3.0. Flavopiridol highly reduced the expression of Ku70 protein, a major participant of DNA repair, as well as Ku-DNA end binding activity, in the nucleus. Gamma-H2AX foci analysis showed that the foci in cells treated with radiation only (2 Gy) can be visualized for 4 h following radiation after which their number rapidly declined, the foci in cells exposed to flavopiridol and radiation were present for 24 h after radiation, indicating the prolonged presence of radiation-induced DNA damage in flavopiridol-treated cells.
Conclusions: Treatment with flavopiridol strongly enhanced sensitivity of Seg-1 esophageal adenocarcinoma cells to radiation, involving inhibition of DNA repair as an underlying mechanism. These findings suggest that flavopiridol has the potential to increase the efficacy of radiotherapy for esophageal cancer.
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502 POSTER

Small inhibitory DNA (siDNA) enhancing tumor sensitivity to radiotherapy by baiting DNA-PK repair proteins

J. Sun[1], N. Berthault[2], S. Gay[2], M. Roy[2], C. Roulin[2], C. Alberti[3], X. Sastre-Garau[4], J. Cosset[5], L. Larue[3], M. Dutreix[2]. *[1]DNA Therapeutics SA, Evry, France; [2]UMR2027 CNRS-Institut Curie, Section Recherche, Orsay, France; [3]UMR146 CNRS-Institut Curie, Section Recherche, Orsay, France; [4]Institut Curie, Department of Pathology, Paris, France; [5]Institut Curie, Department of Radiotherapy, Paris, France*

Background: Radiotherapy, used alone or in association with surgery and/or chemotherapy, remains in 2006 one of the main anticancer therapies. Unfortunately, the cumulative toxicity of the combined therapies frequently limits their efficacy. In this work, we present a novel strategy to enhance the effect of radiotherapy on radioresistant tumors without directly increasing damage to genetic material.
Materiel and Methods: Small inhibitory DNA (siDNA) molecules – called Dbait – that mimic DNA double-strand breaks (DSB) were designed and synthesized. They were tested for their ability to interfere with various functions of DNA-PK in cell extracts and in transfected cells with the goal of inhibiting the DSB repair pathway in irradiated tumors and so promoting tumor regression. The efficacy of these molecules in sensitizing tumor cells to irradiation was evaluated in nude mice xenografted with several radioresistant human tumor cell line.
Results: In vitro, Dbait specifically activates DNA-PK's kinase activity and inhibits non-homologous recombination and DNA repair by non-homologous end joining (NHEJ), thereby increasing cell death in response to irradiation. The requirements for Dbait activity were similar in all the assays (activation of the protein kinase, inhibition of DNA fragment ligation in a cell-free assay, inhibition of plasmid integration and enhanced sensitivity to γ-irradiation in cultured cells). We found that the optimal Dbait molecule was a double-stranded DNA, at least 32-bp long and with at least one free end. The sequence had no influence on the activities tested indicating that the effects of Dbait were due to the molecular structure as a substrate mimetic rather than to targeting of a specific sequence. In vivo, a combination of Dbait treatment and radiotherapy induces regression of tumors in nude mice xenografted with various radioresistant tumors, in a dose-dependent manner.
Conclusion: The use of siDNA as a DSB substrate mimetic which baits and hijacks the enzyme complexes that repair DSBs is a novel and original pathway-targeting approach. This work provides evidence of a potential new adjunct molecular therapy to radiotherapy for treating radio-resistant malignant tumors. Further work is required to confirm whether our DNA bait strategy presents a paradigm shift from single gene/protein targeting to multi-gene/protein targeting (pathway), in order to fight against treatment-resistant cancer.

