treatment with decitabine and TSA increased docetaxel sensitivity in MCF-7 and MDA-MB-231 cells ($P < 0.05$). An array of 84 genes identified 5 genes decreased in resistant cells whose expression was upregulated after decitabine and TSA treatment ($P < 0.05$). Western analysis confirmed expression changes in only one gene, SERPINE1, in docetaxel-resistant cells.

Conclusions: Docetaxel resistance is associated with changes in the DNA methylation machinery. Inhibiting DNA methylation and histone deacetylation, in combination, overcomes resistance of breast cancer cells to docetaxel. Our findings indicate that decreased SERPINE1 expression is associated with docetaxel resistance.

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O-12 TRANSLATIONAL EXPLORATION OF PIK3/Akt pathway activation in early invasive breast cancer

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The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is a frequently deregulated pathway in breast cancer (BC). Akt arguably relays a plethora of extracellular signals to modulate diverse biologic effects, including cell proliferation, growth, motility, and survival, downstream of PI3K activation. However, complexity and diversity in the upstream/downstream arms of this pathway challenge considerably the clinical evolvement of effective therapies.

This study aims to study expressions of PIK3CA and phospho-Akt1 (pAkt) in BC, with respect to proteins upstream/downstream of Akt activation, clinicopathologic parameters, and disease outcome. PIK3CA and pAkt (ser473) were evaluated by immunohistochemistry on tissue microarrays containing 1202 early invasive BC with long term follow-up.

In this study, pAkt overexpression was associated with patients' age, estrogen and androgen receptors, cytokeratin (CK)18, CK19 and PTEN expression. Loss of pAkt was correlated with higher grade, CK5/6, p53 and Ki-67 labelling index. Luminal-like tumours displayed more pAkt positivity than triple negative/basal-like subtypes. However, pAkt overexpression was not associated with breast cancer-specific (BCSS) or metastasis-free survival (MFS). Four combinatorial phenotypes were identified based on PIK3CA and pAkt expression, with considerable proportions being $PIK3CA^{-}/pAkt^{+}$ or $PIK3CA^{+}/pAkt^{-}$. These phenotypes were significantly associated with BCSS ($p = 0.001$) and MFS ($p = 0.002$).

Although pAKT is an oncogene that correlated with poor prognostic variables, it was not a prognostic marker. Combinatorial phenotypic groups of PIK3CA/pAkt denoted, at translational level, functional complexity within the upstream and downstream network of Akt activation with significant impact on patients' outcome. These findings may help developing adequate therapeutic regimens against specific components of this key signalling pathway.

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O-13 *D-gLUCURONYL C5-EPIMERASE* INHIBITS BREAST CANCER CELLS PROLIFERATION THROUGH THE TUMOUR SUPPRESSOR GENES ACTIVATION

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D-Glucuronyl C5-epimerase (GLCE) is one of the key enzymes of the biosynthesis of heparan sulphate proteoglycans. Down-regulation of *GLCE* expression in human breast tumours and cancer cell lines suggested a possible involvement of the gene in breast carcinogenesis. To test the hypothesis, we ectopically expressed GLCE in breast cancer cells MCF7 and showed that re-expression of *D-glucuronyl C5-epimerase* significantly inhibited proliferative activity of MCF7 cells according to CyQUANT NF Cell Proliferation Assay and did not affect the viability of the cells in Colony Formation Test. The antimitotic effect of *D-glucuronyl C5-epimerase* in human breast cancer cells probably is realised via the activation of tumour suppressor genes SYK (+8.1-fold), BRCA1 (+3.5-fold), p53 (+3.3-fold) and E2F1 (+3.00-fold) and change of a balance of pro- and anti-apoptotic factors BCL2 (+4.2-fold), NFKB1 (+2.6-fold) and TNF (+4.6-fold) (PathFinder RT Profiler PCR Array). Also, *GLCE* re-expression in MCF7 cells considerably changed expression of some genes involved in angiogenesis (IL8, IFNB1, TNF and TGFB1) and invasion/metastasis (SYK, NME1, S100A4) suggesting a possible antimetastatic effect of *GLCE in vivo*.

In summary, the ability of the *D-glucuronyl C5-epimerase* to suppress proliferation of breast cancer cells through the affecting different key genes involved in cell cycle regulation, angiogenesis and invasion/metastasis molecular pathways supposes the gene as a new potential candidate for diagnosis and treatment of breast cancer.

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O-14 EXPRESSION AND ACTIVATION OF Akt AND NFkB IN BREAST CANCER PATIENTS

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Background: It is postulated that Akt activates the Nf-kB pathway to promote tumour growth and survival in breast cancer cells.

Material and methods: Tissue microarray technology was employed to analyse tissue from 426 breast cancer patients. Immunohistochemistry was performed using antibodies for pAkt (phosphorylated at serine 473), NF- kB and pNFkappaB (phosphorylated at serine 536). Expression was assessed using the weighted histoscore method by two independent scorers.

Results: Median age was 62 years, median tumour size was 20 mm, 48% were pathologically graded G2 and 31% G3 and 48% were lymph node positive. Ninety-eight patients had unilateral

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:

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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.