CLA on p53, p21WAF1 and bcl-2 expression in addition to the cytotoxic effects of CLA on breast tumour growth.

Methods: Oestrogen receptor positive and negative breast cancer cells (MCF7, MDA-MBA-231) were grown in supplemented RPMI media, containing CLA at concentrations (0 μM to 200 μM) for 24 hours. The effects on cell growth were assessed using MTT assay. Following treatment with CLA, northern blotting and ELISA were performed to determine the effects on the expression of p53, p21WAF1 and bcl-2.

Results: Exposure to CLA resulted in a dose dependent reduction in growth of MCF-7 cells - 20% at 6.25 μM, 50% at 100 μM and 65% at 200 μM ($p < 0.001$). Similar results were obtained for MDA-MBA-231 cells. Northern blot analysis showed that CLA treatment caused a dose dependent increase in wild-type p53 expression (MCF-7 cells) by 284% at 12.5 μM, 347% at 100 μM, and 523% at 200 μM of CLA ($p < 0.01$). There was no change in bcl-2 expression. The expression of p21WAF1, a key downstream regulator of p53, was raised to 203%. CLA did not change the expression of mutant p53 (present in MDA-MBA-231 cells) or p21WAF1, but did increase the expression of bcl-2 by 103% at 12.5 μM, 201% at 50 μM, and 207% at 100 μM of CLA ($p < 0.01$). Similar over-expression of the corresponding proteins were noted by ELISA.

Conclusions: This is the first demonstration which shows that CLA exerts its anti-tumour effects by increasing the expression of the wild-type p53 and the p21WAF1 gene. However, in cells with mutant p53, CLA inhibits cell growth, through a p53-independent pathway.

O-83. HYPERSENSITIVE K303R OESTROGEN RECEPTOR VARIANT NOT FOUND IN DUCTAL CARCINOMA *IN SITU*

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Non-atypical hyperplasia of the breast (hyperplasia of usual type) is believed to be a non-obligate precursor of breast cancer. As such, genetic abnormalities or mutations in such lesions may play a role in progression toward malignancy. One recently described mutation, occurring in about one third of hyperplasia tested is an A908G (K303R) change in the oestrogen receptor a gene that creates a hypersensitivity to oestradiol (Fuqua *et al.*, Cancer Res 2000, 60: 4026–4029).

We have examined a significant number of DCIS, by sequencing PCR products from microdissected samples.

No evidence of the A908G mutation was found, either individually or together with the wild-type allele. Enough cases of DCIS (44) were studied to make this results statistically significant ($P < 0.001$; Fisher Exact Test).

Retention of the A908G mutation in more advanced lesions, such as ductal carcinoma *in situ* (DCIS), would provide evidence that this mutation is involved in breast cancer progression. That the mutation was not found, leads us to believe that either the mutation is not retained during progression, that it may be involved only in the progression of lower grade DCIS, or that this mutation is limited to HUT that fail to progress via DCIS.

O-84. OESTROGEN WITHDRAWAL REDUCES EPITHELIAL CELL PROLIFERATION IN OESTROGEN RECEPTOR (ER) POSITIVE BUT NOT NEGATIVE DUCTAL CARCINOMA IN-SITU (DCIS)

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Aims: Over 50% of DCIS is ER negative which will not respond to hormone therapy. To investigate the effect of hormone manipulation on epithelial proliferation we studied 100 women who had undergone diagnostic core biopsy followed by surgery for DCIS 14–21 days later. ER status and ki67 (a measure of epithelial cell proliferation) was determined by counting 1,000 cells after immuno-histochemical staining on paired sections of the core biopsy and operative specimens for each woman. In ER negative DCIS epithelial proliferation did not change between diagnosis and treatment. Only in ER positive patients who stopped HRT was a fall in epithelial proliferation observed (see table).

Group	ER (No)	Median core Ki67 (IQR)	Operative Ki67 (IQR)	P value*
1. Never taken HRT	– (24)	9.8 (5.6–18.5)	11.3 (7.5–15.5)	0.92
(control)	+ (32)	8.2 (4.9–14.9)	7.7 (4.4–11.8)	0.49
2. Continued HRT	– (4)	11.4 (6.1–28.8)	16.0 (6.9–28.8)	1.00
	+(17)	8.8 (3.8–16.8)	8.8 (2.0–15.4)	0.76
3. Stopped HRT	– (6)	15.9 (10.5–24.4	16.4 (10.7–23.7)	0.94
	+ (15)	**9.3 (2.3–17.1)**	**3.3 (0.8–8.9)**	**0.04**

*Mann-Whitney 2-tailed test

Conclusions: Oestrogen withdrawal reduces proliferation in ER positive but not in ER negative DCIS. Therefore adjuvant anti-oestrogen therapy is likely only to benefit ER positive patients.

O-85. CHROMOSOMAL ALTERATIONS ASSOCIATED WITH TRANSFORMATION OF A TAMOXIFEN-SENSITIVE CELL LINE INTO ITS DRUG RESISTANT CLONE

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Introduction: Tamoxifen resistance is a serious problem in the management of breast cancer. Knowledge of the genetic pathways leading to tamoxifen resistance may allow the development of novel therapeutic strategies.

Aims: To determine the genetic changes between MCF-7 a tamoxifen sensitive human breast cancer cell line and its resistant clone CL-9 using comparative genomic hybridisation (CGH).