503 POSTER

Phase I pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of an oral small molecule inhibitor of Poly ADP-Ribose Polymerase (PARP), Ku in patients (p) with advanced tumours

J.S. de Bono[1], P.C. Fong[1], D. Boss[2], J. Spicer[1], M. Roelvink[2], A. Tutt[3], P. Mortimer[4], M. O'Connor[4], J.H.M. Schellens[2], S.B. Kaye[1]. *[1]Royal Marsden Hospital, Drug Development Unit, London, UK; [2]Netherlands Cancer Institute, Medical Oncology, Amsterdam, Netherlands; [3]Institute of Cancer Research, Breakthrough Breast Cancer, London, UK; [4]Kudos Pharmaceuticals, Cambridge, UK*

Background: PARP is a DNA strand break and damage repair enzyme. Ku inhibits PARP-1 with a mean IC50 of 2 nM. Inhibition of PARP leads to defective DNA repair and induces selective cytotoxicity in cells with defective homologous recombination through, for example, loss of BRCA 1/2 function (Farmer et al, Nature 2005; 434(7035): 917−21). This is a first in human Phase I trial of Ku. PD studies included functional evaluation of PARP-1 activity in surrogate and tumor tissue.
Methods: Ku was administered daily for 14 of every 21 days to p with advanced solid tumors refractory to standard treatment. Cohorts of 3–6 p were treated, with a starting dose of 10 mg/day. The dose was doubled in the absence of drug related grade 2 CTC-AE toxicity. Drug related toxicity in cancer patients known to carry a BRCA mutation is being compared to toxicity in other patients.
Results: To date 21 p (mean age 55y [25−82y; 12 females]) with solid tumours have received 54 courses (range 1–8). 3 of these p have either a known BRCA mutation (2 p) or a strong family history suggesting BRCA mutation (1 p; refused BRCA testing). Dose levels evaluated to date include 10, 20, 40, and 80 mg once a day; and then 60 and 100 mg twice a day. No dose limiting toxicity has been reported with only grade 1 drug related toxicity being observed to date. PK support dose proportionality with a mean elimination half-life of 6.7 hours (Range: 6.3–6.9), a mean clearance of 4.37 L/h (Range: 3.1−6.3) and a mean volume of distribution of 41.0 L (Range: 29.5−60.6). PD studies indicate inhibition of PARP functional activity in peripheral blood mononuclear cells with increasing inhibition observed with increasing dose of Ku. Initial studies in tumor biopsies performed pre-treatment and on day 8 revealed PARP inhibition of around 50% at doses above 40 mg/day. A p with metastatic ovarian carcinoma, with previously platinum-responsive disease but became platinum-resistant later and a strong family history suggesting BRCA mutation has had an objective partial response by RECIST criteria with a CA125 fall of >70%. Two p having soft tissue sarcoma and renal carcinoma respectively and progressing disease pretreatment achieved stable disease for 24 weeks.
Conclusions: Dose escalation continues with more BRCA carriers planned. PARP inhibition in both surrogate and tumor tissue is achievable with minimal toxicity in cancer patients, and has not resulted in any short-term toxicity difference in BRCA mutation carriers.

504 POSTER

Centrosome abnormalities occur early and coexist with genomic instability during cancer progression in Barrett's esophagus

Y. Yang, I. Ghiran, H. Shields, S. Xiang, J. Fruehauf, M. Upton, T. LaMont, C. Li. *Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA*

Centrosomes play important roles in processes that ensure proper segregation of chromosomes and maintain the genomic stability of human cells. Centrosome defects have been found in aggressive carcinoma of multiple origins. The contribution of centrosome defects to esophageal adenocarcinoma (EadCA) and its precursor Barrett's esophagus (BE) has not been evaluated. We have previously shown that genomic instability (GIN) precedes alterations in tumor suppressor p53 and APC in BE-associated tumorigenesis. The aim of this study was to determine centrosome alterations during cancer progression in BE.
We analyzed specimens from endoscopic biopsies or esophagectomies in patients with BE (10 cases) or with BE-associated esophageal adenocarcinoma (10 cases), with normal gastro-esophageal junction (5 cases) as controls. A mouse monoclonal γ-tubulin antibody or a rabbit polyclonal pericentrin antibody was used for centrosome staining. Chromosomal enumeration probe Cep 7, 11, 12, 17 and 18 were detected by fluorescence in situ hybridization (FISH). In normal controls, centrosomes appeared uniform in size. In contrast, centrosomes showed structurally and numerically abnormal in the majority (90%) of EadCA. In pre-cancerous lesions, centrosome abnormalities were observed in 57% of non-dysplastic Barrett's epithelium, 67% of low-grade dysplasia (LGD), and 83% of high-grade dysplasia (HGD), respectively. Interestingly, centrosome abnormalities coexisted with GIN.
These results, for the first time, demonstrate that centrosome abnormalities occur early and coexist with GIN during cancer development and progression in BE. These findings suggest that the centrosome may be a biomarker for predicting patients at risk for cancer and a potential therapeutic target.

