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POSTER

Pre-clinical studies of concomitant taxanes and ionizing radiation therapy: local and systemic anti-tumor effectsL. Liebes¹, B. Ng¹, S. Demaria², M. Devitt³, J. Babb⁴, S. Formenti³.¹New York University Medical Center, NYU Cancer Institute, New York, USA; ²New York University Medical Center, Pathology, New York, USA;³New York University Medical Center, Radiation Oncology, New York, USA; ⁴New York University Medical Center, Radiology, New York, USA

Background: A pre-clinical breast cancer model was used to test whether a taxane combination and local radiation can induce protective anti-tumor immunity in tumor-bearing hosts. The hypothesis tested is that local treatment by ionizing radiation (RT) or concomitant chemotherapy and RT, may have systemic anti-tumor effects.

Methods: The effects of combined RT and taxanes chemotherapy used an immune competent, syngeneic murine mammary carcinoma model. On day 0, BALB/C mice were inoculated with 1×10^5 TSA murine mammary carcinoma cells (left flank, "primary" tumor, PT). On day 5, 1×10^5 TSA cells were inoculated in the right flank ("secondary" tumor, ST). Tumor-bearing mice were randomized on day 11 into 6 treatment groups: untreated control, 2Gy RT, 8 mg/kg i.v. Docetaxel \pm 2 Gy RT and 8 mg/kg i.v. Paclitaxel \pm 2 Gy RT. Each group consisted of 5 animals. The taxanes were administered intravenously IV 48hr prior to the ionizing radiation treatment. The RT was delivered at a single dose of 2 Gy on day13 exclusively to the PT. Tumor dimensions were measured every third day, over 6-weeks. A mixed model regression statistical analysis was used to assess anti-tumor effects.

Results: Both paclitaxel and docetaxel showed similar effects in slowing the growth of the irradiated PT compared with the un-treated control. The combination of RT and either taxane treatment showed additive effects on tumor growth delay when compared to either modality alone. With respect to the growth of the ST, the RT to the primary tumor did not show any effect and was comparable to that of the untreated controls. Both docetaxel and paclitaxel chemotherapy treated groups showed comparable growth delay responses of the non-irradiated tumors. The effect of left-side RT on ST growth did have a significant effect in the paclitaxel group ($p = 0.0138$). Relative to animals without radiation exposure, the animals with PT RT had lower mean tumor volumes initially but tended to grow at a faster rate, resulting in comparable mean tumor volumes for the two groups by study termination. Chemo and RT had significant additive effects on tumor growth rate and the estimated rates of increase in log tumor volume for the 3 RT treatment regimens were 0.144 (no RT), 0.106 for paclitaxel and 0.113 for docetaxel.

Conclusions: These results suggest that concurrent chemoradiation exerts an effect outside of the RT field that is superior to that of chemo alone. Supported by a grant from Aventis.

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Influence of Irinotecan and SN-38 on the irradiation response of WHO3 human esophageal tumour cells under hypoxic conditions

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Background: Irinotecan and its metabolite SN-38 are known for their cytostatic properties against lung, colorectal, cervical and ovarian carcinomas. The 2 drugs have also been reported to be effective radiation sensitizers in various carcinoma cell lines under aerobic conditions. The performance of the drugs under anaerobic conditions has not been investigated and is the subject of the present investigation.

Materials and Methods: WHO3 human esophageal carcinoma cells were plated in 5 ml glass tubes at 600 cells per tube and incubated under standard cell culture conditions. After 24 hours, 3.1 μ M Irinotecan or 0.046 nM SN-38 were added and the tubes were incubated for 30 minutes in a modular shaker/chamber under a constant stream of 5% CO₂/95% air or 5% CO₂/95% N₂ to generate aerobic conditions, when the gas supply was terminated. After a further incubation period of 1 hour the tubes were irradiated with 1–10 Gy of 8 MeV photons and incubated for another 4 hours when the medium was replaced and cells were grown under aerobic conditions for 6 days. Cell survival was determined by the MTT assay using appropriate solvent controls and correction for drug toxicity alone. Survival data were fitted to the linear quadratic equation to obtain mean inactivation doses which were then used to calculate dose modifying factors.

