Immunohistochemistry (IHC) was used to measure oestrogen receptor α and β expression,

Results: Mean age was 69.5 years (range 43–99) in treated and 65.1 years (range 40–91) in untreated. Median follow up was 83.45 months (range 1–96). Mean survival was 74.1 months in treated group and 70.9 months in untreated. In neoadjuvant group, there was a trend to better survival in ERβ negative tumours than positive ($p = 0.1$). In the non-neoadjuvant group, this trend was also seen ($p = 0.04$). Correlation of ERα and β with survival showed best prognosis in ER alpha+/Beta− and worse prognosis in Alpha−/Beta+ tumours ($p = 0.01$).

Conclusion: Our results confirm ERβ expression to be associated with worse prognosis, survival and resistance to endocrine therapy.

O-77. Oestrogen receptor variant expression as potential selectors for adjuvant endocrine therapy in breast cancer patients

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Disease-free interval differs amongst invasive breast cancer patients, who had not received any prior adjuvant systemic therapy, with oestrogen receptor (ER) positive cancers treated by endocrine therapy at the time of relapse. This suggests the presence of biological factors inherent in tumours which affect responsiveness to subsequent endocrine therapy. ER splice variants result from exon deletions and can repress wild-type receptors and modulate anti-oestrogen activity. We hypothesise ER splice variants contribute to the differences seen with therapeutic response in these patients.

We have characterised variant ER expression in primary invasive breast cancer patients ($n = 17$) that either responded or not responded to endocrine therapy at the time of relapse. Breast tumour cells were isolated from formalin-fixed archival tumour sections using laser microdissection. Total RNA was extracted and expression of ERα, ERαΔ2–3, ERαΔ3, ERαΔ5, ERβ1 and ERβ2 was quantified using real-time PCR. Gene expression was normalised against 18s rRNA expression.

Expression of ER wild-type and variants were detected in most breast tumours although levels differed. ERαΔ2–3 and ERαΔ5 expression was significantly higher in those tumours that responded to endocrine therapy compared with those tumours that did not respond. No difference was seen with ERα, ERαΔ3, ERβ1 or ERβ2 expression between non-progressive and progressive tumours. The potential role of these ER splice variants warrants further investigation particularly in the prediction of a tumour to respond to endocrine therapy.

O-78. Importance of methodology in plasma oestradiol measurements: applications in breast cancer research and management

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Measurement of plasma oestradiol levels is important in the development and application of endocrine treatments for breast cancer and may be valuable in the evaluation of breast cancer risk. However the accurate assessment of oestradiol at the low levels found in postmenopausal women is complicated by the presence in plasma of high concentrations of cross-reacting, water-soluble conjugated steroids. Application of inappropriate methodology can lead to the introduction of substantial bias, which limits the accuracy, and interpretation of results. In a study designed to assess the effect of the aromatase inhibitor anastrozole on plasma oestradiol levels, we measured oestradiol using two commercially available direct methods (Beckman Coulter Access Immunoassay System and Diagnostic systems laboratories DSL-39100 radioimmunoassay) and two indirect methods (radioimmunoassay with ether extraction). Anastrozole inhibits aromatase, the only source of postmenopausal oestradiol, by a mean 97%. The two direct assays gave oestradiol values that fell after treatment, by a mean 25% and 34%, respectively. In contrast using a sensitive indirect assay 88% suppression was found. Values obtained with this assay have been validated against those obtained using tandem mass spectrometry. The results of this study indicate that at least 70% of the oestradiol measured by the direct assays was an artefact. Application of an extraction step prior to the use of the DSL-39100 kit led to the elimination of this bias. The relationship between plasma oestradiol and breast cancer risk may potentially have an important and widespread application in association with anti-hormonal strategies for breast cancer prevention. However it is important to recognise the deficits in some types of methodology for the quantification of oestradiol in its application to postmenopausal women.

O-79. Zinc-dependant activation of C-SRC, EGFR and IGF-1R mitogenic pathways in Tamoxifen-resistant

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Zinc is essential to normal cell growth and present in elevated levels in breast cancer tissue. Recent evidence suggests zinc can activate growth factor signalling pathways such as MAPK and EGFR. Tenovus have developed a tamoxifen-resistant breast cancer cell line which has evaded growth inhibition by tamoxifen by utilising both the EGFR and IGF-1R signalling pathways. This tamoxifen-resistant cell line has increased intracellular zinc levels and affymetrix array analysis shows increased levels of the ZIP family of zinc influx transporters. Treatment of breast cancer cells with 0–100 μM zinc demonstrated a dose- and time-dependent activation of EGFR at tyrosines 1068 and 845, abolished by both the zinc chelator TPEN and the c-Src kinase inhibitor Su6656. This activation is present in the absence of stimulation by EGF and is accompanied by a parallel zinc-dependant activation of c-Src. We demonstrate downstream activation of ERK1/2 and IGF-1R signalling by the addition of zinc. Fluorescent microscopy visualised $EGFR^{Y845}$ in cells after zinc treatment and the results confirm activation and plasma membrane localisation of activated EGFR after treatment with zinc. Interestingly, activated EGFR shows co-localisation with Vinculin in focal adhesions and an increased motility and invasiveness of tamox-

ifen resistant cells after zinc treatment. These results suggest a mechanism whereby cells can continue to grow in the presence of tamoxifen and may provide a useful new therapeutic target for anti-hormone resistant breast cancer.

