*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)**

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**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**

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**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**

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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

tumor model. Inhibition of both aromatase and sulfatase activities should offer a new therapeutic approach to the treatment of hormone-dependent breast cancer.

**509** POSTER

**Evaluation of in vitro toxicity and efficacy of ferutinin, a natural promising chemopreventive compound**

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The biological activity of the natural phytoestrogen ferutinin have not been extensively examined as yet, in spite of the interest about plant-derived products as possible chemopreventives. In this study, the efficacy of ferutinin on several in vitro experimental endpoints correlated with tumour onset and progression has been compared to that of the well characterized soy isoflavone genistein. Effects of ferutinin and genistein have been examined on cell proliferation and growth inhibition, anchorage-independent growth and Matrigel invasion, cell growth in estrogen depleted media, programmed cell death. Like genistein, ferutinin acts as an estrogen agonist in the E-screen assay and exerts a biphasic effect on cell growth and proliferation in ER-positive MCF-7 cells, with an induction of proliferation at lower concentration (1 μM) and an antiproliferative dose-response effect at higher concentrations (10–100 μM). In MDA-MB-231 ER-negative cells, the dose-related inhibition of cell growth induced by ferutinin or genistein was evident, even if no biphasic effect was shown. In the agar clonogenic assay, ferutinin did not induce any significant increase in colony growth of MCF-7 cells at the assayed doses, while it showed a strong dose-related antiproliferative activity at high concentrations (10–100 μM). The biphasic effect of genistein on anchorage-independent growth was evident. The effect of the phytoestrogens on the malignant phenotype was evaluated in the in vitro Matrigel invasion assay. Ferutinin (1−100 μM) induced a dose-dependent inhibition of the invasive ability of MDA-MB-231 cells. The effect of ferutinin on cell death was also evaluated. The morphology of dying cells suggested the induction of a different mechanism of cell death induced by ferutinin, possibly alternative to apoptosis. The monodansylcadaverine (MDC) assay for autophagic cell death revealed some MDC-positive structures, which could be classified as autophagic vacuoles, in cells treated with high ferutinin concentrations (80 and 40 μM). The results show that ferutinin is more effective than genistein in the assayed in vitro endpoints in human breast cancer cells, suggesting a possible use of this natural compound as a chemopreventive or chemoterapeutic drug, even if the mechanisms of action and the benefit/risk ratio need to be further evaluated.

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**510** POSTER

**Estrogenic effect of ellagic acid in the estrogen sensitive breast cancer cells**

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**Background:** Ellagic acid is a dietary polyphenol present in abundance in strawberries and pomegranate. The antiproliferative activity of ellagic acid is documented and has been extensively studied in colorectal cancer, prostate cancer, and endometrial cancer cells. Ellagic acid exerts its effects via activation of various signaling pathways. Some study showed that the antiproliferative effect of ellagic acid is through mitochondrial pathway in colorectal cancer cell line. But another study reported that ellagic acid has antiproliferative effect by estrogen receptor α. We hypothesized that ellagic acid could be used as a new anticancer drug or new selective estrogen receptor modulator to manage the breast cancer. In the present in vitro study, we have compared the effect of ellagic acid on the proliferation of estrogen receptor negative or positive human breast cancer cells. In addition, the effect of ellagic acid on estradiol-induced stimulation of receptor-positive breast cancer cells was studied. Next, we evaluate the expression of pS2 and c-fos which is the down stream gene of ER.
**Material and Methods**: The receptor-positive breast cancer cell line MCF-7 and the receptor-negative cell line MDA-MB231 were used. The ellagic acid were tested in the concentration range of 10 um to 100 um. In MCF-7, 17α-estradiol and the mixture of ellagic acid and 17α-estradiol were also evaluated. Cell proliferation was measured after 0 hours, 24 hours, 4 8hours and 72 hours using the MTT assay. Apoptosis was confirmed by flowcytometry. The western blotting for pS2 and c-fos was done.
**Results:** Ellagic acid was able to significantly inhibit the cell proliferation of MDA-MB-231. But it caused cell proliferation in MCF-7. Flowcytometry showed that ellagic acid caused apoptosis in MDA-MB-231. It provokes cell-cycle arrest in S phase in MDA-MB-231. The western blotting for pS2 and c-fos was done to evaluate the estrogenic effect of ellagic acid on estrogen-receptor positive cell line. The expressions of pS2 and c-fos were higher in MCF-7 which was treated by ellagic acid.
**Conclusions**: The present data indicate that ellagic acid can inhibit the proliferation of receptor-negative human breast cancer cells. But it also stimulates the proliferation of receptor-positive breast cancer cells. Ellagic acid has different pathway in ER positive and ER negative cell lines. It might be used as anticancer drug in ER negative breast cancer, but it would be forbidden in ER positive breast cancer.