Results and Conclusions: The oxygen enhancement factor (OER) for WHO3 cells (controls) was found to be 2.1 indicating that the cell system responds in the expected manner. Presence of the drugs under hypoxic conditions restored the radiosensitivity of WHO3 cells in a dose dependant manner by factors of 1.5–2.1 suggesting an additive cytotoxic effect for Irinotecan which was also seen under aerobic conditions. Addition of

SN-38 in the subtoxic range of 10–2 nM produced dose modifying factors of 1.6–2.1 and a marked sensitisation of anoxic tumour cells to irradiation approximating the radiation sensitivity under aerobic conditions. It is concluded that the topoisomerase inhibitors Irinotecan and in particular the metabolite SN-38 may be clinically useful in the radiotherapy of tumour pathologies which are notoriously hypoxic.

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Effects of Fulvestrant on oestrogen receptor levels during long-term treatment of patients with advanced breast cancerE. Gutteridge¹, J. Robertson¹, K. Cheung¹, S. Pinder², A. Wakeling³.¹Nottingham City Hospital, Department of Surgery, Nottingham, UK;²Nottingham City Hospital, Department of Histopathology, Nottingham, UK; ³AstraZeneca Pharmaceuticals, Macclesfield, UK

Background: Fulvestrant (Faslodex[®]) is an oestrogen receptor (ER) antagonist with no agonist effects. Fulvestrant (50 mg, 125 mg, 250 mg intramuscular [IM] injection) for 14–21 days prior to surgery for primary breast cancer caused a dose-dependent reduction in ER protein levels (Cancer Res 2001;61:6739–46). Here, we determine the long-term changes in ER levels occurring in patients receiving fulvestrant as first-line endocrine therapy for locally advanced/metastatic breast cancer.

Material and methods: 29 patients with lesions suitable for biopsy were recruited. Fulvestrant was given as a monthly 250 mg IM injection until disease progression (PD). Biopsies were taken pre-treatment (at diagnosis), after 4–6 weeks and 6 months of treatment, and at PD. Changes in ER levels were assessed by immunohistochemistry and analysed using SPSS statistical software. Tumour response was assessed every 3 months according to UICC criteria.

Results: 24 patients had evaluable disease at 6 months: 5 had PD and 19 had clinical benefit (CB; complete response, n=1; partial response, n=5; stable disease \geq 24 weeks, n=13), giving a CB rate of 79.2% (19/24 patients) and an objective response rate of 25.0% (6/24 patients). Overall, ER downregulation was seen at 4–6 weeks ($p=0.016$) and at 6 months ($p=0.011$) compared with pre-treatment samples. There was a trend for continued downregulation from 4–6 weeks (mean H-score 170.8 [range 50–280]) to 6 months (mean H-score 132.33 [range 0–260]). No significant reduction in ER ($p=0.461$) was seen in patients who progressed *de novo* (n=5). In patients achieving CB, ER downregulation was statistically significant at both 4–6 weeks ($p=0.029$) and 6 months ($p=0.011$). In all patients, ER was detectable at the time of PD.

Conclusions: This is the first dataset to show continued downregulation of the ER beyond the presurgical period and demonstrates that fulvestrant can maintain ER suppression for at least 6 months. Fulvestrant showed good clinical efficacy at the current clinical dose (250 mg). The presence of ER at PD supports the use of further endocrine therapies. Ongoing studies are investigating whether alternative dosing regimens result in earlier/increased ER downregulation and further improvement of clinical efficacy.

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An essential role for AIB1 phosphorylation in tamoxifen resistanceJ. Shou¹, R. Cook², G. Chamness¹, K. Osborne¹, R. Schiff¹. ¹Baylor College of Medicine, Breast Ctr, Medicine, Molecular & Cell Biology,Houston, USA, ²Baylor College of Medicine, Department of Immunology, Houston, USA

AIB1 is a steroid nuclear receptor coactivator. In addition, its effect also extends to unrelated transcription factors such as those involved in the cAMP or cytokine pathways. It is overexpressed in human breast, prostate, and ovarian cancers. There is compelling evidence that overexpression of AIB1 reduces tamoxifen's antagonistic activity in cultured cells. Breast cancer patients with overexpression of AIB1 along with high HER2 signaling are much more clinically resistant to tamoxifen in comparison to patients with only one of these factors elevated. Therefore we hypothesized that signaling from HER2 overexpression leads to phosphorylation and activation of AIB1, which contributes to development of tamoxifen resistance.

