Conclusion: Her-2 overexpression is rarely if ever, present in patients who belong to the Excellent/Good Prognostic Groups (ie small, grade 1–2 tumours). Survival of in MPG I, II or PPG is significantly worse irrespective of the application of adjuvant therapies.
These data demonstrate that it is not useful or cost effective to apply testing to all cases of early breast cancer but rather those falling into poorer prognostic groups.
Also that chemotherapy does not correct the survival discrepancy between Her-2 positive and negative cases.

O-90 Prognostic significance of vascular endothelial cell growth factor (VEGF) -A, -C and -D in breast cancer and their relationship with angio- and lymphangiogenesis

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Vascular endothelial cell growth factors (VEGF)-A, VEGF-C and VEGF-D have potent angiogenic and lymphangiogenic functions in experimental models however their role in the progression of human breast cancer is unclear. The aims of the current study were to examine the relationship between the expression of the aforementioned VEGFs with the angiogenic and lymphangiogenic characteristics of breast cancer and to assess their suitability as potential prognostic factors.
Paraffin embedded sections of 177 primary invasive breast cancer, with complete clinical follow up information for 10 years, were stained for VEGF-A, -C, -D, podoplanin (to assess lymph vessel density (LVD)) and CD34 (to assess microvessel density (MVD)) using standard immunohistochemical approaches. The expression of the VEGFs was correlated with clinicopathological criteria, LVD, MVD and patients' survival.
High expression of VEGF-A, -C and -D was detected in 40%, 37% and 42% of specimens respectively. High expression of VEGF-A and VEGF-C, but not of VEGF-D, was associated with a higher LVD ($P = 0.013$ and $P = 0.014$ respectively), a higher MVD ($P < 0.001$ and $P = 0.002$ respectively), the presence of lymph node metastasis ($P < 0.001$ and $P < 0.001$ respectively), distant metastasis ($P = 0.010$ and $P = 0.008$ respectively) and shorter OS ($P = 0.029$ and $P = 0.028$ respectively).
In conclusion, breast cancers that express high levels of VEGF-A and VEGF-C are characterized by a poor prognosis, likely though the induction of angiogenesis and lymphangiogenesis. Examination of expression of VEGF-A and VEGF-C in breast cancer may help to identify a subset of tumours that have a higher probability of recurrence and metastatic spread.

O-91 Prognostic estimation: re-analysis of data from cases diagnosed in 1990–99 by Cox proportional hazards method

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The original Nottingham Prognostic Index (NPI) was based on a multivariate analysis by the Cox proportional hazards method, of 9 potential prognostic factors and described in 1982. Based on the 3 factors retaining independent significance the NPI is Grade (I-III) + LN Stage (1–3) + maximum diameter (cm $\times 0.2$).
The survival figures have been re-examined for each prognostic group for cases diagnosed in the 1990's (presented at this meeting) and the NPI still separate to six groups with significantly differing survivals, with wide separation between the best and worst groups.
The new analysis now identifies 5 factors showing independent significance and their relative contribution to h azard: the structure of the formula is preserved but a revised index may be calculated as: LN status (LN neg scores 1, LN neg LVI+ 1.5, LN 1–3 scores 2, LN 4+ scores 3) + Grade (I scores 0.7, II 2, III 3, III basal 3.4) + Size (cm x 0.2) + 0.6 for HER2neu positivity.
Using the revised index 20% of women have a change in their prognostic group (ie) a difference in their survival estimate of between 4 and 15%. However the change loses the simplicity of the NPI and for most women does not make a sufficient difference to alter treatment decisions.

O-92 UPARAP/ENDO180 expression in invasive breast carcinoma and its relation to patient outcome

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Introduction: Local growth, invasion, and metastasis of malignancies of invasive breast carcinoma involve extensive degradation and remodelling of the surrounding, collagen-rich connective tissue. Urokinase plasminogen activator receptor-associated protein (uPARAP)/Endo180 is an endocytic receptor recently shown to play a critical role in the uptake and intracellular degradation of collagen by mesenchymal cells. However, the expression of this protein and its clinical significance in breast cancer is unknown.
Methods: immunohistochemistry was used to investigate the expression of (uPARAP)/Endo180 in tissue microarrays of a large (n = 880) well-characterized series of human breast carcinomas using blinded semiquantitative scoring, in addition to a set of well known biological markers in breast cancer.
Results: (uPARAP)/was expressed in (5.7%) of invasive breast cancer, and in (78.8%) in the stroma surrounding these tumours. Positive expression of Endo 180 in the tumour cells was significantly correlated with negative steroid receptor as, ER (P = 0.013), and AR (P = 0.001), negative luminal cytokeratines like CK7/8(P = 0.041). further more, a positive correlation was found between Endo180 expression and basal subtype of breast carcinoma (P = 0.003). In addition to the association between its expression and shorter disease free interval (P = 0.01).
Conclusion: The association between (uPARAP)/Endo180 expression in malignant cancer cells with basal phenotype and its association with poor patient outcome could explain the aggressive behaviour of these types of tumour.

O-93 ALCAM is an independent predictor of survival in unselected breast cancer

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Introduction: Breast cancer is a heterogeneous disease and their complexity is demonstrated by the anomalies seen in their classification using histological-based criteria. There is a need to develop a molecular classification system to predict tumour behaviour, thus providing information on patient outcome and therapeutic response. To achieve this, key molecules associated with cancer biology need to be assessed as putative biomarkers.
Aim: To assess Activated Leukocyte Cell Adhesion Molecule (ALCAM) as a prognostic indicator of survival in breast cancer.
Materials and Methods: Tissue microarray (TMA) sections containing 196 well-characterised unselected breast tumours were immunohistochemically stained to detect

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.