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