Discussion: The importance of one-time surgery must be underscored, as it is associated with less psychological burden and better cosmetic results. MRI and secondary US are invaluable adjuncts in the preoperative work-up of ILC. Reoperation rate is decreased by implementing a clinical algorithm. Follow-up of patients will be presented in the future, to evaluate the impact of the clinical tool on recurrence.

Table 1. Invasive lobular carcinoma − Clinical tool

(1) Tumor/breast index (favoring or not BCS) (IRM > US > clinical > mammography)	1 − BCS uncertain 10 − Mastectomy
(2) MRI findings	0 − Unique lesion 1 − Other lesions
(a) US confirms other lesions	0 − Unique lesion 1 − Other lesions
(b) Biopsy of other lesion(s) (preoperative OR intraoperative biopsy of wire-guide marked lesions)	0 − Unique lesion 1 − Suspect lesion 2 − Malignant
(c) Multicentric	1 − Uncertain 5 − Certain
(d) Multifocal	1 − Uncertain 3 − Certain
(3) Intraoperative assessment of margins	1 − Negative 8 − Positive
(4) Excision of margins possible	0 − Yes 1 − No
Score	⩾10: Mastectomy ⩽9: BCS possible
Final pathologic assessment	Negative margins: One-time surgery possible Positive margins: Reoperation

*In decreasing order of sensitivity, diagnostic tools to determine extent of lesion.

219 Proffered paper oral

Lobular cancer is different, but its surgical management is changing over time: analysis of an institutional database over a 10-year period

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Background: Lobular carcinoma is a common form of breast cancer, and is known to be more often multicentric and bilateral, leading surgeons to pursue a more aggressive approach. Aim of the present report is to compare patients with invasive ductal (DC) and lobular carcinoma (LC) to study clinical differences over a recent time interval.

Materials and Methods: A retrospective review of 1417 breast cancer patients operated at one Institution from October 1999 to October 2009 was performed. Patients with particular types of histology other than ductal or lobular (n = 38), with stage IV at presentation or in the immediate post-operative work-up (n = 95), enrolled in neo-adjuvant chemotherapy protocols (n = 46), or patients with no information on initial stage of disease (n = 56) were excluded, leaving 1182 patients for analysis. Patients were thereafter analysed according to two study intervals (1999–2004 and 2004–2009) to report differences in surgical trends. Median follow-up was 4 years.

Results: There were 171 LC (14.5%) and 1011 DC patients in the study period. Median age was 63 years for DC and 65 years for LC patients. Median diameter was 1.7 cm for both groups. Diagnoses of LC were more frequent in the second half of the study period (55/465 vs. 116/662, $p < 0.01$). Tumors were diagnosed at an early T stage (<2 cm in diameter) in 670/1011 DC and 105/171 LC (p=NS). Multicentricity was reported in 108/1011 (10.6%) DC and in 31/171 (18.1%) LC patients ($p < 0.01$). A positive margin of resection at initial surgery was documented in 71/1011 (7%) DC and in 21/171 (12.3%) LC patients, respectively ($p < 0.001$). Mastectomy was performed in 72/171 (42%) LC patients, and in 401/1011 (39.7%) DC patients, respectively (p= NS). Although the rate of mastectomy decreased over time in both groups, this was more pronounced for LC patients (from 31/55 in the period 1999–2004 to 43/116 in the period 2004–2009, $p < 0.05$). There was a trend for more controlateral cancer surgery in the LC group (38/1011 vs 11/171, $p = 0.1$). An ipsilateral relapse was diagnosed in 19 (1.8%) DC and 2 (1.2%) LC patients (p=NS). Systemic relapses (14/171 vs. 40/1011) or deaths (13/171 vs. 48/1011) were not significantly different in both groups.

Conclusions: Although LC is more often multicentric, associated with a positive margin of resection and a trend for bilaterality, it is not associated with a higher incidence of local recurrence. LC is diagnosed more frequently in recent years, and it is now treated by surgeons similarly to its DC counterpart, with of a more significant reduction of the mastectomy rate in this group.

Thursday, 25 March 2010 15:30–17:00

CLINICAL SCIENCE SYMPOSIUM

Bone microenvironment and bone disease

221 Invited

Bone targeted therapy for early breast cancer

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Background: Bisphosphonates (BPs) and denosumab (an antibody to RANK-ligand) are potent inhibitors of bone resorption that reduce the risk of skeletal complications and prevent treatment induced bone loss. However, their use in cancer may provide more than just supportive care and modify the course of the disease by disrupting the metastatic process, and reduce the risks of disease recurrence. Whether this reflects just the indirect inhibitory effects on the vicious cycle within the bone marrow microenvironment or directs effects on the cancer is an area of debate and uncertainty.

Pre-clinical evidence: *In vitro* studies have shown that BPs induce tumour cell apoptosis and inhibit tumour cell adhesion, invasion, proliferation, and angiogenesis. *In vivo* animal studies have shown that bone targeted therapies can prevent establishment of bone metastases and inhibit progression of established lesions. BPs appear to enhance the anti-tumour activity of cytotoxic drugs in a sequence dependent manner.

