O-33 Is the difference between tamoxifen and anastrozole in adjuvant trials applicable for primary endocrine therapy of early operable primary breast cancer in the elderly?

A. Agrawal*, M. Ying, L. Winterbottom, D.A.L. Morgan, I.O. Ellis, K.L. Cheung. *University of Nottingham and Nottingham City Hospital, UK*

Background: The optimal management of predominantly estrogen receptor (ER) positive primary breast cancer (PBC) in the elderly remains controversial but use of tamoxifen as "primary endocrine therapy" is well established largely due to high proportion of elderly patients either refusing or being unfit for surgery. Yet unexplored in literature, we compare use of tamoxifen with anastrozole in this clinical setting.
Methods: Over 2 years period, elderly patients (>70 years) with UICC assessable ER+ operable PBC (<5 cm), had tamoxifen for ⩾6 months, unless they progressed prior. Anastrozole was used when there were contraindications to tamoxifen (eg, thromboembolic, gynecological risks).
Results: See the table.

Total = 93 patients	Anastrozole (n = 58)	Tamoxifen (n = 35)	p-value
Clinical Benefit (CR+PR+SD) at 6m	96.6% (56/58)	100% (35/35)	0.525
Objective Response (CR+PR) at 6m	43.1% (25/58)	31.4% (11/35)	0.282
Median Duration of response (months)	14.9 (6.0–57.0)	18.4 (7.0–39.2)	0.187
Median Time to progression (months)	15.2 (12.1–25.9)	16.5 (12.1–20.8)	0.304
Median ER H-score	240 (10 to 300)	260 (80 to 300)	0.398

CR = complete response; PR = partial response; SD = stable disease

At a median follow-up of 14.8 (6–57) months, 8 patients have progressed. Treatment was well tolerated in both groups and no patients withdrew due to side-effects.
Conclusion: In this observational non-randomised study, there was no significant difference in efficacy between tamoxifen and anastrozole, in contrast to results from adjuvant trials. Superiority of aromatase inhibitor was less apparent perhaps due to highly rich ER+ tumors (on semi-quantitative scoring of immunhistological assays) in this age-group.

O-34 Nuclear and cytoplasmic ER beta2 expression identifies distinct prognostic outcome in breast cancer patients

A.M. Shaaban, A.R. Green, M.B. Peter, S. Karthik, Y. Alizadeh, I.O. Ellis, J.F. Robertson, E.C. Paish, P.T.K. Saunders, N.P. Groome, V. Speirs*. *Leeds Institute of Molecular Medicine, Universities of Nottingham, Edinburgh and Oxford Brookes, UK*

Previous conflicting results regarding the prognostic significance of ER alpha in breast cancer may be explained by contribution of isoforms, of which 5 exist. Our aim was to elucidate the prognostic significance of ER beta2 by immunohistochemistry in a large cohort of breast carcinomas with long term follow up. Seven hundred and fifty seven cases, represented on tissue microarrays were stained with a specific, well-characterised ER beta2 antibody (Serotec) and scored either as continuous variables or using the Allred system. Nuclear and cytoplasmic staining was evaluated and correlated with histopathological characteristics, overall (OS) and disease free survival (DFS). Nuclear ER beta2 expression correlated significantly with OS ($P = 0.006$) and DFS ($P = 0.013$). ER beta2 also predicted response to endocrine therapy ($P = 0.036$), correlated positively with ER alpha, PR, AR, BRCA1 and inversely with metastasis and vascular invasion. Tumours co-expressing ER beta2 with ER alpha had better OS and DFS than ER alpha or ER beta2 alone. Cytoplasmic ERbeta2 expression, whether alone or in combination with nuclear staining, predicted significantly worse OS. Notably, patients with only cytoplasmic ER beta2 expression had the worst outcome of all ($P = 0.0014$). This data is currently being validated in an independent TMA dataset. Our data indicate that ER beta2 is a powerful prognostic indicator in breast cancer and also highlights for the first time, the importance of cytoplasmic as well as nuclear expression in dictating outcome. Measuring nuclear and cytoplasmic ER beta2 in clinical breast cancer could thus provide a more comprehensive picture of patient outcome, complementing ER alpha.

