breast carcinoma, the associations between *CCND1* amplification/cyclin D1 overexpression, clinicopathological variables and clinical outcome remain controversial.
Aim of the study: The aims of this study are fourfold: (i) to correlate cyclin D1 expression with gene amplification; (ii) to analyse the correlations between *CCND1* amplification and overexpression with clinicopathological features and patients' outcome in invasive breast cancer; (iii) to define the prevalence of cyclin D1 overexpression and *CCND1* amplification in ER positive breast carcinomas and its relation to patient outcome; (iv) to define the prevalence of cyclin D1 overexpression and *CCND1* amplification in the breast cancers with basal-like immunophenotype.
Material and Methods: *CCND1* amplification and protein expression were assessed on a tissue microarray containing 880 unselected invasive breast cancer cases, by means of chromogenic in situ hybridisation (CISH) using the SpotLight *CCND1* amplification probe (Zymed, South San Francisco, CA), and immunohistochemistry, with the rabbit monoclonal antibody SP4 (Zymed).
Results: A total of 59/613 tumours (9.6%) showed *CCND1* amplification and 224/514 (43.6%) showed strong Cyclin D1 expression. A strong correlation between *CCND1* amplification and cyclin D1 expression was found ($P < 0.001$). Basal-like cancers less frequently show *CCND1* amplification and cyclin D1 overexpression when compared to cancers pertaining to the other molecular subgroups ($P < 0.001$). Both *CCND1* amplification and cyclin D1 expression were associated with positive ER status. *CCND1* gene amplification was an independent prognostic factor for patients with ER positive breast cancer.
Conclusion: Our results demonstrate a strong correlation between *CCND1* amplification and its protein expression. However, protein expression is more pervasive than gene amplification and associated with ER expression.

O-81 Interactions of tumorantigen-reactive T-cells derived from bone marrow and tumor-cells in breast cancer patient

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Breast Cancer is an immunogenic tumor which is usually recognized by the cellular immunosystem via tumor-associated antigens (TAA) presented by antigen-presenting cells like dendritic cells. Although we were able to find tumorantigen-reactive $CD8^+CD45R0^+$ T-memory cells (TMC) by using interferon-γ-ELISPOT-analysis in 67% of primary breast cancer patient's bone marrow there seems to be a minority of non-responers. In comparison to classic tumor characteristics non-responders can be find more often in non-differentiated, hormone-receptor negative tumors and in metastatic breast cancer patients.
In a phase-1 trial of a cellular immunotherapy with reactivated tumorantigen-reactive autologous TMC derived from bone marrow we measured $CD4^+$ T-cell (TC) responses in stimulation cultures *ex vivo* to examine whether there are other immunological answers in non-responder. TC were activated by dendritic cells pulsed with TAA from MCF-7 lysate under IL-2 co-stimulation. We were able to show that next to a classic TH1-response with high levels of IFN-α there seems to exist TH2-responses mediated by high levels of TGF-β1 and low levels of IFN-α. The relation of both cytokines was directly related to the detection of tumorantigen-reactive TC and to tumor grading. Multiplex-cytokine analysis was able to confirm these findings. In patients with tumorantigen-reactive TC a combined active and passive vaccination trial was done.
These results may play an important role in further active and passive vaccination strategies.

O-82 Evidence for a tumour suppressive function of IGF1-binding proteins in human breast cancer

