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-