

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 qwx3), 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

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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 ER $\alpha$ , which is frequently expressed and an established target for anti-hormonal therapy, ER $\beta$  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-tumorigenic and results in expression of numerous tumour suppressor genes. The objectives of this study were to evaluate whether HDAC inhibition influenced expression of ER $\alpha$  and ER $\beta$ , 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 (ER $\alpha$  positive, ER $\beta$  negative) and its counterpart MCF-7/ER $\beta$  expressing stably transfected ER $\beta$ . 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 ER $\alpha$  and ER $\beta$  expression in these cells.

**Results:** Expression of ER $\beta$  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 ER $\beta$  in the wild-type MCF-7 cells as shown by qRT-PCR. Treatment with 0.1  $\mu$ M drugs resulted in a 17-fold and 2-fold increase in ER $\beta$  mRNA levels with MS-275 and TSA, respectively. Higher concentrations of drugs (1  $\mu$ M) were also observed to down-regulate ER $\alpha$  mRNA levels.

**Conclusion:** These data suggest that HDAC inhibition may induce re-expression of ER $\beta$  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 ER $\beta$  re-expression.

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POSTER

### Reactive oxygen species modulate the phosphorylation status of estrogen receptor alpha

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**Background:** Estrogen receptor  $\alpha$  (ER $\alpha$ ) 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 ER $\alpha$ , respectively. This activity may lead to ligand-independent activation of ER $\alpha$ , downregulation of ER $\alpha$  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 u/ml). Cells were harvested at different time points after an addition of GO and expression of ER $\alpha$  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-ER $\alpha$  was 475%  $\pm$  282% and of p-S167-ER $\alpha$  was 998%  $\pm$  580% (mean  $\pm$  SD, N=4). ER $\alpha$  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 $\pm$ 8.5% (mean  $\pm$  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 ER $\alpha$  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 ER $\alpha$  may be a mechanisms associated with development of anti-estrogen resistance.

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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<sub>50</sub> values for both aromatase, using [1 $\beta$ -<sup>3</sup>H] androstenedione as substrate, and steroid sulfatase inhibition, using [<sup>3</sup>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<sub>50</sub> 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