Formulation research

505 POSTER

Improved effectiveness of nab-paclitaxel versus docetaxel in various xenografts as a function of HER2 and SPARC status

N. Desai[1], V. Trieu[1], M. Hawkins[1], P. Soon-Shiong[1]. *[1]Abraxis BioScience, Inc., Santa Monica, CA, USA*

Background: Docetaxel (Taxotere®) showed improved survival and time to progression over paclitaxel (Taxol®) in a randomized phase 3 study in metastatic breast cancer, but toxicity was greater for docetaxel *[Jones,*

JCO 2005;23:5542]. *nab*-Paclitaxel (Abraxane®; ABX) showed significantly higher response rates and greater safety than Taxol *[Gradishar, JCO 2005;23:7794]*. This study compared the safety and efficacy of docetaxel (TAX) and ABX in the preclinical setting.
Methods: Overall toxicity of TAX and ABX was compared in a dose-ranging study in nontumored athymic mice (8/group) at doses of 0, 7, 15, 22, 33, and 50 mg/kg for TAX and 0, 15, 30, 60, 120, and 240 mg/kg for ABX. Antitumor activity was compared in MX-1 breast carcinoma xenograft at equidose (15 mg/kg qwkx3), and in LX-1 lung, PC3 prostate, HT29 colon, and MDA-MB-231 breast at 15 mg/kg for TAX and 50 and 120 mg/kg for ABX q4dx3 (saline as control). HER2 and SPARC status were determined by immunohistochemistry using a monoclonal antihuman HER2 antibody and a polyclonal antihuman SPARC antibody (scored 0 [neg] to 4 [strong pos]). HER2 status also was confirmed by RT-PCR.
Results: ABX was nontoxic (no appreciable wt loss) up to 120 mg/kg; toxicity was observed at 240 mg/kg. The maximum tolerated dose (MTD) of ABX was 120–240 mg/kg. TAX showed dose-dependent weight loss at 15−50 mg/kg, with MTD (~20% wt loss) at 15 mg/kg. In HER2-negative xenografts (LX-1 and MX-1), ABX was superior to TAX. For MX-1, ABX 15 mg/kg (ABX15) was more effective than TAX15 ($P < 0.0001$), with tumor growth inhibition (TGI) of 79.8% and 29.1%, respectively. For LX-1, both ABX120 (TGI 98%) and ABX50 (TGI 84%) were superior to TAX15 (TGI 61%) ($P < 0.0001$ and $P = 0.0001$, respectively). In HER2-positive xenografts (HT29, PC3, and MDA-MB-231), ABX efficacy relative to TAX increased with increasing SPARC expression. For HT29 (high SPARC expressor), both ABX120 (TGI 65%) and ABX50 (TGI 50%) were superior to TAX15 (TGI 36%) ($P < 0.0001$ and $P = 0.006$, respectively). For PC3 (medium expressor), ABX120 (TGI 99%) was equivalent to TAX15 (TGI 97%) (P=ns), and ABX50 (TGI 94%) was less effective than TAX15 ($P < 0.0001$). For MDA-MB-231 (low expressor), both ABX120 (TGI 99%) and ABX50 (TGI 94%) were less effective than TAX15 (TGI 97%) ($P < 0.0001$ for each).
Conclusion: In 4 of the 5 xenograft tumors, ABX was equally or more effective at sub-MTD (120 mg/kg) than TAX at its MTD (15 mg/kg). Effectiveness of ABX was influenced by HER2 and SPARC status.

