 5-fluorocytosin (5-FC) into the known chemotherapeutic agent 5-fluorouracil (5-FU). YCD was cloned for regulated expression from the SCLC specific promoter Insulinoma-associated 1 (INSM1) and transiently transfected into different cell lines, which were exposed to increasing concentrations of 5-FC. Transfected SCLC cells were greatly sensitised to 5-FC and significant cell death was achieved while cancer cell lines of other origins were unaffected to treatment. Furthermore the YCD gene was fused with the yeast uracil posphoribosyltransferase (YUPRT) gene, which augments the conversion of 5-FU into active cytotoxins. The fusion construct (YCD-YUPRT) demonstrated significantly increased sensitivity towards 5-FC in treated SCLC cell lines inducing cytotoxicity comparable to treatment with the 5-FU toxin alone.
Due to limited efficiency of gene delivery in vivo an important feature of suicide gene therapy is the bystander effect where suicide gene/prodrug-produced toxins diffuse to untransfected neighbouring cells. In the cytosine deaminase-based suicide gene therapy 100% cell death was achieved after 5-FC treatment when only 50% of cells expressed the YCD or YUPRT gene. Further it was established that the YCD-YUPRT/5-FC strategy caused extensive cell death when as few as 10% cells expressed the transgene. This contrast previously obtained results with the suicide gene Herpes simplex virus thymidine kinase (HSVtk) and the prodrug penciclovir (PCV) where cell death was restricted to HSVtk-transfected cells.
As succesfull cancer treatment relies on multi-targeting treatment the combination of HSVtk and YCD-YUPRT suicide gene therapy was tested. At low prodrug concentrations an additive effect of the systems was obtained while the YCD-YUPRT mediated toxicity dominated at high 5-FC concentrations. Further testing of these and other suicide systems in vivo will conclude on the significance of combinatorial suicide gene therapy for SCLC.

307 POSTER

Effects of triple knockdown of cIAP-1, c-IAP-2 and XIAP on prostate cancer cell susceptibility to apoptosis

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Background: Treatments for hormone resistant prostate cancer are currently effective with resistance to apoptotic cell death a common mechanism of resistance in this advanced form of the disease. Manipulation of the apoptotic resistant phenotype represents an important strategy for increasing the response of hormone refractory prostate cancer cells to therapy. Previous studies in our laboratory have identified elevated expression of the inhibitors of apoptosis proteins (IAP) in prostate cancer cell lines and primary material. Knockdown of XIAP is associated with increased susceptibility to chemotherapy induced apoptosis. We hypothesis that simultaneous knockdown of cIAP-1, cIAP-2 and XIAP would further increase the sensitivity of both type 1 and type 2 triggers of apoptosis.
Material and Methods: PC-3 androgen independent prostate cancer cells were treated with optimum concentrations of siRNA to knock down cIAP-1, cIAP-2 and XIAP which was confirmed by western blotting. Following knock down these cells were treated with TRAIL, Etoposide, and Tunicamycin and assessed for apoptosis by PI DNA staining, Annexin V staining and PARP cleavage. Caspase 3 activity was assessed by western blotting and inhibition of apoptosis with the zVAD.fmk pan-caspase inhibitor. Clonogenic assays assessed the ability of the cells to recover following IAP knockdown and ID-1 protein expression was assessed by western blotting as a marker of proliferation.
Results: Triple knock of the IAP only sensitised for TRAIL induced apoptosis in the PC-3 cells with corresponding increases in caspase activity and PARP cleavage which was inhibited by ZVAD.fmk. Individual knock down of the IAP has no significant effects. Triple knock down alone decreases clonogeneic survival of the PC-3 cells which was correlated with a decrease in ID-1 expression.

Conclusions: Triple knock down of cIAP-1, cIAP-2 and XIAP not only sensitised the androgen independent prostate cancer cells line, PC-3, to TRAIL induced apoptosis but also altered their proliferation rates mediated via a decrease in ID-1 expression. The inability of IAP knock down to alter type 2 triggers of apoptosis demonstrates a maintained mitochondrial staibility. IAP knock down may facilitate the bodies natural immune-surveillance mechanisms to counter cancer progression by receptor mediated apoptosis but also therapeutic approaches with TRAIL. Overcoming cancer cell resistance to therapeutic approaches represents an important combined treatment strategy.