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Introduction: The role of the IGF system in various human malignancies has been well established. The aim of this study was to determine the levels of mRNA expression of IGFBP 1, 3 and 7 genes in benign and malignant breast tissue and correlate this with various prognostic parameters. **Methods:** Breast cancer tissue (n = 127) and normal background tissue (n-33) were prospectively collected and analysed for levels of IGFBP1, 3 and 7 mRNA using real time Q-PCR. mRNA levels were then analysed against tumour grade, nodal status, NPI/TNM stage and tumour type.
Results: For IGFBP 1 and 3, mRNA expression was higher in normal tissue. This was reversed for IGFBP 7. This wassignificant for IGFBP1 comparing NPI 3 with NPI 1 ($p = 0.050$) and the normal group ($p = 0.040$). With TNM analysis, there was less IGFBP1 mRNA comparing TNM 3 with normal ($p = 0.017$), TNM 1 ($p = 0.047$) and TNM 2 ($p = 0.019$). This was also found when comparing TNM 4 samples with normal tissue ($p = 0.017$), TNM 1($p = 0.046$) and TNM 2 ($p = 0.019$). For IGFBP3 mRNA, there was less mRNA when comparing TNM3 with TNM 1 ($p = 0.017$) and TNM 2 ($p = 0.050$), and also less mRNA expression when comparing TNM 4 with TNM 1 ($p = 0.030$). For IGFBP7 mRNA, both TNM 1 ($p = 0.0077$) and TNM 2 ($p = 0.015$) had significantly more expression than TNM 3 samples.
Conclusion: This study strongly supports the role of IGFBP 1, 3 and 7 as potential tumour suppressor genes in human breast cancer, which may open up exciting therapeutic possibilities in the future.

O-83 A possible paracrine protective effect of Insulin like binding protein 7 in mammalian breast cancer

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Aims: The role of the IGF (Insulin like growth factor) system in various human malignancies has been well established. The study examined levels of mRNA expression of IGFBP (IGF binding protein) 3 and 7 genes in malignant breast tissue and it's associated 'adjacent non cancerous tissue' (ANCT) and correlated this with various prognostic parameters.
Methods: Breast cancer tissue and ANCT pairs were prospectively collected and analysed for levels of IGFBP 3 and 7 mRNA using real time Q-PCR. mRNA levels were analysed against tumour grade, nodal status, NPI stage, size, recurrence and disease free survival (DFS). Full ethical approval was obtained.
Results: Data were analysed using non parametric formulae throughout. The number of validated results were, $BP7^{anct} = 90$, $BP7^{tumour} = 84$, $BP3^{anct} = 57$, $BP3^{tumour} = 58$. Correlating ANCT IGFBP7 expression with NPI, significantly more binding protein was expressed adjacent to good prognostic tumours (NPI 1) when compared with poor prognostic tumours (NPI 3), ($p = 0.016$). This pattern was repeated for tumour grade, with greater

expression adjacent to low grade tumours ($p = 0.047$) and for recurrence with significantly greater expression adjacent to tumours who remained recurrence free ($p = 0.006$). Survival analysis using Kaplan-Meier curves also revealed improved DFS associated with high ANCT IGFBP7 levels, which was statistically significant ($p = 0.004$). Results for IGFBP3 in either tumour or normal tissue or IGFBP7 in tumour tissue were not significant.

Conclusions: These data suggest that the level of local IGFBP7 adjacent to neoplastic breast tissue may act to restrict the progression of the malignant phenotype in a paracrine fashion, possibly even conferring a survival advantage. This needs to be evaluated further with larger series.

O-84 Characterisation of specific micro-RNA expression profiles in fresh frozen human breast tissue

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MicroRNAs negatively regulate the expression of specific mRNA targets at the post-transcriptional level and have been shown to display aberrant expression in several cancers including breast cancer. The expression profiles of miR-26b and miR-30a-3p were characterised in human breast tissue using real-time quantitative PCR. Significant deregulation of miR-26b has been shown in human breast tumour tissues. Transcripts targeted by miR-30a-3p include angiogenesis-related mRNAs. MiR-30a-3p has not previously been shown to be deregulated in breast cancer.

Following informed consent, malignant ($n = 33$) and benign ($n = 5$) primary breast tumour tissues and normal ($n = 5$) breast tissues were obtained at the time of surgery at University College Hospital, Galway. Malignant breast tumour samples were grouped according to the metastatic status of the patient five years from initial diagnosis into metastasis-free (MF, $n = 13$), bone-metastasis positive (BM, $n = 11$) and visceral and bone metastasis positive (VBM, $n = 9$) groups. Stem-loop gene-specific primers were used for cDNA synthesis and gene expression was measured using TaqMan® microRNA assays. Following relative quantification using qBASE software, statistical analysis was performed using Minitab (v.15).

