min intravenously on days 1 and 8 of a 21-day cycle or TPC (cytotoxic, hormonal, or biologic monotherapy, or supportive care only). Exploratory OS sub-analysis was carried out.
Results: Of the 254 pts enrolled in the TPC arm, 156 received a class of agent they had not previously received, and 98 had been re-challenged with a therapy of the same type. Re-challenged pts were in the following TPC groups: taxanes (n = 38/41; 92.7%), anthracyclines (n = 24; 100%), vinorelbine (n = 5/65; 7.7%), capecitabine (n = 4/45; 8.9%), hormonal therapy (n = 4/8; 50%), and other (n = 23/25; 92.0%); no-one receiving gemcitabine (n = 46) was re-challenged. When the re-challenged pts were excluded from the analysis, eribulin significantly improved OS vs TPC (HR 0.74; 95% CI 0.58, 0.94; nominal p = 0.014) with median OS of 13.1 and 10.5 months, respectively. Analysis of eribulin vs the 98 re-challenged pts in the TPC arm showed median OS of 13.1 vs 10.7 months, respectively (HR 0.92; 95% CI 0.68, 1.23; nominal p = 0.556).
Conclusions: Eribulin demonstrated OS improvement for pts with locally recurrent or MBC, even when re-challenged pts were removed from the TPC arm.

5007 ORAL

The MicroRNAs-30 Family Interferes With the Formation of Breast Cancer Bone Metastases by Targeting Osteomimetic Genes

M. Croset[1], R. Bachelier[1], N. Allioli[2], S.S. Hong[3], R. Agami[4], P. Clezardin[5]. *[1]INSERM UMR1033 Université de Lyon, Faculté de Médecine Lyon-Est, Lyon Cedex O8, France; [2]IGFL CNRS UMR5242 Université de Lyon, Faculté de Médecine Lyon-Sud, Lyon, France; [3]INRA UMR754, Université Lyon 1, Lyon, France; [4]The Netherlands Cancer Institute, Division of gene regulation, Amsterdam, The Netherlands; [5]INSERM UMR1033 Université de Lyon, Faculté de Médecine Lyon-Est, Lyon, France*

MicroRNAs (miRNAs), a class of endogenous small non coding RNAs have been involved in tumorigenesis and metastatic dissemination through their activity as negative regulators of gene expression. Alterations of miRNA expression have been reported in the different steps of initiation, progression and dissemination of breast tumours. Breast cancer cells preferentially invade and grow as secondary tumours in bone. To adapt and thrive in this environnement metastatic breast cancer cells express genes normally expressed by the osteoblastes and acquire a bone cell pseudo-phenotype named osteomimicry.
To investigate if miRNAs interfere with this specific steps of bone metastasis formation, we compared the expression profiles of the human breast cancer cell line MDA-MB-231 with that of a subpopulation (MDA-BO2) that metastasize to bone with high efficiency, using TaqMan Low Density Array analysis. Then, target genes of the differentially expressed miRNAs within the two cell lines were predicted by looking at sequence complementarities of the 5′ seed regions of the miRNAs within the 3′UTR of the genes using miRanda, PicTAR and TargetScan softwares. By doing so, it appeared that the miRNAs-30 family which is composed of 5 members: miRNA-30a, 30b, 30c, 30d and 30e, was substantially downregulated in MDA-BO2 cells. Therefore, the expression of these miRNAs-30 in MDA-BO2 cells were restored through retroviral transduction using the pmiR-Vec plasmid vector to perform functional studies. The mRNA expression of connective tissue growth factor (CTGF), connexin 43, integrin β3 and transcription factor runx2, which all harbor phylogenetically conserved miRNAs-30 binding sites in their 3′-UTR, was decreased in MDA-BO2 overexpressing miRNAs-30 (miRNAs-30-BO2), when compared to pmiR-Vec-BO2 clones. The expression of cadherin-11, for which we found a perfect base pairing of the miRNAs-30 “seed region” in the coding sequence, was also decreased. MDA-BO2 modified clones were inoculated into the tail vein of nude mice and bone metastases were radiographically detected and enumerated. A 50% decrease in the extent of osteolytic lesions was observed in animals bearing miRNAs-30-BO2 tumours versus mice inoculated with pmiR-Vec-BO2 clones or with MDA-BO2 wild-type clones.
These results strongly suggest that miRNAs-30 act as a regulator of breast cancer bone metastases formation.