In this study, we found that exogenous overexpression of AIB1 can further increase tamoxifen's agnostic activity in HER2-overexpressing MCF7/HER2-18 (HER2-18) cells compared to parental cells. Retardation of AIB1 mobility on Western blots was found in HER2-18 cells treated with estrogen, tamoxifen, or the growth factor heregulin, and in *in vivo* HER2-18 tumors treated with tamoxifen, indicating that tamoxifen treatment may also lead to AIB1 phosphorylation in these cells. Recently we have identified a specific serine residue (S) located between the nuclear receptor interaction domain and CBP interaction domain in AIB1 that can be phosphorylated

by Akt and PKA in *in vitro* kinase assays. Substitution of this serine with glutamic acid (S to E) greatly increases estrogen receptor (ER) dependent gene transcriptional activity, while substitution with alanine (A) (S to A) greatly reduced AIB1 coactivity of ER and androgen receptor dependent gene transcription, but not progesterone receptor dependent gene transcription in HeLa cells. The S to A mutation in AIB1 doesn't change its cellular distribution with or without estrogen treatment, as examined by fluorescence microscopy. This specific phosphorylation, however, may mediate p300's enhancement of ER-dependent transcription, since the S to A mutation in AIB1 significantly attenuated this activity of p300. Moreover, the specific phosphorylation may have a role in the tamoxifen's agnostic activity, since the S to A mutation partially diminishes tamoxifen-induced ER-dependent gene transcriptional activity, as compared with tamoxifen-induced ER-dependent gene transcriptional activity in the presence of wild type AIB1.

Taken together, the serine phosphorylation site we have identified in AIB1 is critical to AIB1 functions and may serve as a link between the nuclear receptor pathway and other important signaling pathways such as the PI-3-kinase and cAMP pathways. Our data provide a mechanistic explanation for our previous clinical observation that high levels of *both* HER2 and high AIB1 are required for tamoxifen resistance, and serve as a molecular basis for strategy to block signaling from HER2 overexpression to AIB1.

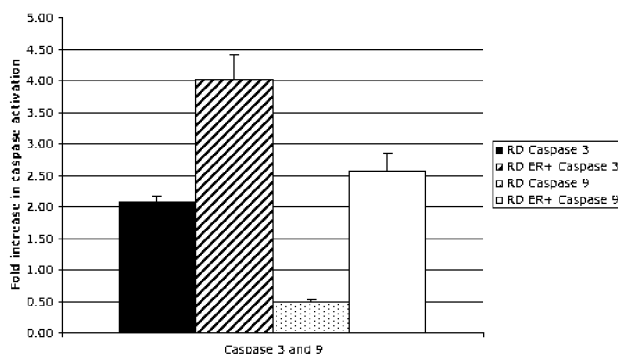
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ER-beta expression in rhabdomyosarcoma cells is essential for activation of caspase mediated apoptosis after treatment with tamoxifen

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Introduction: Tamoxifen (TAM), a selective estrogen receptor modulator, has a significant effect on the recurrence and survival rates of patients with breast cancer. TAM has also been shown to induce responses in desmoid tumors and aggressive fibromatosis in children. We have previously shown that there is a correlation between the endogenous expression of estrogen receptor beta (ER-beta) in rhabdomyosarcoma (RMS) cells and sensitivity to treatment with TAM. In this study we demonstrate that the sensitivity to TAM is significantly increased by expression of ER-beta in ER-beta negative RMS cells, and that the increased cell-death is caspase-mediated.



Caspase activation 12 hours after treatment with 7.5 μ M tamoxifen. The bars show fold increase and standard deviation for caspase activation compared with an untreated control. For both caspase 3 and caspase 9 the difference between RD and RD ER+ was statistically significant ($p < 0.002$ and $p < 0.001$).

Methods: RD, an ER-beta negative embryonal RMS cell-line, was cultured until 50% confluent, then transfected using a psg5-ER-Beta transfection vector. TAM was added 20 hours after transfection at the following concentrations: 5 μ M, 7.5 μ M and 10 μ M. Successful transfection was verified by western blot analysis using a monoclonal antibody (ER-beta 14C8) to verify high expression levels of ER-beta after transfection. After treatment for 12 hours, caspases 3 and 9 activation were assessed, using a fluorometric assay. After 20 hours, alamar blue viability assay was performed. All experiments were done in triplicate. For statistical analysis, Student's t-test was applied to compare two groups and a p-value of less than 0.05 was considered significant. MCF7, a breast cancer cell line, was used as positive control, and RD transfected with empty psg5 vector served as a negative control.

