Table 1

Reported side effects	Tamoxifen (23 patients)	Faslodex (19 patients)
Amenorrhoea/Disturbances in menses	2 (7%)	1 (5%)
Light headed	3 (13%)	none
Hot flushes	4 (17%)	3 (16%)
Loose stools	1 (4%)	3 (16%)
Strong smelling urine	none	2 (11%)
Headache	none	6 (32%)
Joint pain	none	2 (11%)
Increased bruising (generalised)	none	2 (11%)
Nausea	1 (4%)	2 (11%)
Tiredness	none	2 (11%)

Adverse events were graded using NCIC CTG Expanded Common Toxicity criteria. All adverse events reported as grade 1–2.

score per injection was 1.6. Injection related morbidity included itching at injection sites (11%), pain at injection sites in the days following injections (47%), swelling at injection sites (16%), redness at injection sites (11%), bruising at injection sites (21%), and skin sensitivity at injection sites (11%). 10 out of 23 patients in the Tamoxifen arm reported no side effects. None of the Tamoxifen patients stopped treatment early due to side effects. Although all the patients in the Faslodex arm had one or more side effects these did not result in any patient contacting staff, before their routine review.

Conclusion: Faslodex at 750 mgs was remarkably well tolerated considering all patients had 3 injections. The side effects and tolerability are comparable with studies of single injections of Faslodex. In light of the effectiveness of this drug reducing proliferation, further exploration is warranted.

O-71. Upregulation of CD44S and variants in anti-hormone resistant breast cancer cells

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Induction of anti-hormone resistance can occur by a variety of mechanisms and is often accompanied by progression to a more invasive-metastatic phenotype. Microarray technology of anti-oestrogen resistant cells revealed that the multifunctional receptor CD44 was upregulated in anti-oestrogen resistance in our model system. Comparison of the expression of CD44 standard(s) and variants by PCR and immunocytochemistry (ICC) was undertaken in wild type MCF-7 cells and its resistant sublines, TAMR cells (Tamoxifen resistant), FASR cells (Faslodex resistant) and the doubly resistant TAMR-Iressa resistant cells cultured under basal conditions and in the presence of anti-hormonal regimens. By both PCR and ICC the CD44 standard protein was significantly upregulated in comparison to the wild type MCF-7 in both the TAMR and FASR cells and to a lesser extent in TAMR-TKI resistant cells. Increased levels of CD44v3, v5 and v6 were observed in the FASR cells and TAMR cells although to differing degrees in these sublines, v3 and v5 in the former and v6 in the latter. Additionally CD44s and variant expression could be regulated by oestradiol and tamoxifen in the MCF-7 cells and by EGFR ligands and Iressa in the TAMR cells. The glycoprotein CD44 and variants are involved in cell/cell and cell/matrix interactions, acting as a scaffold for MMPs and growth factors, also as co-receptors to mediate signalling by c-met and the c-erbB family and via binding to ERM proteins and ankyrin to influence the cytoskeleton and cell motility. It is likely that upregulation of CD44 and particularly its variants in resistant cells will contribute to a worsening phenotype and may represent an additional drug target in anti-hormone resistance disease.

O-72. Identification of oestrogen-regulated genes in human breast cancer cells using DNA microarrays

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Oestrogens are well known to be important in the aetiology of breast cancer. However, the complete repertoire of oestrogen-regulated genes, as well as the exact roles of oestrogen in breast cancer remains unknown. The aim of this study was to identify novel oestrogen-regulated genes in breast cancer using DNA microarrays.

Human breast cancer cells were grown to confluence and then withdrawn from oestrogen for 7 days before treatment with oestradiol. Three oestrogen-responsive breast cancer cell lines (MCF-7, EFM-19 and EFF-3) were studied. RNA was extracted with TRIZOL reagent and Affymetrix protocols followed prior to hybridization of cRNA to Affymetrix Hu U133 Plus 2.0 Genechips. Microarray data was analysed with Microarray Suite 5.0 Software. Quantitative real-time PCR (Q-RT-PCR) was then performed for oestrogen receptor-alpha, trefoil factor 1, and 10 novel oestrogen-regulated genes.

Of the >47000 genes analysed, 2091 (MCF-7), 727 (EFM-19) and 245 (EFF-3) genes respectively varied 2-fold or more after oestradiol stimulation. Known oestrogen-regulated genes were identified including TFFI, PgR and CXCLI2. Numerous novel oestrogen-regulated genes were also identified, including 10 genes that were consistently regulated by oestrogen in all three cell lines. Q-RT-PCR has validated microarray data and confirmed the oestrogen-regulation of 10 novel genes at the transcript level.

O-73. Regulation of oestrogen receptor beta in MCF-7 breast cancer cells with Trilostane by comparative gene expression microarray analysis

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Trilostane (Modrenal™), has direct non-competitive inhibitory effects on oestrogen receptor (ER) function. These include a reduction in ERα binding to oestrogen response elements (EREs), inhibition of activating protein-1 (AP-1) reporter activity, and altered oestrogen binding kinetics. The net effect is an inhibition of proliferation of breast cancer cells. In contrast, tamoxifen, a competitive inhibitor of ER, blocks oestrogen action on EREs, but may enhance AP-1 mediated activity. These studies were designed to compare the effects of trilostane and 4-hydroxytamoxifen (OHT) on gene expression in MCF-7 breast cancer cells using gene microarrays representing 20 000 human genes.