Materials and Methods: CGH is a technique by which we can detect amplifications or deletions in the genome in a single hybridisation experiment. DNA was extracted from both MCF-7 and CL-9. The CGH assay was performed using: MCF-7 DNA - normal placental DNA, b) CL-9 DNA - normal placental DNA, c) MCF-7 DNA - CL-9 DNA. Images were captured with a CCD camera and analysed. Metaphases were prepared from the cell lines and analysed by cytogenetics and also by chromosomal painting.

Results: CGH analysis for the tamoxifen sensitive and resistant clone showed many areas of concordance but important differences were seen in amplification of chromosomes 2p16.3-23.2, 2q21-34, 3p12.3-14.1, 3p22-26, 3q, 12q13.2-22, 13q12-14, 17q21.3-23, 20q11.2-13.1 and 21q11.2-21 as well as the deletion of chromosomes 6p21.1, 6p23-25, 7q11.1-31, 7q35-36, 11p15, 11q24, 13q33, 17p, 18q12-21.1, 19p, 19q13.3, 22q13.1-13.2. These findings were confirmed by cytogenetics and chromosomal painting.

Discussion and Conclusions: Transformation from a tamoxifen sensitive to a resistant phenotype could be explained by changes at the molecular level. Definite alterations in the genetic profile were seen in the tamoxifen resistant cell line involving regions harbouring potential genes e.g. *TGF-β* at 19q13.3, *MDM-2* at 12q14.3-15. These may be involved in the development of tamoxifen resistance and need further evaluation. This study has shown that the development of tamoxifen resistance is associated with changes at the chromosomal level.

O-86. COMPARISON OF OESTROGEN RECEPTOR α AND β mRNA AND PROTEIN IN MALIGNANT AND NON-MALIGNANT BREAST

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The identification of a second oestrogen receptor (ER), ERβ, has resulted in interest in its role in the response of breast cancers to endocrine therapy. In this study we have studied ERβ mRNA and protein in malignant and non-malignant breast and compared it to ERα to define its significance in breast cancer.

62 cancers (38 with adjacent normal), 32 normal tissues and 8 benign lesions were studied using RT-nested PCR for mRNA expression and immunohistochemistry for protein expression. The identity of expressed sequences was confirmed by automated sequencing. 41/62 tumours (66%) expressed wt ERβ mRNA in comparison to 90% for wt ERα and 8 only expressed an exon 5-deleted variant. All but one cancer expressed either ERα or ERβ alone or both genes (34 cases). Surrounds showed similar expression to the corresponding tumours and all 8 benign lesions expressed wt ERα and 7 expressed ERβ. For the carcinomas weak/moderate staining for ERβ protein was detected in 1–25% of tumour cells in 3 of 4 Grade I, 8/27 grade II and 7/29 grade 3 cases. ERα protein was detected in 70% and showed a significant association with grade. Non-involved, normal and benign tissues showed moderate to strong staining of 10–75% of both myoepithelial and epithelial cells. For the 32 normal tissues examined, there was no relation ship to menstrual cycle phase. For surrounds, 13 premenopausal cases showed similar staining to the normal controls, whereas the postmenopausal group (16 cases) showed significantly more expression of ERβ in the ducts ($p = 0.002$, Kruskal Wallis).

In comparison to ERα there is loss of ERβ in breast carcinomas but there is still a weak association with better grade.

O-87. TAMOXIFEN AND ARIMIDEX DO NOT INHIBIT ANGIOGENESIS IN VITRO

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Angiogenesis is vital for tumour growth and metastases and has been identified as an independent prognostic factor for recurrence in breast cancer. The aim of this study was to examine the anti-angiogenic properties of endocrine therapies, Tamoxifen and Arimidex using a human *in vitro* model of angiogenesis.

In this model human endothelial cells are co-cultured with human fibroblasts in a specially designed medium (TCS CellWorks Ltd., UK). The effects on tubule formation of Tamoxifen (0.5 μM, 1.0 μM), Arimidex (0.1 μM and 0.05 μM) and Suramin, a known anti-angiogenic agent, were assessed following staining with CD31 monoclonal antibody in six separate plates.

Results: Tamoxifen and Arimidex do not inhibit tubule formation as compared to the control ($p > 0.05$). Tamoxifen (0.5 μM) shows a 12% and 16% increase in total tubule length alone, and in combination with Arimidex (0.05 μM), respectively. This pro-angiogenic effect did not reach statistical significance. Culture with suramin resulted in significant inhibition of total tubule length as compared to the control and all drug doses ($P < 0.001$).

Control	Tamoxifen		Arimidex		Suramin
	1 μM	0.5 μM	0.1 μM	0.05 μM	
935.8 (145)	895.6 (128.3)	1055.1 (139.3)	819.7 (178.5)	925.1 (158.2)	336.7 $(132)_{6)}$

*tubule length shown in mm; Data = Mean (Standard Deviation), Stats = ANOVA and Tukey HSD tests

Conclusion: This model shows that neither Tamoxifen, nor Arimidex have an anti-angiogenic effect on endothelial cell tubule formation, in contrast to previous *in vitro* studies.

O-88. SERUM AND TISSUE CerbB-2 ANTIGEN LEVELS PREDICTS OUTCOME AND RESPONSE TO HORMONAL THERAPY OF BONE METASTATIC BREAST CANCER

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Bone metastases are considered oestrogen receptor positive and