(45%), which was 87% of SN visualized at scintigraphy. SN had tumour cells in 7 patients (35%), which influenced the treatment.
SNB can identify SN at a high rate in recurrent breast cancer if scintigraphy visualizes nodes and the findings may influence further planning of treatment. SNB should be considered as an important option in recurrent breast cancer.

O-77 Incidental malignant breast disease in routine breast reduction specimens

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Breast reduction surgery is a relatively common procedure in the UK. The finding of clinically unsuspected malignancy complicates subsequent management, particularly regarding assessment of margins and lesion size. We have performed a retrospective audit of breast reductions performed at Addenbrookes Hospital. Between 2000–2006 978 reductions were performed, 40% (389) in patients over 40. 61 patients had had previous breast cancer, almost all in the over 40 age group (57/61 patients).
In the remaining 917 cases, previously unsuspected in situ or invasive breast cancer was found in 3 patients, all over 50 years of age (3/135; 2%). One woman in the 40–50 year age group presented with malignant axillary lymphadenopathy shortly after surgery; retrospective histological review revealed a single focus of lymphovascular invasion. No unsuspected cancers were found in patients under 40. Lesions associated with an increased risk of malignancy (lobular in situ neoplasia, atypical ductal hyperplasia or flat epithelial atypia) were found in a further 13 women, 10 over 40 years of age.
In total, 4.4% of women aged over 50 had unsuspected invasive or in situ carcinoma or a high risk lesion, compared to 4.0% of women aged 40–50 and <1% of women aged <40.
Based on these findings, screening mammography may be appropriate in women over the age of 40 prior to breast reduction surgery. Breast reduction specimens from women over 40 should be sampled more thoroughly. In younger women, where the chances of finding an incidental carcinoma are negligible, fewer blocks may be sufficient.

O-78 Microvascular breast reconstruction: lessons learnt following our first 255 flaps

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Introduction: Autologous reconstruction is recognized as the "gold standard" and presently remains the preserve of specialist Plastic Surgery Centres. It can be performed rapidly and safely; patient satisfaction is high and it has economic advantages over implant-based procedures.
Over the last five years we performed 255 microvascular reconstrcuctions, consisting of DIEP flap, muscle-sparing TRAM and superior gluteal artery perforator (S-GAP) flaps. The purpose of this study was to review outcome and developments in techniques that have led to increasing success.
Patients and Methods: Between 2001 and today, 222 patients underwent microvascular reconstruction (189 unilateral, 33 bilateral, 180 immediate, 75 delayed), requiring 255 flaps. A retrospective review was made of each case, including anastomotic technique and post-operative recovery.
Results: The overall number of cases has been rising every year, with 30 microvascular reconstructions in the first 3 months of 2007. Furthermore, an increasing demand of bilateral microvascular breast reconstructions could be observed. Of the two hundred and fifty five flaps, a total of 10 flaps were lost, 8 in the first 3 years. Two (S-GAP) out of 145 flaps (1.4%) were lost over the last three years. Ten flaps were re-explored, 4 successfully. Important advances included team operating, the use of a venous coupler and venous salvage techniques.
Conclusion: In our experience autologous breast reconstruction is a very successful procedure both in regard of patient satisfaction and outcome. Flap survival, patient recovery and complications were all greatly improved with experience and use of innovative techniques. This paper supports the need to provide these techniques in committed centres.

O-79 Skin-sparing mastectomy with immediate reconstruction: To leave or to take the nipple-areolar complex (NAC)

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Background: Nipple-preserving mastectomy and immediate reconstruction has further improved the aesthetic outcome of skin-sparing mastectomy. Formal indications are still not published and besides tumour location evaluated preoperatively, intra-operative frozen section is chosen by some groups to help in the final decision to remove or leave the NAC complex.
Methods: Forty one cases of skin-sparing envelope mastectomy and immediate reconstruction with intention to preserve the NAC were evaluated. In all cases the preoperative evaluation showed by imaging (Mammography and ultrasound) a distance of at least 10 mm to the nipple–areolar complex. Cases selected were mainly extensive DCIS, multifocal invasive disease and a tumour/breast size relation or location favouring a worst cosmetic result with conservative treatment. Intra-operative evaluation of the retroareolar region, was done in all cases to decide the preservation or resection of the NAC complex. The methods of reconstruction used were variable and described as implant-only reconstruction (5; 12%), latissimus dorsi muscle with implant (32; 78%), and TRAM flap (4; 10%).
Results: In the studied sample there were 17 (41.5%) cases of extensive DCIS, 8 (19.5%) cases of multifocal invasive disease and 16 (39%) cases where tumour/breast size relation or location led to a mastectomy option. The frozen section analysis revealed invasion in the retroareolar position in 7 (17, 3%) cases, 5 (71%) of extensive DCIS. There were no false-negative results in the final report. Additional partial or complete removal of the NAC complex was undertaken in 4 cases (9.8%) due to necrosis (only 1 needed complete ablation and replacement).
Conclusion: In patients who are intended to undergo nipple-preserving envelope mastectomy, intra-operative frozen section examination of the retroareolar region is important to help in the final decision even when imaging pre-operative methods show a safe distance to the NAC complex. In our series it precluded a second intervention in 7 (17.3%) cases.

O-80 *CCND1* amplification and cyclin D1 expression in breast cancer and their relation with proteomic subgroups and patient outcome

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Introduction: Despite strong evidence regarding the role of *CCND1* amplification and protein overexpression in

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