308 POSTER

Synergistic anti-tumour activity of oncolytic Reovirus and cisplatin in a B16.F10 mouse melanoma model

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Background: Reovirus type 3 Dearing (RV) has demonstrated oncolytic activity in numerous in vitro systems, in vivo murine models and early clinical trials, In this study, we examine the in vitro and in vivo oncolytic activity of RV against the mouse melanoma cell line B16.F10 in combination with cisplatin (CP), a pseudoalkylating chemotherapeutic which causes DNA cross-linking and is active in a wide range of cancers.
Material and Methods: The effect of RV and CP was assessed in vitro for synergistic tumour kill and mechanism of tumour death. For in vivo evaluation, subcutaneous B16.F10 tumours in C57Bl/6 mice were treated with intratumoural RV and intraperitoneal CP either alone or in combination. Tumour volume was estimated thrice weekly. Tumours and organs were harvested post-treatment for viral retrieval and histology; serum samples were tested for induction of neutralising anti-reovirus antibody (NARA).
Results: A synergistic interaction (combination index value (CIV) of less than one) was observed between RV and CP (CIV: ED50 0.42±0.03; ED75 0.30±0.02; ED90 0.24±0.01). Flow cytometric analysis showed a marked increase in apoptotic cells following combined exposure, compared to single agent exposure. Reduced tumour growth and extended median survival time was observed in mice treated with RV/CP combination therapy compare to single agent treatments. Mean relative tumour volumes ±SD day 12 – Control all reached endpoint, RV alone 8.92±6.94, CP alone 9.87±2.80, RV plus CP 3.86±2.24. Median survival (days) – Control 6, RV 12, CP 8, Combination 17.
Live virus was recovered from the tumours of all RV only treated animals and from the liver and heart of 1/6. In contrast live virus was detected in only 50% of tumours from combination treated mice but in the liver of 4/6 mice. CP did not affect the NARA response to RV.
Conclusions: Taken together, these results indicate that the addition of chemotherapeutic agents can significantly enhance the anti-tumour efficacy of RV therapy and justify formal clinical evaluation.

Her

309 POSTER

Fully human anti-HER3 mAb U3-1287 (AMG 888) demonstrates unique in vitro and in vivo activities versus other HER family inhibitors in NSCLC models

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Background: HER3 is a member of the Human Epidermal Growth Factor Receptor (HER) family and is an important component of HER family driven tumorigenesis. Though HER3 lacks intrinsic kinase activity, it is a scaffold for PI3K/AKT signaling for the HER family via heterodimeric interactions. HER3 signaling may be a resistance mechanism for EGFR and HER2 inhibitors. We report unique in vitro and in vivo activities of U3-1287 (AMG 888), the first fully human Anti-HER3 mAb vs. current HER family inhibitors in NSCLC models. Combinations with EGFR inhibitors are also explored.
Methods: To determine the inhibition of HER3 oncogenic signaling, A549, Calu-3 and H1975 NSCLC cells were treated with up to 10 μg/ml of U3-1287 (AMG 888), C225 (Anti-EGFR), c2C4 (Anti-HER2) or control mAbs 1 hour prior to heregulin-beta (HRG) or vehicle stimulation. Since HER3 is a heterodimerization partner for HER family members, U3-1287 (AMG 888) was combined with EGFR inhibitors in vitro. To determine in vivo efficacy, mice bearing ~200 mm^3 A549 NSCLC xenografts were treated 2×/week with anti-HER or control Abs. A549 xenograft tumors were analyzed for the inhibition of pHER3 by Western blotting. The anti-tumor effects of U3-1287 (AMG 888) with an EGFR inhibitor was tested in the Calu-3 and H1975 NSCLC xenograft models.
Results: Treatment with U3-1287 (AMG 888) resulted in an inhibition of ligand-induced pHER3, basal pHER3 and pAkt in A549, Calu-3 and H1975 NSCLC cell lines, respectively. In NCI-H1975 cells, combining U3-1287 (AMG 888) with the anti-EGFR mAb C225 resulted in greater pHER3 and pAkt inhibition in vitro than with either single agent alone. Administration of U3-1287 (AMG 888) resulted in tumor stasis in the (EGFR TKI resistant) A549 NSCLC xenograft model vs control and other HER mAbs and tumor inhibition in Calu-3 and NCI-H1975 xenografts compared to IgG treated mice ($p < 0.05$). Combinations with the anti-EGFR mAb C225 resulted in tumor growth inhibition that was greater than either single agent alone in CaLu-3 ($p < 0.001$) and NCI-H1975 ($p < 0.001$) xenografts.
Conclusions: U3-1287 (AMG 888) inhibits basal and ligand-induced HER3 oncogenic signaling in NSCLC cell lines in vitro and basal pHER3 in vivo. NSCLC xenografts are sensitive to U3-1287 (AMG 888) treatment as single agent or in combination with an anti-EGFR mAb, including an EGFR TKI resistant model. These data provide preclinical evidence for the potential clinical application of U3-1287 (AMG 888) in NSCLC.