MCF-7 cells were treated with oestrogen alone (10 nM)

or together with either trilostane (10 μM) or OHT (1 μM) for 72 hours. Cy5 or Cy3 fluorescently-labelled cRNAs were synthesised from experimental RNA samples and hybridised with oligonucleotide microarrays. Red (Cy5) or green (Cy3) fluorescence was analysed and treatment comparisons were performed.

In the presence of oestrogen, trilostane and tamoxifen had different actions on the expression of key sets of genes, including those coding for cell adhesion molecules, cell cycle and growth factor pathway components, and matrix-related proteins. Of particular interest was the preferential up-regulation of ER beta isoforms by trilostane. ER beta is thought to be down-regulated in tamoxifen-resistant tumours and is also a negative modulator of oestrogen's actions in both ERE- and API-dependent transcription. Therefore, this observation may provide an explanation for the clinical benefit seen with trilostane in patients who have relapsed on tamoxifen.

O-74. Caveolin-l expression in Tamoxifen-sensitive and resistant MCF-7 breast cancer cells

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We have previously shown that growth of a tamoxifen-resistant MCF-7 breast cancer cell line (Tam-R) is mediated by the epidermal growth factor receptor (EGFR)/mitogen-activated protein kinase (MAPK) signalling pathway. Components of this signalling pathway are found within caveolae as a result of binding to the principal coat protein, caveolin-l. Recent evidence suggests a reciprocal negative regulation exists between caveolin-l expression and EGFR/MAPK signalling activity and that hyperactivation of the MAPK pathway, as a result of caveolin-l downregulation, can drive cell transformation. To investigate whether caveolin-l plays a role in the development of tamoxifen resistance we have studied the relationship between caveolin-l and the oestrogen receptor (ER) and EGFR/MAPK signalling pathways in tamoxifen-sensitive (WT) and -resistant (Tam-R) MCF-7 breast cancer cell lines. RT-PCR, Western blotting and Immunocytochemistry were used to assess caveolin-l expression prior to and following pharmacological manipulation of ER and EGFR/MAPK pathways. An inducible caveolin-l expression system was used to evaluate the effects of caveolin-l expression in Tam-R cells. Caveolin-l mRNA and protein was expressed in WT, But not Tam-R cells. In WT cells, inhibition of ER up-regulated caveolin-l expression and reduced cell growth, whereas, blockade of EGFR activity increased caveolin-l expression but had little effect on proliferation. Blockade of EGFR/MAPK signalling similarly up-regulated caveolin-l expression in Tam-R cells. However, increased caveolin-l expression in both WT and Tam-R cells had no effect on EGF-induced activation of the MAPK pathway. These findings suggest a negative regulatory role for EGFR/MAPK signalling on caveolin-l expression but no reciprocal regulation of this pathway by caveolin-l in WT and Tam-R cells. Thus, caveolin-l appears to play no significant role in the development of tamoxifen resistance in this cell line.

O-75. Expressions of Cyclin B1 and CKS2 in breast cancers go down after short-term treatment with aromatase inhibitors

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Introduction: Changes in gene expression following short-term treatment may be helpful in identifying markers of tumor response to treatment. The aim of this study was to quantify changes in gene expression during treatment with aromatase inhibitors (AIs).

Materials and Methods: 21 post-menopausal breast cancer patients with estrogen receptor positive operable primary breast cancers received either letrozole or anastrozole for 14 days prior to surgery. Tumour samples were available before and after treatment. mRNA for mammaglobins 1 and 2 (MGB1 and MGB2), Cyclin-B1 (CB1), CDC28 pk 2 regulatory sub-unit (CKS2), Pleiotrophin (Ptph) and Lipin-2 (Lip2) were measured by real time PCR. 2-fold changes were regarded as meaningful.

Results: Results are summarized in the table.

	MGB1	MGB2	CB1	CKS2	Ptph	Lip2
Up	7	6	1	1	9	2
Stable	7	7	12	9	9	19
Down	7	6	8	11	3	0

Changes in MGB1 positively correlated with those in MGB2 ($p < 0.01$); similarly, CKS2 with Cyclin-B1 ($p < O.O1$).

Conclusion: Early changes in mRNA expression with treatment could be detected in all studied genes except for Lip2. Consistent changes were detected in CB1 and CKS2L, which were concordantly decreased in about a half of the studied cases. As these genes closely interact in promoting of cell cycle progression it is suggested that their dynamic measurements may be markers of early response to AIs in estrogen receptor positive postmenopausal breast cancer patients.

O-76. Does oestrogen receptor β expression influence survival in breast cancer?

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Background: Whilst it is ERα that is used routinely in assessment of breast cancer patient oestrogen receptor status, studies are now suggesting expression of ERβ to be also of great importance for therapy assessment. There is also a suggestion that ERβ levels may have greater influence in the biological behaviour of breast cancer, where the levels of ERα are low.

Methods: Archival formalin fixed paraffin embedded tissue was used from a group of 199 patients from 1996. There were three surgeons. The major difference in clinical practice was that two treated patients with neoadjuvant endocrine therapy (20 mg daily Tamoxifen 3 weeks pre-operatively) and other did not. All treatment was based on multidisciplinary meetings following national guidelines. Long-term survival was collected using the hospital breast cancer Database and used as end point.