O-35 Pattern of oestrogen receptor (ER)-positivity in elderly breast cancer: comparison with younger age

K.L. Cheung*, D.A.L. Morgan, A.W.S. Wong, H. Parker, I.O. Ellis. *University of Nottingham and Nottingham City Hospital, UK*

ER-positivity in breast cancer increases with age. Some clinical data show that radiotherapy can be safely avoided following lumpectomy in elderly patients taking tamoxifen; and that there is negligible benefit of adjuvant chemotherapy for patients approaching 70 years. Biological differences (with ER being an important one) may be contributory. This study aimed to analyse the pattern of ER-positivity in the elderly population.
Among 2,061 patients >70 years with operable primary breast cancer managed in a dedicated clinic from 1987, there were 1,557 tumours which had ER data available. Their pattern was compared with that in 2,674 tumours from younger (⩽70 years) counterparts treated during the same period. Histochemical (H)-score was measured using standard immunohistochemical assay by the same team of pathologists.
The patterns of ER-positivity are shown:

H-score	⩽35 years (n = 109)	>35–50 years (n = 903)	>50–70 years (n = 1,662)	>70 years (n = 1,557)
0	52.3%	29.5%	19.6%	18.2%
>0–50	5.5%	5.1%	3.2%	1.7%
>50–100	5.5%	12.5%	8.2%	4%
>100–200	31.2%	47.6%	46.2%	31.7%
>200–300	5.5%	5.3%	22.8%	44.4%
Total	100%	100%	100%	100%

In all age groups there is a marked biphasal distribution of ER-positivity, but in patients >70 years this is more marked, with a preponderance of highly ER-positive tumours, and a substantial minority being ER-negative (H = 0), with very few in intermediate groups. Endocrine therapy is clearly appropriate for the highly ER-positive majority, but management of those ER-negative ones is a challenge.

O-36 The potential role of ER beta in stromal regulation of mammary carcinogenesis

C. Green*, T.A. Hughes, D.J. Scott, A.M. Hanby, A.M. Shaaban, V. Speirs. *University of Leeds, UK*

Breast tumour proliferation, invasion and metastasis are influenced by the surrounding stroma and fibroblasts comprise the major constituent of this. The aim of this study was to establish if, and how, stromal mammary

fibroblasts affect mammary epithelial cells using a coculture cell model. Benign (HB2) and malignant (MCF7) cells were stably transfected with a GFP expression plasmid, to enable subsequent differentiation from mammary fibroblasts in a coculture model, using flow cytometry. Cell proliferation was compared between epithelial and fibroblast cocultures when grown alone or in coculture using an endothelial cell line as a control. Malignant epithelial cell proliferation was enhanced by coculture with primary mammary fibroblasts, whereas benign epithelial cells showed dramatic proliferative inhibition. Two separate experiments were performed using each cell type and statistically significant p values were obtained: HB2 $p = 0.05$, $p < 0.001$; MCF7 $p = 0.001$, $p = 0.019$. The endothelial cocultures inhibited both mammary epithelial cell lines to a similar degree, suggesting that this was a mammary-specific event. Fibroblasts expressed multiple isoforms of ER beta by RT-PCR; additionally ER beta1 and beta2 were detected using Western blotting. Using a specific ER beta agonist (DPN) we noted changes in fibroblast proliferation and migration in response to wound healing and this is currently being extended to our coculture model using siRNA to knockdown ER beta. In conclusion, fibroblasts exert differential proliferative effects on mammary epithelial cells determined by their tumourigenicity and this may be mediated by ERbeta.

O-37 Fulvestrant versus exemestane following prior non-steroidal aromatase inhibitor therapy: efficacy and tolerability results from effect

J.F.R. Robertson*, on behalf of the EFECT trial investigators. *Nottingham City Hospital, UK*

