ALCAM protein expression. A modified H-score system was used to enumerate the intensity and proportion of cytoplasmic and membranous staining in the neoplastic component, using a number of tumour stratification approaches. Univariate and multivariate analysis was used to examine the association between ALCAM expression and clinicopathological parameters.
Results: ALCAM under-expression was significantly related to increased histological grade ($p = 0.010$) and distant metastases ($p = 0.035$) but not recurrence ($p = 0.995$). ALCAM under-expression was associated with significantly worse overall survival ($p = 0.022$) and an odds ratio of 0.56 compared to 2.12 for the Nottingham Prognostic Index (NPI).
Conclusion: Loss of the cell adhesion molecule ALCAM is a significant predictor of poor survival in breast cancer. A possible explanation for this observation is enhancement of metastatic tumour spread resulting from the loss of cell-to-cell adhesion affected by ALCAM.

O-94 Stat3 expression is a poor prognostic marker for invasive breast cancer

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Introduction: Signal Transducer and Activator of Transcription 3 (STAT3) is a cell cycle regulatory transcription factor over-expressed by some breast cancers. STAT3 activation contributes to cell survival and resistance to apoptosis and radiation treatment due to its relation with the expression of survivin and Bcl-2. Inhibition of its action results in apoptosis and radio sensitisation of breast cancer cells. The aim of this study is to determine if the expression of STAT3 is a significant prognostic marker in invasive breast cancer.
Methods: Consecutive patients with invasive breast cancer undergoing surgical excision during a 12 month period were selected. STAT3 expression was assessed using a novel immunohistochemistry (IHC) technique on paraffin embedded samples and follow up data of the cohort was recorded.
Results: Of the 205 cases assessed, 151 cases were STAT3 negative and 54 cases expressed STAT3. STAT3 expression was associated with node positive disease ($p \leqslant 0.001$), ER negative tumours ($p \leqslant 0.01$), a high NPI ($p \leqslant 0.001$) but not with HER2 expression ($p \geqslant 1.0$). The 5yr survival was poorer in STAT3 positive patients compared to STAT3 negative patients (72.3% c.f. 85.3%) ($p = 0.04$). The co-expression of STAT3 and HER2 decreased 5yr survival by 16.5% compared to HER2 positive STAT3 negative patients (see table 1).

Table 1. Co-expression of HER2 and STAT3 and 5 yr survival

	HER2 Negative	HER2 Positive
STAT3 Negative	88.1%	76.5%
STAT3 Positive	80.6%	60%

Conclusion: The elevated expression of active STAT3 is a marker of poor disease outcome and should now be considered as a prognostic marker. The STAT3 status of HER2 positive breast cancers and its implication for survival is an important finding.

O-95 TOPO II is an independent predictor of survival in unselected breast cancer

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Introduction: The identification of new biomarkers is contributing to improvements in the predicting the response to treatment in breast cancer. Topoisomerase II alpha (*TOP2A*) is involved in the relaxation of DNA during replication and transcription. Several studies suggest that amplification of the *TOP2A* gene associates with response to anthracycline-based chemotherapy, however few studies have analysed the prognostic impact of TOP2A in a population based cohort of breast cancer patients.
Aim: To assess *TOP2A* as a predictor of survival in patients with breast cancer, comparing fluorescent (FISH) and chromogenic (CISH) in situ hybridisation techniques in high throughput tissue microarrays (TMA).
Materials and Methods: Tissue microarray (TMA) sections containing 183 and 269 well-characterised unselected breast tumours were subjected to fluorescent (FISH) and digoxigenin (CISH)-labelled *TOP2A* in situ hybridisation, respectively. *TOP2A* amplification was defined by 50% or greater of at least 30 neoplastic nuclei showing 5 or more signals in the CISH-treated sections, or by a ratio of >2 for cancer cells probed by FISH, compared to a chr17 centromeric probe. Uni- and multivariate analysis was used to test for association between *TOP2A* and clinical parameters.
Results: FISH and CISH showed excellent agreement ($p = 0.0001$), with 20 and 25 tumours showing amplification by *TOP2A* FISH and CISH, respectively. *TOP2A* amplification associated with high tumour grade (>0.05), metastases (>0.03), recurrence ($p > 0.015$), disease-free interval ($p = 0.002$), and overall survival (OR = 2.69).
Conclusion: *TOP2A* is an independent predictor of survival, equalling or bettering the Nottingham Prognostic Index. *TOP2A* gene status assessment by FISH and CISH techniques show excellent correlation.

