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

usually respond to hormonal therapy. Expression of the cerbB2 oncoprotein is claimed to predict cancers, which recur early and respond poorly to hormonal therapy.

Aims: To detect whether raised serum levels of serum cerbB2 antigen predicts poor response to hormonal therapy as judged by UICC criteria and to determine the incidence of cerbB2 expression in primary tumours associated with bone metastasis.

Methods: Serum from 65 women with metastatic breast cancer bone metastasis undergoing hormonal therapy was assessed for cerbB2 oncogene (EIA, Bayer, UK). In addition paraffin sections of 35 of the 65 women were retrieved and immunohistochemistry performed NCL-CBL11 (Novocastra) antibody to determine cerbB2 expression. Serum levels of >25 ng/ml were considered significantly raised.

Results: Significantly more women with bone metastases which progressed on hormonal therapy had raised serum levels of *cerb*B2 compared to women with stable or responding disease ($p < 0.0001$).

Response	Number	Serum > 25 ng/ml	cerbB2
CR/PR	17	0	2 (12%)
Stable	25	0	4 (16%)
Progressive	23	14 (61%)**	10 (43%)*

Chi square test **$p < 0.0001$, *$p < 0.05$

Conclusion: Expression of cerbB2 occurs frequently in bone metastases, which explains progressive disease on hormonal therapy. Raised serum levels of cerbB2 predict response to hormonal therapy.

O-89. THE INFLUENCE OF OESTRADIOL ON INTEGRIN β1 EXPRESSION AND FUNCTION IN BREAST CANCER AND THE EFFECTS OF LONG-TERM OESTROGEN DEPRIVATION

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Introduction: Integrins are transmembrane proteins that play a leading role in tumour metastasis. Breast cancer risk is associated with prolonged exposure to oestrogens. The aims of this study were to (i) assess the effects of oestradiol (E2) on integrin β1 expression and function and (ii) evaluate variation in expression of integrin β1 during oestrogen deprivation.

Methods: Integrin β1 expression was measured on MCF-7 cells incubated in E2 by western blot. Integrin function was measured by cell adhesion to matrix proteins. MCF-7 cells were cultured in oestrogen deficient medium for over 100 weeks and integrin β1 expression on these long-term oestrogen-deprived (LTED) cells was measured at regular intervals by western blot.

Results: Overnight incubation in 10^{-7} M E2, resulted in the up-regulation of integrin β1 by 1.7-fold, conversely 10^{-11} M E2 down-regulated expression by 29%. The effect of 10^{-9} M E2 on integrin β1 expression could be seen as early as 15 minutes, with peak effect at 2 hours. E2 enhanced cell adhesion to both collagen IV and fibronectin, with a significant maximal effect at 10^{-10} M. Integrin β1 expression was significantly up-regulated during weeks 1 to 25 of oestrogen deprivation. During this time the cells were noticeably more difficult to trypsinise during passage. After 25 weeks the cells appeared to adapt to oestrogen deprivation with little or no change in expression between weeks 26 to 109.

Discussion: Cell adhesion is a pre-requisite for successful cell invasion and metastasis. We have demonstrated that E2 has an effect on both integrin β1 expression and cellular function. Our data suggest that integrins may play an integral role in the adaptation of tumours to steroid-independence.

O-90. FACTORS AFFECTING COSMETIC OUTCOME AFTER BREAST CONSERVING SURGERY

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120 patients who underwent breast conserving surgery from 1996/97 completed a questionnaire in July 2000 which asked for an assessment of cosmetic outcome on a scale of 1–10 and also included a 15 question body image questionnaire. 100 replies were complete and suitable for analysis. 86% of patients had an excellent or good cosmetic result using the 10 point scale, 81% had good or excellent scores using the body image score. There was a significant correlation between the two scores, $p < 0.0001$. Volume of tissue excised and the volume of the breast excised (calculated by using specimen volume and breast volume determined from the CC mammogram) both correlated with cosmetic outcome ($p < 0.0001$) with the percentage of breast excised correlating better than simple volume. Patients who had more than 12% of their breast excised were significantly more likely to have a poor cosmetic outcome ($p < 0.01$). Patients who underwent axillary sampling had a significantly better cosmetic outcome than if they had an axillary clearance or axillary sampling and radiotherapy, $p = 0.001$. Of the 4 consultant surgeons who performed all but 13 of the operations, 1 had significantly more good or excellent results, $p = 0.026$ and 1 had significantly more poor results, $p = 0.014$. Differences between surgeons were directly related to different volumes of tissue excised between surgeons, $p = 0.006$.

Percentage breast volume excised is the main determinant of cosmetic outcome after breast conserving surgery. As different surgeons remove different amounts of tissue, the surgeon performing the surgery has a significant impact on the final cosmetic result.

O-91. COSMESIS AND SATISFACTION AFTER BREAST CONSERVING SURGERY (BCS) CORRELATES WITH THE PERCENTAGE OF BREAST VOLUME LOST

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Cosmesis after BCS is an important outcome that correlates with