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.
This work was supported by Esophageal Cancer MRP grant, The University of Texas M.D. Anderson Cancer Center and Aventis Pharmaceuticals.

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,*