expression can confer resistance to multiple natural product drugs, it has not been shown to be associated with thechemotherapeutic resistance of ovarian cancer. Using anonymized specimens fromthe Gynecologic Oncology Group (GOG) Tumor Bank, we have observed that the ABCC1 gene is overexpressed in pre-treatment ovarian cancer, compared to matched-normal ovarian epithelium, and we have identified a number of splice variants ofthis gene that appear to be uniquely expressed in these tumor specimens,suggesting that they may play some role in the therapeutic insensitivity ofovarian cancer. In support of this idea, we have also seen such splice variantsin a leukemic cell line selected for resistance to teniposide, andcross-resistant to etoposide, a drug used to treat ovarian cancer. (Supportedin part by grants from the National Cancer Institute [to WTB] and in part by theGOG [the Core Lab in Molecular Pharmacology, to WTB])

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AKT as a target for enhancing breast cancer chemotherapy and radiotherapy

Z. Fan[1], L. Ke[1], C. Knuefermann[1], M. Schmidt[1], W. Jin[1], Y. Lu[1], K. Ang[2], L. Milas[3]. [1]*Experimental Therapeutics,* [2]*Radiation Oncology,* [3]*Experimental Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, USA*

The phosphatidylinositol-3 kinase (PI-3K) pathway, regulated by its upstream oncoproteins such as the growth factor receptors with tyrosine kinase activity or Ras, play a critical role in promoting cancer cell proliferation and inhibiting cancer cell death. Akt, the cellular homologue of the viral oncogene v-akt, is an important mediator of such effects of the PI-3K. However, it has not been established whether increased activity of Akt could directly render breast cancer cell resistance to chemotherapy or radiotherapy. In this study, we demonstrated a causal role of Akt in conferring resistance to chemotherapy- and radiotherapy-induced apoptosis on MCF7 human breast cells. MCF7 cells were stably transfected with a farnesylated Akt expression vector, which we demonstrated was constitutively active. We assessed the effect of the farnesylated Akt on the sensitivity of MCF7 cells to several chemotherapeutic agents that are currently used for breast cancer patients, and on the sensitivity of MCF7 cells to radiotherapy as well. Compared with control vector-transfected MCF7 cells, MCF7 cells expressing farnesylated Akt (MCF7Akt-farn) showed significantly greater resistance to the cytotoxic effects mediated by paclitaxel, doxorubicin, etoposide, 5-fluorouracil, or camptothecin, and showed a markedly increased clonogenic survival rate following 5 Gy irradiation (from 7.3% in MCF7 control vector-transfected cells to 16.9% in the constitutively active MCF7Akt-farn cells). We next examined the effects of inhibiting the PI-3K pathway with the specific inhibitor LY294002 on MCF7 cells that were stably transfected with HER2 or the constitutively active RasG12V mutant; both showed PI-3K-dependent (LY294002-sensitive) increase in Akt activity. Compared with control vector transfected cells, MCF7HER2 or MCF7RasG12V cells showed increased resistance to these chemotherapeutic agents and to gamma irradiation. Co-treatment of these MCF7HER2 or MCF7RasG12V cells with LY294002 markedly inhibited Akt activity, and sensitized these transfectant cells to the treatment with chemotherapeutic agents or with radiotherapy. Our results suggest that Akt plays an important role in conferring resistance to conventional chemotherapy and radiotherapy on breast cancer cells and therefore may be a target for improving the therapeutic outcome of breast cancer treatments.

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Rapamycin, an inhibitor of mTOR, reverses chemoresistance in PTEN negative prostate cancer xenografts

V. Gruenwald, M. Hidalgo. *The Sidney Kimmel Comprehensive Cancer Center at J, Experimental Therapeutics, Baltimore, USA*