Oral Presentations (Sat, 24 Sep, 11:15–13:20)
Breast Cancer – Early Disease

5008 ORAL

Long Term Results of Video-assisted Breast Surgery (VABS)

K. Yamashita[1], S. Haga[1], K. Shimizu[1]. *[1]Nippon Medical School, Department of Surgery, Tokyo, Japan*

Background: The breast conserving surgery and the sentinel node (SN) biopsy became to be recognized as the standard treatment for early breast cancers. We have reported about cosmetic effectiveness and lower infestation of the video-assisted breast surgery (VABS) for the breast diseases. We devised the trans-axillary retro-mammary (TARM) approach of VABS. It needs only one skin incision in the axilla and can treat any tumour even in the medial or lower side of the breast without making any injuries on the breast skin. And it can preserve skin touch sensation. We evaluated the aesthetic results and the curability of this surgical method.
Methods: We have performed VABS on 300 patients since December, 2001. The newly devised trans-axillary retromammary-route approach (TARM) was performed on 120 patients of early breast cancer, stage I and II. After endoscopic sentinel node biopsy, we elongated the axillary skin incision to 2.5 cm. We marked the surgical margin 2 cm apart from the tumour edge by injecting blue dye into subcutane and retromamma. We dissected major pectoral muscle fascia to detach retromammary tissue under the tumour. The working space was made by lifting traction sutures through the gland. We cut the proximal side of the gland vertically at dye marking points, and dissect skin flap over the tumour by tunnel method. Then we cut each sides of the gland vertically and removed it through the axillary port by using the tumour collection bag. The breast reconstruction was done by filling absorbable fiber cotton. The postoperative aesthetic results were evaluated by our original score system, ABNSW.
Results: Breast conserving surgery was performed on 286 patients (26 after preoperative chemotherapy) and skin-sparing total mastectomy on 14. We do not use the special disposable apparates. The operative cost is very low as the conventional one. There was no significant difference in operational infestation. There was no serious complication after surgery. Surgical margin was minimally positive in 2 patients. The original shapes of the breast were preserved well. All patients expressed great satisfaction. The follow-up time is 112 months at maximum and 72 months on average. There is two locoregional recurrence and 12 distant metastases (brain: 4 with 2 cancer deaths, lung: 3, liver: 3, bone: 5). 5-year survival rate is 97.3%. With regard to TARM, The skin incision was made only in the axilla without any wound on the breast. It could be applied for any tumour existing in the medial or caudal side of the breast (A and B regions). The reconstruction by filling absorbable fiber cotton need no excessive detachment of the skin beyond the surgical margin of the mammary gland. The postoperative esthetic results were excellent and good. The sensory disturbance was minimal, observed only in the detached area within the surgical margin.
Conclusions: VABS can be considered as a surgical procedure with good locoregional control and can provide aesthetic advantages for patients with breast disease.

5009 ORAL

Near-infrared Fluorescence Sentinel Lymph Node Detection in Breast Cancer Patients – the GREEN LIGHT Studies

B.E. Schaafsma[1], J.R. van der Vorst[1], M. Hutteman[1], F.P.R. Verbeek[1], J.S.D. Mieog[1], G.J. Liefers[1], P.J.K. Kuppen[1], J.V. Frangioni[2], C.J.H. van de Velde[1], A.L. Vahrmeijer[1]. *[1]Leiden University Medical Center, Oncologic Surgery, Leiden, The Netherlands; [2]Beth Israel Deaconess Medical Center, Medicine and Radiology, Boston, USA*