Clinical evidence: In the early disease setting, improvements in disease free survival have been seen in some, although not all clinical trials of adjuvant bisphosphonates. Additionally, supportive exploratory clinical data have been reported from the cohort of patients who received neoadjuvant chemotherapy +/− zoledronic acid in the ongoing AZURE trial. A smaller residual invasive tumour size and a higher rate of pathological complete response was observed in those patients receiving zoledronic acid in addition to chemotherapy. A neoadjuvant biomarker study, ANZAC is evaluating the effects of chemotherapy followed by zoledronic acid on proliferation, angiogenesis markers and apoptosis. Studies to evaluate the potential for denosumab to prevent metastasis are also in progress.

Conclusions: The potential anti-tumour and disease-modifying role of adjuvant bone targeted therapies holds great promise. Bone targeted agents may have anticancer activity but results from recently completed large adjuvant studies must be awaited before their routine use in metastasis can be recommended.

222 Invited

New developments in the treatment of metastatic bone disease

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Classical “skeletal-related events” (SREs) include need for radiotherapy, pathologic fractures, need for bone surgery and spinal cord compression. Complications of bone metastases (BM) also include hypercalcemia, severe pain and deterioration of quality of life for which specific monitoring tools have just been developed. Taken from data in placebo groups of randomized bisphosphonates (BPs) trials, the mean skeletal morbidity rate in breast cancer, i.e. the mean number of objective SREs per year, varies between 2.2 and 4.0. It is lower nowadays because of an earlier use of BPs. BPs are able to interrupt the vicious circle typical of tumor osteolysis by acting directly on osteoclasts and maybe also on tumor cells. BPs reduce the skeletal morbidity rate by 25% to 40%. It has been shown in a 2-year controlled comparative trial between pamidronate and zoledronic acid (ZA) that this latter compound has a superior efficacy as the likelihood of getting a SRE during therapy is reduced by a further 20%. The administration of a high “loading” dose of ibandronate could be especially useful in patients with severe bone pain but this concept should be tested in controlled trials. ZA 8 mg is not more effective than the 4 mg dose level which suggests that we have reached some form of a ceiling effect with classical BP schemes to reduce SRE incidence. Recently presented data (Body et al., CABS 2009) in patients with highly aggressive or advanced disease indicate that ZA not only prevents SREs, but has also a positive effect on survival. Denosumab is a fully human monoclonal antibody that inhibits RANK Ligand, a key factor that mediates the increased osteoclast activity typical of BM. The effects of denosumab (120 mg sc q4 weeks) and ZA (4 mg q4 weeks adjusted for Creat. Clear.) on the incidence of SREs in patients with BM from breast cancer have been compared in a double-blind double-dummy phase III trial. Denosumab significantly delayed the time to first on-study SRE compared with ZA (hazard ratio [HR] 0.82; 95% CI: 0.71–0.95; P = 0.01) in this 34-month study. Denosumab also significantly delayed the time to first and subsequent on-study SRE (multiple event analysis)

compared with ZA (HR 0.77; 95% CI: 0.66–0.89; P = 0.001). Rates of adverse events were similar for both treatment arms. Osteonecrosis of the jaw occurred infrequently (2.0% denosumab, 1.4% ZA; P = 0.39). AEs potentially associated with renal toxicity occurred in 4.9% of the denosumab arm and in 8.5% of the ZA arm. In conclusion, an antitumor effect of ZA has been recently suggested in breast cancer whereas denosumab appears to be superior to ZA in delaying or preventing SREs in breast cancer.

Thursday, 25 March 2010 15:30–17:00

CLINICAL SCIENCE SYMPOSIUM

Stroma and microenvironment

223 Invited

A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer

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To identify factors that predict the response to chemotherapy, we have developed an approach based on the use of multiple regression to reduce the complexity of gene expression data sets. We tested the approach on tumour biopsies from individuals with estrogen receptor-negative breast cancer treated with 5-fluorouracil, epirubicin and cyclophosphamide (FEC) in the EORTC 10994/BIG 00−01 trial. We report that increased stromal gene expression predicts resistance to preoperative chemotherapy with FEC. The predictive value of the stromal signature was validated in two independent cohorts that received chemotherapy but not in an untreated control group, indicating that the signature is a predictive marker for drug response rather than a prognostic marker for innate tumour aggressiveness. Several models could explain the result, the simplest being reduced access of drugs to the tumour cells or crosstalk between tumour and stroma leading to secretion of survival factors by the stromal cells. These findings suggest that antistromal agents may offer a new way to overcome resistance to FEC.

