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.