Results: No detectable expression of constitutive ER-beta was seen in RD. However, western blot analysis showed strong expression of ER-beta in RD cells transfected with ER-beta psg5. The sensitivity to TAM was increased significantly in RD ER-beta expressing cells (RD ER+) compared with

RD ER-beta negative (RD) cells for TAM concentrations of 5 μ M (92.0% vs 111.8%, $p < 0.0001$), 7.5 μ M (66.9% vs 89.8%, $p < 0.0001$) and 10 μ M (29.4% vs 60.9%, $p < 0.0001$). Fold increase in caspase 3 activation was significantly higher in RD ER+ compared with RD for TAM concentrations of 5 μ M TAM (1.5 vs 0.92, $p < 0.02$) and 7.5 μ M (4.0 vs 2.1, $p < 0.002$) (figure 1). Caspase 9 activation was also significantly higher in RD ER+ as compared with RD for 5 μ M TAM (1.2 vs 0.5, $p < 0.001$) and 7.5 μ M (2.6 vs 0.5, $p < 0.001$) (figure 1).

Conclusion: Our findings suggest a biologic mechanism for caspase mediated apoptosis after Tamoxifen treatment, that is dependent on ER-beta expression. These observations provide a basis for development of further studies to examine the *in vivo* effects of TAM in RMS, and to incorporate its use in clinical trials in the treatment of rhabdomyosarcoma.

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Fulvestrant does not markedly inhibit human cytochrome p450 isozymes: results from *in vitro* studies

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Background: Fulvestrant (Faslodex[®]) is an oestrogen receptor (ER) antagonist with no agonist effects that is indicated for the treatment of postmenopausal women with ER-positive breast cancer who have progressed following prior antioestrogen therapy. Patients in this setting are often receiving polypharmacy for comorbid conditions, which increases the likelihood of adverse drug-drug interactions. Here we present a preclinical study designed to investigate the potential of fulvestrant to inhibit the metabolism of model substrates of the cytochrome P450 isozymes CYP 1A2, 3A4, 2A6, 2C8, 2C9, 2C19, 2D6, and 2E1.

Material and Methods: Human hepatic microsomal protein preparations were incubated with substrates of cytochrome P450 isozymes 1A2 (phenacetin), 3A4 (testosterone), 2A6 (coumarin), 2C8 (paclitaxel), 2C9 (tolbutamide), 2C19 (mephenytoin), 2D6 (dextromethorphan), and 2E1 (chloroxazone), at approximate K_m concentrations for each enzyme and fulvestrant (0.003 to 10 μ g·mL⁻¹). P450 enzyme activities were determined by spectrophotometry (coumarin 7-hydroxylase), HPLC with on-line radiochemical detection (paclitaxel 6 α -hydroxylase), HPLC with UV detection (chloroxazone 6-hydroxylase; phenacetin O-deethylase; tolbutamide 4'-hydroxylase; S-mephenytoin 4-hydroxylase), or HPLC-fluorimetric assay (dextromethorphan O-demethylase). Enzyme activities were compared with control samples in which fulvestrant had been replaced with water. The effect of pre-incubation with a NADPH-regenerating system on the inhibitory potential of fulvestrant was also assessed (2A6, 2C8 and 2E1 incubations). Selective inhibitors of the various CYP isozymes were used as controls in this model system.

Results: Fulvestrant did not markedly inhibit the activity of any of the human P450 isozymes (<20% inhibition at the highest concentrations of fulvestrant) included in this study. Pre-incubation with NADPH had no effect on the inhibitory potential of fulvestrant, while the degree of inhibition observed with the control inhibitors was comparable to that observed in previous studies.

Conclusions: *In vitro* data suggest that fulvestrant is unlikely to cause clinically significant reversible inhibition or mechanism-based inactivation of CYP isozymes 1A2, 3A4, 2A6, 2C8, 2C9, 2C19, 2D6, and 2E1. Fulvestrant would not therefore be expected to cause significant drug interactions through P450-mediated metabolism of co-administered agents

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An androgen-receptor (AR)-negative human prostate cancer xenograft induces mouse-derived osteogenesis

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Background: The burden of prostate cancer (PC) bone metastases (BM) correlates with survival and a deeper understanding of the pathobiology may offer therapeutic opportunities. PC BM are typically osteoblastic but experimental models rarely recapitulate this phenotype.

Goals: To study malignant epithelial-osteoblast interactions that may be critical determinants of the pathophysiology of PC progression we sought to develop a representative model of osteoblastic bone metastases.

Methods: A SCID mouse xenograft was generated subcutaneously from an exophytic osteoblastic bone metastasis from a patient with androgen-independent PC. Tumors grew progressively and were passaged