Materials and Methods: A triple-negative breast cancer cell line (GPR30−1) was established from a clinical specimen under an IRB-approved tumor banking protocol. GPR30 expression in this cell line was demonstrated by immunohistochemical staining and RTPCR. The effect of GPR30 knock-down was assayed on GPR30−1 cells that were transfected with a 29mer short hairpin RNA (shRNA) constructed against GPR30 (Origene Technologies, Inc, Rockville, MD). Control cells were transfected with a non-effective 29mer shRNA cassette. Transient expression of green fluorescence protein (GFP) allowed selection of transfected cells by fluorescence-activated cell sorting (FACS). Proliferation of untransfected GPR30−1, control, and GPR30 knock-down cells was tested in normal medium, 20 and 100 micromolar TAM using the MTS assay.

Results: Control-transfected GPR30−1 cells had an equal proliferation rate to untransfected cells in normal medium (ratio 1.04:1.0). In normal medium, GPR30-knockdown cells had a reduced proliferation rate (ratio 0.34:1.0) compared to negative control or untransfected cells. Untransfected and control-transfected GPR30−1 cells showed a 53% and 43% decrease in proliferation after 24 hours in low-dose (20 micromolar) TAM compared to a 92% reduction in GPR30-knockdown cells. All cells showed a greater than 90% decrease in proliferation in high-dose (100 micromolar) TAM.

Conclusions: Knock-down of GPR30 in GPR30−1 cells lowers the proliferation of cells in normal medium and when exposed to TAM compared to normal and control-transfected cells. These results are consistent with a tamoxifen resistance effect of GPR30. GPR30 may represent a therapeutic target in breast cancer.

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Cytotoxicity of docetaxel, epirubicin and carboplatin on hormonal receptors positive and triple negative breast cancer cell lines

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Background: Breast cancer patients are stratified into three main groups: those expressing hormonal receptors (HR), which respond to therapies targeting estrogen receptors; HER2 positive tumors that are candidate for targeting therapy with trastuzumab or lapatinib; triple negative (TN) tumors, for which the only systemic therapy available is standard chemotherapy. Some studies suggest an increase susceptibility of TN to platinum-derived chemotherapy, with a pathological remission rate of 21%.

The purpose of this study was to evaluate cytotoxic capacity of docetaxel, epirubicin and carboplatin in MCF7 (HR positive) and HCC 1806 (TN) breast cancer cell lines.

Material and Methods: Human breast cancer cell lines MCF7 and HCC1806 were purchased to ATCC and cultured according to recomended procedures. Both cell lines were incubated in absence and presence of the docetaxel, epirubicin and carboplatin in several concentrations ranging from 50nM to 150μM. The sensitivity of the cell lines to the drugs studied was analyzed using the MTT colorimetric assay, performed 24, 48 and 72 hours after incubation. Cytotoxicity was expressed as the percentage of inhibition of cell proliferation correlated with untreated cultures. Dose-response curves were established and the half maximal inhibitory concentration (IC50) was calculated in Origin7 software.

Results: Epirubicin on HCC 1806 at 24h had a higher IC50 than on MCF7 (2.3 *vs.* 1.8μM), but this performance reached a similar level at 72h. Focusing on docetaxel, IC50 was higher on MCF7 than on HCC 1806, showing a better performance considering cell death for HCC 1806. Considering carboplatin, the IC50 was considerably elevated on MCF7. In HCC 1806, carboplatin proved IC 50 of 44.8 μM and 8.6 μM at 48 and 72h, respectively.

Comparing epirubicin *vs.* carboplatin cytotoxicity on MCF7, IC50 was always high in carboplatin studies, with IC50 of 53.5 μM at 72h for these particular cells. Also on HCC 1806, carboplatin showed a worse activity than epirubin, emphasized by higher IC50 for carboplatin at 48h (44.9 μM) and 72h (8.6 μM) than epirubicin.

Conclusions: Epirubicin had a similar effect on HR positive and TN cell lines. On the contrary, docetaxel proved a better performance on TN than on HR positive cells, the last showing elevated IC50. Carboplatin reached less cytotoxicity than epirubicin either in HR positive and TN breast cancer cell lines.

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Long-term effect of fulvestrant on hormone receptors and proliferation marker in breast cancer

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Background: Fulvestrant has been shown in short-term (maximum of 4 months) pre-surgical studies of breast cancer to decrease expression of estrogen receptor (ER), progesterone receptor (PgR) and proliferation marker, Ki67. We present changes in these markers from 6 months and beyond (median time to progression of 25.8 months) in breast cancer patients treated with fulvestrant.

Materials and Methods: 32 post-menopausal women with locally advanced (n = 22) or metastatic breast cancer (n = 10) with measurable breast lesions had fulvestrant (250 mg. intra-muscularly monthly) as first-line primary endocrine therapy. Immunohistochemistry was performed on sequential breast tumour biopsies taken at diagnosis (before commencing fulvestrant, T1), 6 weeks (T2), 6 months (T3) and at progression (T4) of disease.

Results: Wilcoxon signed rank sum analysis revealed decrease in the levels of all 3 markers at all subsequent time-points from pre-treatment level (significance at $p < 0.05$) (table).

Marker	Median level at T1 (range)	p-value for change		
		T1−T2	T1−T3	T1−T4
ER H score	130 (60–190)	<0.001	<0.001	0.001
PgR H score	30 (0–270)	Non-significant	0.001	0.012
Ki67% stain	18 (1–60)	0.001	<0.001	0.028

There was non-significant recovery of ER and Ki67 at progression (T4) compared with 6 months level (T3). Kaplan-Meier analysis revealed lower pre-treatment (T1) Ki67 predictive of longer TTP but no similar relation was noted with ER and PgR. While not apparent at T1, higher PgR at 6 weeks (T2) predicted for longer TTP ($p = 0.008$).

Conclusions: The remaining expression of ER (partly due to some recovery) may contribute to acquired resistance as ER is still available for cross-talk with growth factors. However, lack of total depletion of ER at progression on fulvestrant may also explain known clinical response to further endocrine therapies. Mix of low and high PgR cases within the responders in this series (median PgR of 30) suggests fulvestrant activity being largely dependent on ER irrespective of PgR. This study confirms decrease in Ki67 (and ER and PgR) expression seen earlier in literature but is the first study which shows statistically significant decrease beyond a median of 2 years.

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A DNA signature to identify high-risk small node-negative breast cancer patients

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Purpose: To identify a DNA signature to predict metastasis of small node-negative breast carcinoma

Experimental Design: The authors used Comparative Genomic Hybridization (CGH) array to analyze 168 pT1T2pN0 invasive ductal carcinoma patients with either good (no event 5 years after diagnosis: 111 patients) or poor (57 patients with early onset metastasis) outcome. A CGH classifier, identifying low and high-risk groups of metastatic recurrence, was established in a training set of 78 patients. This classifier was based on both genomic regions with statistically different alterations between the two groups of clinical outcome and the number of alterations. It was then tested on a validation set of 90 patients and compared to clinicopathological parameters.