224 Invited

Epigenetics of carcinoma-associated myofibroblasts: implications for anti-cancer therapies

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It is increasingly recognized that the non-neoplastic stromal compartment in most solid cancers plays an active role in tumor proliferation, invasion and metastasis. Cancer associated fibroblasts (CAFs) are one of the most abundant cell types in the tumor stroma, and these cells can be strongly pro-tumorigenic. Evidence that CAFs are epigenetically and possibly also genetically distinct from normal fibroblasts is beginning to define these cells as potential targets of anti-cancer therapy. In particular, our lab has shown that CAFs in gastric carcinomas have global DNA hypomethylation, with focal gains of methylation in gene promoters (Jiang, Gonda, et al, Cancer Res, 2008; Gonda et al., Semin Cell Dev Biol., 2009). We now find that this phenomenon generalizes to other common types of human carcinomas including prostate cancers and some types of breast cancer. For anti-cancer therapy we are testing the hypothesis that further decreases in DNA methylation induced in CAFs by the hypomethylating drug 5aza-dC may lead to a functional crisis in these cells, thus impairing their ability to support tumor growth. In a triple transgenic mouse model of aggressive pancreatic carcinoma (p53/Brca1/K-ras) we find that single-agent 5aza-dC has striking anti-tumor efficacy when initiated at the stage of pancreatic intraepithelial neoplasia. Our current experiments are aimed at dissecting whether this effect is due to functional inhibition of CAFs, or to direct inhibition of neoplastic epithelial cell proliferation, or both.

225 Invited

Oxidative stress promotes myofibroblast differentiation and tumour spreading

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JunD regulates genes involved in anti-oxidant defense. We took advantage of the chronic oxidative stress resulting from junD deletion to examine the role of Reactive-Oxygen-Species (ROS) in tumor development. In a model of mammary carcinogenesis, junD inactivation increased tumor incidence and revealed an associated reactive stroma. junD-inactivation in the stroma was sufficient to shorten tumor free survival rate and enhance metastatic spread. ROS promoted conversion of fibroblasts into highly migrating myofibroblasts through accumulation of the HIF-1a transcription factor and the CXCL12 chemokine. Accordingly, treatment with an antioxidant reduced the levels of HIF and CXCL12 and, subsequently, numerous myofibroblast features. Interestingly, CXCL12 accumulated in the stroma of HER2− human breast adenocarcinomas. Moreover, stroma of HER2 tumors exhibited a high proportion of myofibroblasts, which was correlated to high rate of nodal metastases. Finally, this subset of tumors revealed an associated oxido-reduction signature, further demonstrating the relevance of our findings in human cancers. Collectively, our data uncover a new mechanism by which oxidative stress increases the migratory properties of stromal fibroblasts, which in turn may potentiate metastatic dissemination.

226 Proffered paper oral

HOXB9, a gene overexpressed in breast cancer, induces angiogenesis, invasion, and lung metastasis

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Background: The mechanisms underlying tumoral secretion of signaling molecules into the microenvironment, which modulates tumor cell fate, angiogenesis, invasion and metastasis, are not well understood. Aberrant expression of transcription factors, which has been implicated in the tumorigenesis of several types of cancers, can constitute a mechanism that induces the expression of growth and angiogenic factors in tumors leading to their local increase in the tumor microenvironment to favor tumor progression. We recently observed that transcription factor, HOXB9, is deregulated in breast cancer and enhanced expression correlated with high tumor grade. A role for elevated HOXB9 expression in breast tumor progression is demonstrated by its ability to activate the ErbB and TGF-B pathways which influence aggressive tumor phenotypes and to induce angiogenesis in the tumor microenvironment.

Materials and Methods: To quantify HOXB9 expression in breast cancer, we analyzed cDNAs generated from laser captured, purified populations of tumor cells and adjacent normal mammary epithelial cells from 40 clinically and pathologically annotated cases of breast cancer. Next, we introduced the HOXB9 construct into MCF10A to test the functional consequence of HOXB9 overexpression. Furthermore, we stably introduced the activated G12V H-Ras allele into HOXB9-MCF10A cells and investigated their ability to form tumors and the metastatic potential.

Results: Overexpression of HOXB9 was found in 43% of primary breast cancer by RT-PCR and in situ hybridization and correlated with high tumor grade. Ectopic expression of HOXB9 in MCF10A mammary epithelial cells induced EMT accelerating cellular migration and invasion. It also increased the expression of angiogenic factors, which enhance the formation of new vessels in mouse dorsal air sac model. Conversely, genetic ablation of endogenous HOXB9 in MDA-MB-231 breast cancer cells suppresses their motility and angiogenic potential. Further, we confirmed that HOXB9-induced tumor phenotypes arise through the activation of both ErbB-AKT and TGFβ signaling pathways. Finally, in mouse xenograft model, we observed that HOXB9 cooperates with activated H-Ras to transform mammary epithelial cells leading to large, vascularized tumors showing highly metastatic potential to the lung.

Conclusions: Our findings imply that overexpression of HOXB9 in human breast cancer contributes to tumor progression through activation of signaling pathways that alter both tumor-specific cell fates and tumor-stromal microenvironment, leading to increased invasion and metastasis.