Hormonal agents

506 POSTER

Increased sensitivity of ERbeta-expressing MCF-7 breast cancer cells to histone deacetylase inhibitors (HDACi)

D. Behrens[1], J.H. Gill[2], I. Fichtner[1]. *[1]Max-Delbrueck-Center for Molecular Medicine, Experimental Pharmacology, Berlin, Germany; [2]Institute of Cancer Therapeutics, Molecular Pharmacology Group, Bradford, United Kingdom*

Background: Estrogen receptors (ER) regulate growth of normal and malignant cells of the mammary gland. Unlike ERα, which is frequently expressed and an established target for anti-hormonal therapy, ERβ appears to be a tumour suppressor protein and down-regulated in breast cancer. One class of compound currently under investigation as novel anticancer therapeutics are the histone deacetylase inhibitors (HDACi). Histone deacetylases are zinc-containing enzymes involved in modulating chromatin structure and subsequent gene expression. Inhibition of HDAC activity in tumour cells has been shown to be anti-tumourigenic and results in expression of numerous tumour suppressor genes. The objectives of this study were to evaluate whether HDAC inhibition influenced expression of ERα and ERβ, whether ER expression influenced response to HDACi and whether the effects of HDACi upon ER could be attributed to inhibition of certain HDAC subtypes.
Methods: The effect of the trichostatin-A (TSA; pan-HDAC inhibitor) and MS-275 (class-I selective HDACi) was evaluated in the MCF-7 breast tumour cell line (ERα positive, ERβ negative) and its counterpart MCF-7/ERb expressing stably transfected ERβ. Effects of HDACi treatment on proliferation of these cell lines was assessed by MTT assay. Quantitative real-time RT-PCR (qRT-PCR) was utilised to examine the effect of HDACi treatment upon ERα and ERβ expression in these cells.
Results: Expression of ERβ resulted in an about 10-fold increase in sensitivity of MCF-7 to both TSA and MS-275 compared to mock-transfected and wild-type MCF-7 cells. Both TSA and MS-275 induced re-expression of ERβ in the wild-type MCF-7 cells as shown by qRT-PCR. Treatment with 0.1 μM drugs resulted in a 17-fold and 2-fold increase in ERβ mRNA levels with MS-275 and TSA, respectively. Higher concentrations of drugs (1 μM) were also observed to down-regulate ERα mRNA levels.
Conclusion: These data suggest that HDAC inhibition may induce re-expression of ERβ in tumours and as such be a valid treatment for those tumours unresponsive to conventional anti-hormonal therapy. Furthermore, the differential response to TSA and MS-275 may suggest class-I HDAC as one of the molecular targets responsible for ERβ re-expression.

507 POSTER

Reactive oxygen species modulate the phosphorylation status of estrogen receptor alpha

G.E. Weitsman, L.C. Murphy. *Manitoba Institute of Cell Biology and University of Manitoba, Dept. of Biochemistry & Medical Genetics, Winnipeg, Canada*