**511** POSTER

**Tamoxifen induces degradation of the o6-methylguanine DNA methyltransferase protein via the ubiquitin-dependent proteosome pathway in human cancer cells**

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**Background:** Tamoxifen is a synthetic nonsteroidal anti-estrogen triphenylethylene compound. It is part of a class of anti-cancer drugs known as selective estrogen receptor modulators (SERMs), and could block tumor growth by mimicking estrogen and filling up estrogen receptors which prevents the cancerous growth. However, the current view is that the action of tamoxifen is not only mediated by its anti-estrogenic properties. Previous study have demonstrated that a combination chemo/hormonal therapy regimen for the treatment of patient with neoplastic diseases, for example, the combination of the tamoxifen with the CNU-type alkylating agents, leads to synergistic cytotoxic effects. However, the mechanism of action of combined effect had not been elucidated.
**Material and Methods:** MGMT activity assay, Western blot analysis, Northern blot analysis, and Immunoprecipitation were used in this study.
**Results:** Here, we demonstrated that treatment of human colorectal HT-29 carcinoma cells with tamoxifen decreased the expression level of MGMT protein in a dose- and time-dependent manner. This inhibition, independent with estrogen receptor status, was also shown in other common cancer types tested. No difference between MGMT mRNA levels before or after tamoxifen treatment was found. However, MGMT protein half-life was markedly decreased in the presence of tamoxifen compared with that of control. Moreover, the MGMT protein was found to increase its ubiquitinated species after tamoxifen treatment. This tamoxifen-induced MGMT degradation could be reversed by proteosome inhibitors lactacystin and MG-132.
**Conclusion:** We conclude that tamoxifen induced reduction of MGMT protein levels by accelerating protein degradation via the ubiquitin-dependent proteosome pathway. This result provides the evidence for the combination benefit in chemo/hormonal therapy.

**512** POSTER

**Alteration of arachidonic acid metabolism in human breast cancer**

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**Background:** The plasma membranes of most cells contain both polyunsaturated and monounsaturated lipids, which are susceptible to oxidative damage by free-radical processes or electrophilic addition reactions, e.g. reaction of hydroxide ion ($OH^-$) with a double bond. Oxidative stress elevate levels of free radicals that can directly target arachidonic acid, an important mediator of inflammation, bound to phospholipids. This generates a complex mixture of oxidized products, known as isoeicosanoids, that can be cleaved off. In mammary gland tissues lipid peroxidation promotes the production of linoleic acid-derived arachidonic acid, a fatty acid that is most susceptible to lipid oxidation and formation of malondialdehyde (MDA). In this research we have studied alteration of arachidonic acid metabolism, both in model systems and in normal tissue adjacent to breast cancer and not in order to understand features of phospholipids fragmentation pattern in breast cancer.
**Materials and Methods:** Membrane phospholipids fractions from normal human tissue adjacent to breast cancer and fibroadenoma were extracted with chloroform/methanol (2:1), then sonicated on ice. The lipids chloroform extract was used for thiobarbituric acid assay (TBA) to induce a fragmentation pattern of lipids and evidenced the formation of aldehydic products as malondialdehyde (MDA). Moreover the lipids chloroform extract obtained after TBA assay was analyzed by ES-MS and MALDI-TOF spectroscopic techniques.