O-96 Accelerated E-CMF (accE-CMF) chemotherapy with pegfilgrastim support in early stage breast cancer is associated with low incidence of severe lymphopenia

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Background: Accelerated chemotherapy, doxorubicin-cyclophosphamide followed by paclitaxel (accAC-P), with GCSF support, has been associated with high incidence of grade 3 or 4 lymphopenia (>60% patients), possibly contributing to a risk of pnemocystis carinii pneumonia (Tolaney SABCS 2006). We have recently conducted a study to explore the feasibility of accelerating E-CMF, reporting dose intensity and toxicity data (Rea ASCO 2007).
Methods: Patients with early breast cancer were treated with two chemotherapy schedules. Schedule A comprised epirubicin 100 mg/m2 repeated every 14 days (d) with pegfilgratim (P) on day 2 for 4 cycles followed by CMF 600/40/600 mg/m^2 day 1&8 with P on day 9 repeated every 21d for 4 cycles. Schedule B had the same epirubicin schedule followed by six cycles of CMF 800/50/600 mg/m^2 with P on day 2 repeated every 14d. FBC were recorded every 7d. We analysed lymphocyte data from 2 centres, 21 patients had schedule A and 19 schedule B. We also examined day 1 lymphocyte counts from 40 patients treated with conventional E-CMF.

Results: Lymphocyte count declines with successive cycles of accelerated (mean: baseline=2.2; final cycle=1.3, $p<0.001$) and conventional E-CMF (mean: baseline=2.0; final cycle=1.2, $p<0.001$). Mean decline did not differ significantly by E-CMF schedule.

Table: Highest grade lymphopenia per patient

	CTCAE (v3)		
	G2 ($<0.8\times10^9$/L)	G3 ($<0.5\times10^9$/L)	G4 ($<0.2\times10^9$/L)
Schedule A	7/21 (33%)	5/21 (23%)	0/21 (0%)
Schedule B	9/19 (47%)	4/19 (21%)	0/19 (0%)
Conventional E-CMF	9/40 (22%)	0/40 (0%)	0/40 (0%)

Weekly blood counts Day 1 only.

Conclusion: E-CMF is associated with progressive lymphopenia irrespective of schedule, the lymhpopenia observed is modest compared to accAC-P.

O-97 Attitudes towards neoadjuvant endocrine therapy and breast conserving surgery (BCS) in the elderly

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Introduction: There is little published information about the views of women aged over 70 with breast cancer regarding attitudes towards neoadjuvant endocrine treatment and breast conservation and factors that influence their choice of surgery.
Methods: A questionnaire was sent to 180 patients who were aged 70 or over when they had breast cancer surgery (122 mastectomy; 58 BCS). Responses were received from 111 (62%). Of these, 71 patients had a mastectomy (64%) and 40 had BCS (36%).
Results: 50% of patients who had mastectomy said they would have taken neoadjuvant endocrine therapy to facilitate BCS. 46% of them said that the possibility of local recurrence following BCS influenced their decision. Only 20% of patients felt that having to travel a long distance to attend for post operative radiotherapy put them off BCS. Nearly half the patients in both groups said that they were worried about the cosmetic and psychological effects of a mastectomy when they were told they had breast cancer. 98% of patients who had BCS said they were happy with their decision. Of these, 70% were happy/very happy with the cosmetic outcome.
Conclusions: Elderly patients with breast cancer are interested in considering breast conservation and half would be willing to take neoadjuvant endocrine therapy to facilitate this. Few patients are deterred by post-operative radiotherapy.