Background: Estrogen receptor α (ERα) is a well-known target for signaling pathways originating from growth factor receptors. Reactive oxygen species (ROS) induced activation of extracellular response kinase 1/2 (ERK1/2) and protein kinase B (Akt) by epidermal growth factor receptor (EGFR) depends on oxidation of essential cysteines in the active sites of protein tyrosine phosphatase 1B and PTEN (phosphatase and tensin homolog). It has been shown that both kinases can be involved in the phosphorylation of serine 118 (Ser118) and 167 (Ser167) on ERα, respectively. This activity may lead to ligand-independent activation of ERα, downregulation of ERα and may contribute to development of the resistance to anti-estrogen therapy.
Material and Methods: MCF-7 human breast cancer cells after incubation for 6 days in medium supplemented with charcoal-treated serum were treated with glucose oxidase (GO, 0.1 un/ml). Cells were harvested at different time points after an addition of GO and expression of ERα phosphorylated at Ser118 and Ser167 was detected by western blot analysis. Selective inhibitors of ERK1/2 (U0126) and Akt (LY294002) upstream kinases, were used to assess the role of these kinases in phosphorylation of Ser118 and Ser167.
Results: GO treatment induced transient phosphorylation of Ser118 and Ser167 peaking at 90 minutes. The increase in expression of p-S118-ERα was 475% ± 282% and of p-S167-ERα was 998% ± 580% (mean ± SD, N = 4). ERα expression declined with time, resembling the effect of treatment with estrogen. After GO treatment the phosphorylation levels of Ser118 in MCF-7 cells overexpressing Her2 were significantly higher than in control non-Her2 expressing cells suggesting involvement of modulation by Her2. Activation of ERK1/2 and Akt was transient with highest levels observed at 90 and 60 minutes after GO, respectively. Inhibition of ERK1/2 by U0126 (10 uM) decreased the p-Ser118 by 51.7±8.5% (mean ± SD, N = 3) and surprisingly our preliminary data suggest that LY294002 had little if any effect on p-ser167 expression.
Conclusions: Our data show for the first time that ROS can induce post-translational modifications of ERα at Ser118 and Ser167 in human breast cancer cells. Activated ERK1/2 is involved in the phosphorylation of Ser118. Both the phosphorylation and consequent downregulation of ERα may be a mechanisms associated with development of anti-estrogen resistance.

508 POSTER

Development and evaluation of dual aromatase and sulfatase inhibitors with therapeutic potential

S.K. Chander[1], P.A. Foster[1], M.F.C. Parsons[1], H. Tutill[1], L.W.L. Woo[2], B.V.L. Potter[2], M.J. Reed[1], A. Purohit[1]. *[1]Imperial College London, Endocrinology and Metabolic Medicine and Sterix Ltd, London, United Kingdom; [2]University of Bath, Medicinal Chemisitry, Department of Pharmacy and Pharmacology and Sterix Ltd, Bath, United Kingdom*

In postmenopausal women estrogens can be formed in peripheral tissues from androstenedione, by the aromatase enzyme, or from estrone sulfate by the action of steroid sulfatase. Separate inhibitors of aromatase and steroid sulfatase have been developed but the development of dual aromatase and steroid sulfatase inhibitors (DASIs) offers a novel approach to effectively ablating the synthesis of estradiol in the treatment of hormone-dependent breast cancer. Our group has synthesized DASIs by sulfamoylating the phenolic derivatives based on known aromatase inhibitors. In this study we report on the *in vitro* and *in vivo* evaluation of these DASIs. *In vitro* studies used JEG-3 cells to determine IC_{50} values for both aromatase, using [1β-^{3}H] androstenedione as substrate, and steroid sulfatase inhibition, using [^{3}H] estrone sulfate as substrate. *In vivo* evaluation involved the use of intact female Wistar rats that were primed with 200IU s.c. of pregnant mares serum gonadotrophin (PMSG) to stimulate ovarian aromatase activity. Three days later rats were orally dosed with DASI compounds at 10 mg/kg. Three hours later rats were culled (under terminal anaesthesia) and samples of blood and liver taken for analysis. Plasma estradiol levels were determined by RIA as an indicator of aromatase inhibition and steroid sulfatase inhibition was measured using liver tissues. *In vitro*, the IC_{50} values for inhibition of aromatase activity ranged from 0.5 to 105 nM with values for steroid sulfatase ranging from 5.5 to 360 nM. Using the PMSG model to test the ability of DASIs to inhibit enzyme activities *in vivo* potent inhibition of both aromatase (75–100%) and steroid sulfatase (91–100%) was detected with derivatives from all aromatase inhibitor classes. Having identified potent DASIs that are active *in vivo* it will be possible to test their efficacy in an appropriate xenograft