310 POSTER

ERBB2/HER2 proteasome–lysosome trafficking and degradation directed by polyubiquitination topology

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Activity of the overexpressed ERBB2/HER2 receptor tyrosine kinase is related to its cell surface recycling and ability to evade intracellular degradation. Attenuated signaling and receptor degradation may be induced by trafficking that directs internalized receptor to either the ubiquitin-proteasome or lysosome-vacuolar compartments. Ubiquitin covalently links to the epsilon-amine of lysine (K) in target proteins and to one or more of seven K residues in protein-linked ubiquitin, forming branched polyubiquitin chains with different structural topologies capable of directing intracellular trafficking and fate of the tagged protein. In contrast to other receptor tyrosine kinases that are monoubiquitinated and targeted for lysosomal decay, ERBB2 is thought to be ubiquitinated with K-48 branched chains directing its proteasomal degradation. Immunblotting and confocal imaging have recently shown that when the proteasome is inhibited by PS341 (bortezomib) in ERBB2 overexpressing SKBR3 breast cancer cells, total and surface localized ERBB2 receptor decline within 12−24 h and ERBB2 accumulates in a perinuclear compartment having lysosomal characteristics (e.g. LAMP1/2-positive) and ubiquitin immunoreactivity. Polyubiquitinated ERBB2 appears in concert with gain of Hsp70 and loss of Hsp90 chaperone proteins co-associating with the receptor. Co-treatment of cells with lysosome inhibitors (chloroquine, CA-074-Me) has no effect on ERBB2 receptor trafficking but prevents PS341-induced decline in total ERBB2. To explore the role of K-specific polyubiquitination in targeting ERBB2 for proteasomal and/or lysosomal decay, we developed a multiple reaction monitoring (MRM) mass spectrometry (MS) procedure using a 4000 QTRAP to measure relative levels of endogenous K-48, K-63 and K-29 branched polyubiquitin linked to undegraded ERBB2 receptor immunoprecipitated from SKBR3 cells. MRM-MS analysis showed a rapid rise in K-48 ubiquitinated ERBB2 within 4 h of bortezomib treatment, reaching a 16.5-fold increase by 10 h. In a more delayed fashion, K-63 and K-29 ubiquitinated ERBB2 increased 40-fold and 10-fold, respectively, after 10 h of proteasome inhibition. These changes in receptor trafficking and polyubiquitin chain topology suggest that the accumulation of undegraded K-48 ubiquitinated ERBB2 triggers formation of K-63 and K-29 ubiquitinated ERBB2, redirecting receptor to an alternative degradation pathway within the lysosome.

311 POSTER

In vivo antitumor efficacy of TAK-285, a novel ErbB1/ErbB2 dual kinase inhibitor

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Background: HER2 and EGFR are promising targets for effective anticancer drugs. TAK-285, a novel HER2/EGFR dual kinase inhibitor is currently in clinical trials in the United States and Japan. In this study the in vivo efficacy of TAK-285 is demonstrated.
Methods: In vivo antitumor efficacy were examined using xenografts of BT-474 or 4−1ST, which express aberrant levels of HER2. A431, which overexpresses EGFR, was also used. TAK-285 was orally administered twice a day and efficacy was determined by: (growth volume of treated