O-98 Role of the chemokine receptor CXCR4 in breast cancer metastasis

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Breast cancer cells express the chemokine (chemotactic cytokine) receptor CXCR4. There is compelling evidence that CXCR4 is a key mediator of breast cancer progression. Binding of the chemokine CXCL12 to this receptor stimulate cells to migrate out of the vasculature and establish metastasis. We aim to assess the metastatic potential of breast cancer cells by altering its expression of CXCR4.
LMD-MB-231, a low CXCR4 expressing rederived sub-line of the human breast cancer cell line MDA-MB-231, was transfected with CXCR4 by electroporation. Functional assessment of the receptor was performed with calcium flux and chemotaxis assays towards CXCL12. An *in vivo* model was used to evaluate the metastatic potential of the transfected cells (trasfectants) compared to the wild type cells. 200,000 cancer cells of either type was injected intravenously into 2 groups of SCID mice ($n=5$ each). On day 28, the mice were examined microscopically to assess tumour load in the lungs and liver.
Flow cytometry confirmed increased expression of CXCR4 on the stable transfectants. At 50 nM concentration of CXCL12, the transfectants fluxed calcium and demonstrated migration (chemotactic index 1.5) towards the chemokine. *In vivo*, the group injected with the transfectants initially demonstrated increased number of metastasis (haematoxylin and eosin staining). However, cytokeratin (epithelial cell marker) staining did not show any significant difference in metastasis.
We believe the basal levels of CXCR4 in the wild type cells may be enough to cause metastasis. We are now attempting to down-regulate this basal CXCR4 expression and will compare metastasis between the up-regulated and down-regulated cells *in vivo*.

O-99 The mTOR (mammalian target of rapamycin) inhibitor RAD001 (Everolimus) is safe and reduces proliferation in postmenopausal women with breast cancer

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Background: mTOR plays a key role in tumour cell cycle proliferation and survival. RAD001 (everolimus) is a rapamycin derivative that inhibits mTOR and its downstream substrates. This study explored, in vivo, RAD001 action in breast cancer.
Methods: 30 post-menopausal women with early breast cancer were given 5 mg RAD001 once daily for 14 days prior to surgery. Biopsies were taken at diagnosis and at surgery (post 14 days of treatment) and assessed for changes in proliferation (Ki67), pAkt (s473), pS6k (s235/236 and s240/244), p-mTOR, ER, and PgR.
Results: Five patients withdrew during the two week treatment period due to adverse events. All adverse events were grade 1 or 2 on the NCIC-CTC scale.
RAD001 treatment significantly decreased proliferation (Ki67, $p=0.024$). p-Akt was reduced in cases with high pre p-Akt scores but increased in patients with low pre pAkt scores. Pre p-Akt correlated significantly with reduction in proliferation (Ki67, $p=0.001$; Pearsons correlation coefficient 0.688). p-S6k staining was reduced independently of Ki67.
Discussion: RAD001 is safe and tolerable in postmenopausal early breast cancer patients. RAD001 inhibits the mTOR pathway and its downstream effectors, and significantly reduces tumour cell proliferation. High levels of p-Akt at diagnosis correlate with greater reductions in proliferation suggesting p-Akt activation as a predictive marker of mTOR activation and therefore RAD001 efficacy.

O-100 A brief review of the dermatological and gastro-intestinal toxicities of Lapatinib

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Background: Lapatinib is a dual (ErbB-1 and ErB-2) receptor tyrosine kinase inhibitor recently approved by FDA for Her2 positive metastatic breast cancer (MBC) patients (pts) pre-treated with anthracycline/taxane/trastuzumab