recurrence and 15 patients had bilateral recurrence, median time to recurrence was 3.2 years and median follow-up time was 6.2 years. A weak correlation was observed between cytoplasmic pAkt and cytoplasmic pNF-kB (cc 0.166, $P = 0.001$). Cytoplasmic pAkt expression was associated with decreased time to recurrence ($p = 0.025$) and was significantly higher in ER negative tumours compared to ER positive ($p = 0.004$). When cohort was split by PR status, the association with decreased time to recurrence and cytoplasmic pAkt was potentiated ($p = 0.008$). Cytoplasmic pAkt expression correlated significantly with nuclear pAkt expression (cc 0.696, $p < 0.001$). Nuclear pAkt expression was also associated with decreased time to recurrence ($p = 0.043$). In addition the observation with nuclear pAkt was potentiated in ER negative tumours ($p = 0.037$) and PR negative tumours ($p = 0.002$). No significant correlation with time to recurrence was observed for NF-kB or pNF-kB.

Conclusion: In the current cohort pAkt expression was associated with recurrence, however this was independent of the NF-kB cascade.

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O-15 UPREGULATION OF THE ESTROGEN PATHWAY IN ENDOCRINE SENSITIVE BREAST CANCER CELLS WITH HERCEPTIN TREATMENT

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Breast cancer is the leading cause of cancer-related deaths in women in Ireland. Receptor crosstalk has been implicated in the development of resistance to therapies and cancer relapse. We have previously shown that treatment of endocrine insensitive or independent breast cancer cells with herceptin repressed transcriptional activity of the oncogene c-Myc through SMRT activity. However, endocrine sensitive breast cancer cells with low levels of HER2 receptor showed hyperactivation of the estrogen/steroid pathway through recruitment of the cointegrator protein CBP.

The aim of this study was to demonstrate the activation of the steroid pathway in endocrine sensitive breast cancer cells treated with herceptin using the classical ER target gene pS2 as a marker of activity.

MCF-7 cells (high ER, low HER2, endocrine dependent) and LCC-1 cells (high ER, high HER2, endocrine independent) were treated with estradiol (E2), tamoxifen and herceptin. Semi-quantitative RT-PCR and qRT- PCR was performed to quantify pS2 mRNA levels. The impact of treatments on pS2 promoter activity was then assessed. Cells were transfected with the expression vector pSG5- ERα and the luciferse reporter plasmid pGL3-pS2 promoter and the level of transcriptional activity recorded. Increases in pS2 mRNA were found in MCF-7 cells treated with herceptin but not LCC-1 cells. This was replicated at a transcriptional level through luciferase assay.

We have shown that at mRNA and transcriptional levels treatment of MCF-7 cells with herceptin results in upregulation of the steroid pathway. We are currently conducting further molecular studies to further elucidate the signalling pathways involved.

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O-16 JAMA-A: A HOPE FOR BREAST CANCER THERAPY?

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Background: Breast cancer is a very prevalent disease with most cancers originating in the milk ducts, composed of a layer of polarized epithelial cells. Loss of polarity is a hallmark of many cancers including breast. We recently showed a novel correlation between over-expression of the cell adhesion protein JAM-A and poor prognosis in invasive breast cancer patients.[1]

Aim: To determine whether JAM-A regulates proliferation and polarity in breast cancer cells in a manner explaining its association with aggressive cancer phenotypes.

Materials and methods: Proliferation assays were carried out using the isogenic breast cancer cell line series HMT-3522, of S1 (normal) and T42 (invasive) cells in the presence of an inhibitory JAM-A antibody. Both cell types were grown in a 3-dimensional (3D) extracellular matrix culture model. Cultures were exposed to inhibitory JAM-A antibody to determine the consequences of antagonising JAM-A function for 3D polarization and differentiation.

Results: We observed significant anti-proliferative effects in both S1 and T42 cells exposed to JAM-A inhibitory antibody over time. Both S1 and T42 cells treated with JAM-A inhibitory antibody showed significant reductions in 3D spheroidal diameter relative to IgG-treated cells ($p < 0.05$), correlating with observed anti-proliferative effect. Furthermore, invasive T4-2 cells in 3D culture treated with JAM-A inhibitory antibody exhibited a partial normalization of phenotype.

Conclusions: Our results indicate that JAM-A inhibition decreases proliferation and promotes polarisation. Therefore, we speculate that pharmacological antagonism of JAM-A in breast cancer patients may offer a novel therapeutic opportunity.

Reference:

1. McSherry EA, McGee SF, Jirstrom K, Doyle EM, Brennan DJ, Landberg G, et al. JAM-A expression positively correlates with poor prognosis in breast cancer patients. *Int J Cancer* 2009;**125**(6):1343–51.

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O-17 BORDERLINE HER2 PROTEIN POSITIVE BREAST CANCERS HAVE SIMILAR PATIENT OUTCOME REGARDLESS OF HER2 GENE AMPLIFICATION STATUS

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HER2 plays an important role in breast cancer progression and provides predictive and prognostic information. However, prognostic information provided by IHC expression categories and prognostic value added by using in situ hybridisation (ISH) in borderline cases remains unclear.