PTEN is a lipid phosphatase with tumor suppressing abilities, which is frequently mutated or deleted in many different cancers. Loss of PTEN leads to activation of the PI3k/Akt pathway, which promotes cellular survival and has been associated with chemoresistance. We showed in previous studies that resistance to doxorubicin in prostate cancer cells is conferred by loss of PTEN/activation of Akt, and that the mTOR inhibitor rapamycin reverses this chemoresistance upon co-treatment in PTEN negative prostate cancer cells (Proc. Am. Assoc. Cancer Res. 2002; vol. 43: Abstract 4703). In the current study we aimed to determine the *in vivo* effects of rapamycin on response to treatment with doxorubicin in the PTEN negative prostate cancer cell line PC-3. Nudemice were inoculated with PC-3 xenografts and treated when tumors reached 200 mm3 with CCI-779 (10 mg/kg d1-5 i.p.), an ester derivative of rapamycin currently in clinical development, doxorubicin (10 mg/kg d1 i.v.), or a combination of both compounds at the same dose levels and schedules. Response data are now available at 2 weeks of follow up. So far, doxorubicin achieved a tumorstatic effect, whereas CCI-779 showed a 40% tumor reduction, and the combination therapy yielded a 50% decrease in tumor volume. Tumor growth reoccurred for mice treated with CCI-779 alone on day 11, whereas tumor volumes for the combination therapy arm remain low. The study is still ongoing and complete data will be presented at the meeting, including molecular analyses of effectors of the PI3k/Akt pathway of the treated tumors. This encouraging data will expectantly provide the rational to explore in clinical trials whether CCI-779 increases the response to chemotherapy of patients with PTEN negative/Akt active prostate cancers.

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JNK and p38 MAP kinases potentially contribute to tamoxifen resistance of breast cancer via direct phosphorylation of both estrogen receptor and AIB1 coactivator

J. Shou[1,2], J. Wong[2], N. Weigel[2], C. Osborne[1,2], R. Schiff[1,2]. [1]*Baylor College of Medicine, Breast Center, Houston, USA;* [2]*Baylor College of Medicine, Dept. of Molecular and Cellular Biology, Houston, USA*

De novo and acquired endocrine resistance is a major clinical problem in management of breast cancer. Using an *in vivo* xenograft model of breast cancer endocrine resistance, we have recently shown that the development of resistance to tamoxifen (TamR) and to prolonged estrogen withdrawal (-E2R) is associated with cellular stress and increased levels of the stress-related kinases JNK and p38 MAPK. Increased JNK has also been documented in clinical TamR tumors. We therefore hypothesize that JNK and p38 MAP kinases are important determinants in the process of acquiring resistance, presumably through activation of the estrogen receptor (ER) pathway by phosphorylation of both ER and its coactivators. We found, using *in vitro* kinase assays, that all forms of p38 MAPK (alpha, beta, gamma and delta) phosphorylate both ER alpha and ER beta, and that the p38 alpha- and beta-induced phosphorylation of ER can be inhibited by the p38 specific inhibitor SB203580. Using truncated mutants of ER alpha we found that p38 phosphorylates the AF1 domain of the receptor. The ER coactivator AIB1 is often amplified and overexpressed in breast tumors, and we have recently found that AIB1 is an important component of TamR found in Her2-overexpressing tumors. Interestingly, we found that both JNK and p38 MAPK can directly phosphorylate AIB1 *in vitro*. Thus, as has been suggested for growth factor signaling, AIB1 may also be a conduit for kinase-mediated stress signaling to the ER pathway. Our data suggests that increased active JNK and p38, and cross-talk between these pathways and the ER pathway, may play a key role in endocrine resistance through phosphorylation and activation of different components of the ER pathway. We are currently studying whether specific JNK and p38 inhibitors can circumvent endocrine resistance *in vivo* in our xenograft breast cancer model.

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Oligonucleotide chip analysis reveals distinctive gene expression patterns in Tam-sensitive and -resistant human mammary carcinoma xenografts

M. Becker[1], A. Sommer[2], J. Krätzschmar[2], H. Seidel[2], H.-D. Pohlenz[2], I. Fichtner[1]. [1]*Max Delbrueck Center for Molecular Medicine, Experimental Pharmacology, Berlin, Germany;* [2]*Schering AG, Research Laboratories, Berlin, Germany*

Aim of study: The mechanisms by which human mammary tumors fail to respond to Tamoxifen (Tam) therapy are mainly unknown. We undertook a comparative gene expression analysis of a Tam-sensitive and -resistant human breast cancer *in vivo*-model to identify molecular targets being involved in Tam resistance.
Methods: Originating from a Tam-sensitive human mammary carcinoma xenograft (MaCa 3366) we successfully established the Tam-resistant model MaCa 3366/TAM by treatment of tumor-bearing nude mice with Tam for 3 years during routine passaging. Samples from both tumor lines were used for comparative analysis. The 5 treatment groups were: MaCa 3366 and MaCa 3366/TAM each supplemented with E2, with E2 plus short-term treatment with TAM, and MaCa 3366/TAM under permanent Tam treatment. Total RNA from the tumor tissues was pooled per group and hybridized to Affymetrix HuGeneFL chips interrogating approximately 7000 human genes and ESTs. Pairwise comparisons and clustering algorithms were used to identify differentially expressed genes and patterns of gene expression, respectively.