Background: Non-steroidal aromatase inhibitors (AIs; anastrozole and letrozole) are increasingly used as adjuvant and first-line advanced treatments for postmenopausal women with hormone receptor-positive (HR+) breast cancer. As many patients subsequently progress, it is important to identify agents with efficacy after non-steroidal AI failure.
Methods: EFECT is a randomised, double-blind, double-dummy, multicentre, Phase III trial comparing the efficacy and tolerability of fulvestrant (Faslodex®) vs exemestane (Aromasin®) in postmenopausal women with HR+ advanced breast cancer (ABC) progressing/recurring after prior non-steroidal AI therapy. Fulvestrant loading-dose (LD) regimen was used: 500 mg on Day 0, 250 mg on Days 14, 28, and 250 mg every 28±3 days thereafter. Exemestane 25 mg was given once daily. Treatment continued until progression, death or withdrawal.
Results: 693 women were randomised to fulvestrant ($n = 351$) or exemestane ($n = 342$). ~60% of patients had received $\geqslant 2$ prior endocrine therapies and ~60% had visceral involvement. Median time to progression was 3.7 months in both groups (hazard ratio: 0.963; 95% confidence intervals: 0.819, 1.133; $p = 0.6531$). Objective response and clinical benefit rates (CBR) were also similar between groups. Median duration of response was 13.5 months vs 9.8 months for fulvestrant and exemestane, respectively. Fulvestrant and exemestane had good activity in patients with visceral disease (CBR 29.1% vs 27.2%, respectively). Both treatments were well-tolerated, with no significant differences in the incidence of pre-specified adverse events (weight gain, increased appetite, hot flushes, joint disorders, nausea/vomiting, diarrhoea, androgenic effects, injection-site reactions). Steady state plasma levels were reached within 1 month with the LD fulvestrant regimen.
Conclusions: Fulvestrant LD offers an effective and well-tolerated treatment option for postmenopausal women with ABC (including those with visceral disease) who progress/recur on non-steroidal AI therapy.

O-38 Influence of a basal phenotype on the metastatic pattern of breast cancer

A.A. Luck*, A.J. Evans, A.R. Green, E.A. Rakha, C. Paish, I.O. Ellis. *Nottingham City Hospital, UK*

Purpose: To assess whether basal phenotype (BP) influences metastatic pattern and survival in patients with metastatic breast cancer.
Materials and Methods: The BP status of a well-characterised series of consecutive primary operable breast cancers (1800 cases) was ascertained using basal CK5/6 and CK14. Follow-up data including time, site and pattern of distant metastasis and post-metastasis survival were available for 113 women with BP cancers and those were compared with 178 matching cases from women in the non-BP (NBP) group.
Results: Patients with the BP were more likely to present with intrapulmonary (25/48, [52%] BP vs. 15/64, [23%] NBP; $p = 0.0009$) and/or brain metastases (20/113, [18%] BP vs. 3/178, [2%] NBP; $p < 0.0001$). Patients with NBP were more likely to present with bone metastases in the absence of visceral disease (48/102, [47%] NBP vs. 14/62, [23%] BP; $p = 0.0017$). There was no significant difference in the frequency of pleural or liver metastases between both groups. BP was also associated with a shorter median survival with metastatic disease (10.1 months vs. 25 months, $p < 0.001$). Multivariate analysis including other established prognostic variables in metastatic breast cancer shows that BP is an independent poor prognostic factor.
Conclusion: Intrapulmonary and brain metastases are seen more frequently at metastatic presentation in BP breast cancer patients, and the BP is associated with a poorer survival after metastatic presentation. Thus assessment of basal CKs may provide valuable prognostic information that may affect patients' management.

O-39 Morphological and molecular evolutionary pathways of low and high grade breast cancers and theier putative precursor lesions

T. Abdel-Fatah*, D.G. Powe, Z. Hodi, J.S. Reis-Filho, A. Lee, I. Ellis. *University of Nottingham and Institute of Cancer Research, London, UK*

In this study, further support for our proposed route of pathogenesis concerning low grade and high grade breast cancers (LGBC and HGBC) and their precursor lesions was provided using immunophenotyping of tissue microarrays containing 790 lesions for putative tumour suppressor genes, cell cycle regulators, proliferation and differentiation markers. The putative precursor lesions were compared with their matching normal gland and invasive lesions.
Results: The epithelial cells in the flat epithelial atypia, Lobular neoplasia, ADH/ low grade DCIS and the intrinsic LGBC shared a common phenotype of CK19/18/8, ER-α, Bcl-2, and Cyclin D1 positivity. The ER-α/ER-β ratio and Cyclin D1 expression increased from precursor lesions to the invasive LGBC.
Conclusion: Our findings support the concept that FEA is the committed precursor cell lesions of LGBC/ILC. These may represent a family of precursor, in situ and invasive neoplastic lesions belonging to the luminal 'A' subclass of breast cancer. Our results suggest that the committed progenitor cells (PCs) for low grade breast neoplasia are CK19/18/8 positive and exhibit ER-α mediated CCND1 and BCL2 gene expression. Alternatively, breast cancer