The expression of miR-26b was significantly upregulated in BM versus MF groups ($P < 0.01$) suggesting a role for miR-26b in the bone-metastases process. There was a significant downregulation of miR-30a-3p in the VBM versus BM groups ($P < 0.05$). A significant relationship was not found between the expression levels of the individual microRNAs and prognostic factors such as nodal status, tumour grade and steroid hormone receptor status. This study confirms the potential of specific microRNAs in controlling outcome in breast cancer.

O-85 Expression of the putative breast cancer gene BASE; relationship with microRNA 154* and estrogen receptor status

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Introduction: It is estimated that 30% of human genes are regulated by non-coding micro-RNAs, many of which are cancer-related. One such potential gene, *BASE* has been shown by *in-vitro* studies to be estrogen responsive and breast cancer specific. Little is known, however, about the associations or precise regulation of *BASE* expression in breast cancer tissues.

Aims: To quantify expression of *BASE* and its putative targeting microRNA *miR-154** in breast cancers, and examine potential correlations with clinicopathological variables.

Methods: Microarray expression profiling of genes and miRNAs was performed in 16 early-stage, matched breast cancer specimens. Expression of selected differentially expressed micro-RNAs, including *miR-154**, was validated by real-time quantitative PCR in 52 breast tumour samples. Expression of *BASE*, a computationally predicted target of miR-154*, was also validated in 52 breast tumours and breast cancer cell lines. Associations between expression of *BASE*, *miR-154** and clinico-pathological variables were examined.

Results: *BASE* was expressed in 50% of tumour samples. A significantly higher proportion of tumours expressing *BASE* were estrogen receptor *(ER)* positive than *ER* negative ($p = 0.019$). *BASE* expression was also detected in the *ER+ve* cell lines but not in an *ER –ve* cell line. *MiR-154** was expressed in all breast tumour samples. *MiR-154** expression was significantly lower in *ER+ve* than *ER-ve* tumours ($p = 0.001$).

Conclusions: These findings suggest that the expression of both *miR-154** and putative target gene *BASE* correlate with ER status in breast tumours. This highlights the importance of these molecules in breast cancer. Functional analysis to elucidate interactions between these molecules is underway.

O-86 Can differences in cellular antioxidant enzyme status predispose to breast cancer in women without a recognised increased risk?

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Introduction: Up to 10% of patients with breast cancer have a known genetic defect (eg. BRCA-1, BRCA-2) but the aetiological factors in the others remain unclear. We hypothesise that impaired expression of cellular antioxidant enzymes and subsequent reduction in the ability to counter DNA damage due to oxidative stressors could be, at least in part, important in the aetiology of breast cancer.

Method: We obtained whole blood and PBMNC from women with breast cancer ($n = 20$) and from an age matched control group without known risk ($n = 20$). Erythrocyte and plasma glutathione peroxidase-1 (GPX1) activity was determined in both groups using a spectrophotometric method. Aliquots of PBMNC were used to determine gene expression of redox enzymes in untreated, fresh cells using RT-PCR. Further aliquots of PBMNCs were incubated in autologus plasma for 24 hrs and stimulated with hydrogen peroxide (1 mM) for 15 minutes to assess inducibility of the selenium-dependent antioxidant enzymes (GPX1) and (GPX4).

Results: Neither GPX1 activity in plasma or erythrocytes nor mRNA expression in fresh, non-induced PBMNC differed significantly between groups although mRNA tended to be lower in the cancer group. However, GPX4 gene expression in fresh PBMNC was significantly (30%, $p < 0.004$) reduced in the cancer group. Percentage induction of mRNA by hydrogen peroxide was similar (30–40%) for GPX1 and GPX4 in both groups but absolute GPX4 induction was lower in the cancer group due to a lower un-stimulated, starting value.

Conclusion: Breast cancer patients do appear to have a lower redox enzyme expression than non-cancer patients which would be expected to impair their ability to counter free-radical damage to DNA resulting in greater risk of genetic mutations.