We have assessed HER2 status in a large well-characterised breast cancer series prepared as tissue microarray ($n = 1858$) using IHC (HercepTest, DakoCytomation) and chromogenic ISH (CISH; DuoCISH, DakoCytomation) in order to identify relationships with clinico-pathological variables and patient outcome. None of these cases have received anti-HER2 therapy.

There was excellent overall concordance between HercepTest negative (scores 0/1+) and positive (3+) with CISH positive/negative (defined as HER2/Chr17 copy number ratio of $\geqslant 2$; $p < 0.001$). Twelve percent of cases were identified as HER2 positive (those with 3+ HercepTest scores or 2+ with gene amplification). Of the 74 borderline HercepTest 2+ cases, 44 cases (59%) showed HER2 gene amplification. We identified that HercepTest 2+ non-amplified cases were not significantly different from those amplified 2+ or 3+ cases with respect to their clinical outcome (BCSS and DFS).

The overall concordance between HercepTest and CISH analysis for HER2 status was excellent. All HercepTest 2+ cases identified were observed to have poor outcomes similar to those HercepTest 3+ cases regardless of gene amplification status. In the current clinical environment, cases exhibiting IHC 2+ with non-amplified HER2 gene status are not offered targeted HER2 therapy but do exhibit aggressive clinical behavioural characteristics and therefore optimal treatment strategies for these patients need to be determined.

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O-18 PARP1 EXPRESSION IN HORMONE ESTROGEN RECEPTOR NEGATIVE BREAST CANCER: PREFERENTIAL EXPRESSION IN BASAL-LIKE AND HER2-POSITIVE TUMOURS

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Nuclear poly (ADP-ribose) polymerases (PARPs) are a family of global monitor of chromatin structure and DNA damage repair. The relative expression levels of PARP1 protein in estrogen receptor negative (ER–ve) BC remain unclear. Therefore the aim of this study was to investigate PARP1 protein expression in ER–ve BC with relevance to molecular subtypes and disease outcome.

Methods: PARP1 protein expression was assessed, using immunohistochemistry, in a well-characterised series of ER–ve primary operable invasive BC cases ($n = 251$) with long term clinical follow-up. Results were correlated with molecular and clinicopathological parameters and patients' outcome.

Results: PARP1 nuclear expression was classified as high or low using a cut-off of 80 H-score, determined using X-Tile bio-informatics software. One hundred and thirty five (53.8%) of the informative cases were classified showing high expression. Significant positive correlation was found between PARP1 expression and BRCA1 expression ($p = 0.002$) but no association was found with p53. High PARP1 expression was observed in both basal-like (HER2– and CK5/6 and or EGFR+) (60%) and HER2+ (64%) compared with triple negative (TN) non-basal BC classes (45%). PARP1 expression was significantly associated with BC specific survival ($p = 0.01$). When analysis was assessed by the molecular subtype, the association of PARP1 expression with improved survival was restricted to TN tumours.

Conclusion: We have observed a positive correlation between PARP1 protein expression and BRCA1 expression. Although high PARP1 expression is seen in ER–/HER+ and HER– tumours, its association with survival was only found in the ER–/HER2– subtype. Thus, its targeted inhibition may particularly benefit patients with TN BC.

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O-19 COMPARISON OF IMPUTATION METHODS FOR MISSING IMMUNOHISTOCHEMICAL MARKERS IN A STUDY OF BREAST CANCER PROGNOSIS

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Background: Tissue micro-arrays (TMA) are increasingly used to generate data for studies of tumour molecular phenotype however, TMA data are particularly prone to missingness. A variety of methods to deal with missing data are available; these have been extensively evaluated using simulated data, but there has been no empirical evaluations of these methods using real TMA data.

Methods: We pooled data from over 11,000 cases of invasive breast cancer from five studies that collected information on seven prognostic indicators including four molecular markers, together with survival time data. We compared the results of a multi-variate Cox regression using complete case analysis (CCA), mean substitution (MS) and multiple imputation (MI). We also performed an analysis in which missing data were simulated under different assumptions.

Results: Over half the cases had data on at least one of the seven variables and 10.5% had missing data on 4 or more. The hazard ratio estimates based on multiple imputation and mean substitution, were similar with similar standard errors. Hazard ratio estimates and confidence intervals (Table). The results from CCA were less precise (wider confidence limits). Accuracy of the estimates was based on the simulated data with CCA having the least accurate results.

Hazard ratios and confidence intervals using mean substitution and multiple imputation.

Method	ER status	PR status	HER2 status	BCL2 status
Multiple imputation – HR (CIs)	0.43 (.35–54)	0.39 (.30–.50)	1.25 (1.13–1.40)	0.85 (.76–.96)
Mean substitution – HR (CIs)	0.40 (.32–.49)	0.37 (.29–.46)	1.31 (1.17–1.46)	0.82 (.74–.92)