O-80. The introduction of better therapies lengthened survival in advanced breast cancer

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Over the last 20 years new endocrine, cytotoxic and biologically targeted agents have been introduced into breast cancer therapy, Claims for their greater efficacy over the agents used in the 1980's have been based on the result of clinical trials in the adjuvant setting and in advanced disease.

Comparison is made between the time from implementation of therapy to death, for symptomatic distant metastases, in breast cancer diagnosed in 1980-86 ($n = 428$) and in those diagnosed as previous in 1990-99 ($n = 280$),

Although the assumptions here are made that the secondary therapy will have been applied earlier in the 80-86 dataset, this is open to bias.

Therefore another analysis has to be carried out of only those women in these datasets who had distant metastases diagnosed within 5 years of the primary tumour.

Table 1. All Distant Recurrence

Dataset	Median time from DR to death
1980-86	12
1990-96	12

Table 2. Distant recurrences

Dataset	Median time from DR to death
1980-86	10
1990-96	11

Although more systemic therapies appear advantageous in the adjuvant setting there is little evidence of this greater efficacy in the treatment of distant metastases.

O-81. Fulvestrant in pretreated patients with advanced breast cancer: experience from the Institut Bergoni

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Background: Fulvestrant ('Faslodex') is a new oestrogen receptor antagonist with no agonist effects that is licensed for use in patients with advanced breast cancer (ABC) following progression on an antioestrogen.

Methods: The fulvestrant Compassionate Use Programme (CUP) permits use of the drug in patients who have progressed on prior endocrine and chemotherapies for ABC. As part of the CUP, 41 women and one man were treated with fulvestrant between August 2001 and September 2004 according to the guidelines of the French Drug Agency (AFSSAPS).

Results: Patients had a median age of 65 years (range 41–86 years) and all had ABC, including 19 patients with visceral metastases (liver and lung) and 25 patients with bone metastases. Sixteen patients received adjuvant endocrine treatment (tamoxifen) and three patients received adjuvant chemotherapy. Fulvestrant was given after a median of 3 (range: 1–5) prior endocrine treatments and a median of 1 prior (range: 0–5) chemotherapy for ABC. Twelve patients had a partial response (PR) with fulvestrant and 10 had stable disease $\geq$6 months (SD), giving an overall clinical benefit (CB) rate of 52%. Five of the six (83%) patients who received fulvestrant as 2nd-line endocrine therapy for ABC gained CB (2 PR, 3 SD). The remaining 36 patients received fulvestrant as 3rd to 9th-line endocrine ABC treatment (CB rate: 47%; 10 PR, 7 SD). In patients with visceral metastases the CB rate was 58% (6 PR, 5 SD). All patients have now ceased fulvestrant treatment; the median duration of treatment was 5 months (range: 1–38 months). Following fulvestrant, two patients received further endocrine therapy (progestins) and 21 received palliative chemotherapy. Fulvestrant was well tolerated; six patients (14%) experienced adverse events during treatment.

Conclusions: In our experience, fulvestrant is effective and well tolerated in the treatment of patients with ABC following progression on prior therapies. The CB rate appeared highest when fulvestrant was given early in the therapy sequence; however, efficacy was also retained in more heavily pre-treated patients.

O-82. Goserelin plus Anastrozole as first-line endocrine therapy for premenopausal oestrogen receptor positive (ER+) advanced breast cancer (ABC)

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We have previously reported the use of goserelin plus anastrozole as second-line endocrine therapy for premenopausal ER+ ABC. With randomised data showing superiority of third-generation aromatase inhibitors over tamoxifen as first-line therapy, we now report our clinical experience of using anastrozole alongside ovarian suppression (with goserelin) in the same setting in premenopausal women.

Twenty premenopausal patients (mean age = 42 (30–57) years) (advanced primary = 3, soft tissue = 2, bone = 8, pleura/lung = 3, stomach = 1, liver = 2, bone + liver = 1) with ER+ ABC seen over a 3-year period were treated with goserelin 3.6 mg 4-weekly plus anastrozole 1 mg daily as first-line therapy. Endocrine therapy was considered therapy of choice except in two patients with liver metastases who did not have chemotherapy due to pulmonary embolism or patient choice. All had disease assessable by UICC criteria and received therapy for $\geq$6 months (except for those who progressed prior).

Twelve patients (60%) derived clinical benefit (CB) (complete ($N = 1$) or partial ($N = 5$) response, or stable disease ($N = 6$) for $\geq$6 months) while eight progressed before 6 months. For the 12 CBs, the median duration of response is 20+ months (6–36 months). At the time of analysis, therapy is continuing in nine patients. When the two patients with liver metastases for whom chemotherapy was therapy of choice were excluded, the CB rate rose to 66%. Therapy has been well tolerated and no patients came off it because of side effects.