Results: As revealed by cluster analysis the Tam-sensitive and -resistant breast carcinoma line differed regarding their gene expression pattern. When comparing the groups, the expression of relatively few genes was distinctly changed. For example, in the resistant MaCa 3366/TAM 45 genes were up-regulated and 19 genes down-regulated more than two fold compared to the Tam-sensitive MaCa 3366. Among the up-regulated genes are several interferone inducible genes as well as genes that are known to be involved in breast cancer. To further evaluate interesting candidates we developed RT-PCRs for several genes and found confirmation between fold regulation as measured by RT-PCR on unpooled RNAs and in the chip experiments for the different treatment groups.
Conclusion: Recent studies reveal that breast cancer prognosis can be correlated with the gene expression pattern of the primary tumor. Our investigations provide the possibility to derive markers for Tam resistance by differential gene expression profiling in a human breast cancer model of acquired Tam resistance. Genes whose expression is distinctly changed between both lines will be further evaluated as potential targets for diagnostic or therapeutic approaches.

Radiation interactive agents

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Prospective phase I/II trial of the cyclooxygenase-2 inhibitor celecoxib in patients with carcinoma of the cervix

A. Fyles, P. Chan, M. Milosevic, A. Oza, A. Syed, S. Chin, M. Pintiie, M. Levin, L. Manchul, C. Doll. *Princess Margaret Hospital, Radiation Oncology, Toronto, Canada*

Purpose: To evaluate the toxicity and biomarker response of celecoxib as a biologic agent in combination with definitive chemoradiotherapy in women with cervix cancer.
Materials and Methods: Fifteen patients with cervix cancer were entered into the first phase of a study between March 2001 and January 2002. FIGO stages included IB(2), IIB(8), IIIB(4), and IVA(1), and median age was 51 (range 26-62). Celecoxib 400mg orally was given twice daily 2 weeks prior to, and during the course of weekly cisplatin and definitive radiotherapy. Toxicity assessments were performed weekly up to 12 weeks following treatment with analysis of toxicity prior to further accrual. Haematological, genitourinary, gastrointestinal and skin toxicity were recorded using the NCIC-CTC. Hypoxia and interstitial fluid pressure (IFP) assays were performed prior to and 2 weeks after celecoxib (prior to radiation).
Results: Eleven patients completed the prescribed therapy with celecoxib. Only 2 of the 4 remaining patients discontinued celecoxib due to GI toxicity > 3. In total there were 6 patients (40%) with grade 3/4 acute toxicity. Four were related to nausea and vomiting, one to perineal reaction and one to neutropenic sepsis. Although it was difficult to distinguish toxicity from chemoradiotherapy from that of celecoxib, the proportion of patients with severe acute toxicity was similar to our previously reported study with chemoradiotherapy alone (8/24 or 33%, Rodrigues, Int J Radiat Oncol Biol Phys 2001:Vol 51:(3): (Supp 1): 334). GI toxicity was more common in this study whereas haematologic effects were more frequent in the previous study. Four of 10 measurable patients showed a decrease in hypoxic fraction of 3.8-89.7% while 7 of 9 measurable patients had a reduction in IFP of 2.7 to 59%. Biomarker correlation with response to treatment or outcome is not yet available.
Conclusions: Celecoxib is tolerated by patients receiving definitive chemoradiotherapy for cervix cancer. The proportion of grade 3 toxicity did not differ from our previous study but further follow-up will be required to determine late toxicity. Biomarker response to celecoxib can be seen in individual patients. Accrual continues to a planned sample size of 45 patients.

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Pifithrin alpha and beta do not inhibit ionising radiation dependent p53 responses in human wild type p53 ovarian and colon cell lines

M. Walton[1], S.C. Wilson[1], I. Hardcastle[2], P. Workman[1]. *[1]Institute of Cancer Research, Cancer Resaerch UK, Centre for Cancer Therapeutics; [2]University of Newcastle upon Tyne, Dept of Chemistry, Newcastle, UK*

Many toxic side effects of conventional chemotherapy are induced via p53-dependent apoptosis. Inhibitor of p53+/+ function may therefore have utility in protecting normal tissue and allowing increased doses of chemotherapy. The compound pifithrin alpha (PFT alpha, Science 285:1733-1737, 1999) has been reported to be an inhibitor of p53 and shown to abrogates p53+/+ dependent cell cycle arrest, cytotoxicity and apoptosis in rodent model tumour cell lines following ionising radiation (IR) and UV exposure. In view of the possible utility and drug development potential of such compounds we have carried out a detailed study of PFT alpha and its ring-closed product PFT beta in human p53+/+ tumour cell lines. Both PFT alpha and beta were synthesised and the structures confirmed by NMR-MS (accurate mass and FAB), microanalysis and crystal structure of the beta-form. PFT alpha converted rapidly to the beta-form in tissue culture medium, with a half-life of 4.9 min and 82% conversion within 30 min. Consequently drugs were made up immediately prior to use. Initial studies were carried out to determine if PFT alpha and beta would inhibit p53-dependent responses in the human colon tumour cell line HCT116 and the human ovarian tumour lines A2780 and CH1. Cells were treated with various doses of PFT alpha and beta (1-30uM) for 1h prior to IR (5Gy) and harvested 24h following exposure. In both A2780 and HCT116, p53 protein induction and G1/S cell cycle arrest were unaltered as measured by western blotting and flow cytometry, respectively. Clonogenic assays showed that there was no protective effect of PFT alpha when combined with an IC_{50} dose of IR in A2780 cells. Mice bearing CH1 ovarian tumour xenografts (p53+/+) were administered a non-toxic dose of PFT alpha (4mg kg-1 ip) 24h prior to IR (5Gy) and tumours removed 4h following irradiation. There was no evidence of inhibition of p21 or MDM-2 mRNA induction as measured by northern blotting. Studies on the mechanism of action of these compounds are in progress. These data suggest that PFT alpha and beta are not offering marked protection from p53 dependent events following ionising radiation treatment in human ovarian and colon tumour cell lines.

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Cassette dosing iv and ip of novel DNA dependent protein kinase inhibitors

F.I. Raynaud[1], N.F. Smith[1], R.J. Griffin[2], I.R. Hardcastle[2], L. Rigoreau[2], M.L. Stockley[2], J.J. Leahy[2], B.T. Golding[2], G.C. Smith[3], P. Workman[1]. *[1]Institute Of Cancer Research, Cancer Research UK Centre for Cancer Therapeutics, Sutton; [2]The University of Newcastle upon Tyne, Department of Chemistry, Newcastle upon Tyne, United Kingdom; [3]KuDOS Pharmaceuticals Limited, Cambridge, United Kingdom*

DNA-dependent protein kinase (DNA-PK) is involved in the repair of double strand breaks, thus inhibition of this enzyme could potentiate the effects of radiotherapy or chemotherapy by DNA-damaging agents. The aim of this study was to evaluate cassette dosing, the simultaneous administration of several compounds to a single animal, as a method to predict the pharmacokinetics of a series of novel DNA-PK inhibitors in high throughput. NU7053, NU7059, NU7062, NU7119 and NU7163 were administered intravenously to Balb C- mice alone at 5mg/kg and in combination at 1mg/kg each. NU7026, NU7031, NU7046, NU7048 and LY294002 were administered ip alone at 20mg/kg and in a cassette with 10mg/kg of each drug. Pharmacokinetic parameters were evaluated by non-compartmental analysis. When administered iv, the compounds displayed a linear increase in maximum concentration (Cmax) and area under the curve (AUC) with a 5-fold increase in dose from 1mg/kg cassette administration to 5mg/kg single administration. The clearance and half-lives of the compounds were similar following cassette and single dosing. For example, the clearance and half-life of NU7059 following cassette and single dosing was 0.091L/hr and 0.17hr, and 0.081L/hr and 0.16hr respectively. The rank order of the compounds from lowest to highest clearance was similar whether they were dosed as single agents. In contrast, when administered ip, the clearances varied up to 50%. For example the clearance for NU7026 decreased from 0.44L/hr after single dosing to 0.20L/hr after cassette dosing or as single agent. Cmax also varied with more than 3 fold variation for NU7046. As a result, ranking of the compounds was different after single and cassette dosing. Although the compounds used in the cassettes iv and ip were different, this suggests that absorption ip may be limiting for cassette dosing. Cassette dosing iv, however will be used to assess a larger number of compounds from this series in order to increase throughput, thus reducing the number of animals used.