Introduction: Detection of the sentinel lymph node is important in the staging and treatment of breast cancer. Near-infrared (NIR) fluorescence imaging is a technique that can be used to visualize lymph nodes during surgery, several centimeters into the living tissue, in real-time. Currently, indocyanine green (ICG) is the only clinical approved NIR fluorescent contrast agent. Premixing of ICG with human serum albumin (HSA) improved the fluorescence and sentinel lymph node retention in preclinical experiments. The current studies focus on optimizing the use of NIR fluorescence imaging for the sentinel lymph node procedure in 3 clinical trials with a total of 64 patients.
Material and Methods: In all studies, the Mini-FLARE intraoperative imaging system (Frangioni Lab, Boston, USA) was used. First (study 1), the optimal dose of ICG:HSA was studied in 24 consecutive patients. These patients received the standard of care sentinel lymph node procedure (blue dye and ^{99m}Tc-nanocoll) and were injected with 1.6 mL of ICG:HSA (dose groups 50 to 1000 μM).
The potential advantage of premixing of ICG with HSA was then studied in a randomized, double-blind study (study 2), with 18 consecutive patients. Patients were injected with 1.6 mL of 500 μM ICG alone or ICG:HSA. Subsequently, sentinel lymph node mapping was performed using 500 μM ICG in 24 patients, with randomization between the use or omission of patent blue (study 3).
Results: The sentinel lymph node was successfully detected in all patients. In the dose finding study (study 1), a total of 35 lymph nodes were detected (average 1.45), all of which were radioactive, 30 nodes were blue. The optimal dose was between 400 and 800 μM.
No differences were observed when premixing ICG with HSA (study 2), in the number of sentinel lymph nodes identified (average of 1.4 per patient), nor in fluorescence intensity (P = 0.18). No difference in fluorescence was

observed when the patent blue dye was used or omitted (study 3). The sentinel lymph node was detected in all patients using NIR fluorescence before the blue dye could be observed.
Conclusion: These studies demonstrate the successful use of NIR fluorescence and ICG in sentinel lymph node mapping of breast cancer patients. The optimal parameters are a dose of 500 μM ICG that is not premixed with HSA, and the use of patent blue can be omitted.

5010 ORAL

Intraoperative Molecular Detection of Lymph Node Metastases and Micro-metastases − Results of the First UK Centre Using the One Step Nucleic Acid Amplification Assay

M. Babar[1], R. Madani[1], L. Thwaites[2], P. Jackson[2], A. Chakravorty[1], T. Irvine[1], M. Kissin[1], G. Layer[3]. [1]*Royal Surrey County Hospital, Breast Unit, Guildford Surrey, United Kingdom;* [2]*Royal Surrey County Hospital, Histopathology, Guildford Surrey, United Kingdom;* [3]*University of Surrey, Postgraduate Medical School, Guildford Surrey, United Kingdom*

Introduction: One step nucleic acid amplification (OSNA), a highly sensitive intraoperative assay of cytokeratin 19 mRNA, is used for the detection of sentinel lymph node (SLN) macro- and micro-metastases in breast cancer. We present our two year data following the introduction of OSNA in our unit.
Methods: Data was collected prospectively from 2008−10. All eligible patients were offered OSNA. Operations were performed by five consultant breast surgeons. On detection of micro-metastasis (+) and positive but inhibited metastases (i+), a level 1 axillary nodal clearance (ANC) and for a macro-metastasis (++), a level 3 ANC was performed.
Results: 471 patients had 999 SLN analysed, median age being 61. All except one were females. 34% (n = 161/471) had positive SLN who had further ANC. Of these, 48% (n = 78/161) had macro-metastases, 37% (n = 59/161) had micro-metastases and 15% (n = 24/161) had positive but inhibited results. 17% (10/59) of the patients with micro-metastases had positive non-SLN (NSLN), four (4/59, 6.8%) had four positive nodes (SLN+NSLN) thus receiving adjuvant radiotherapy. 8% (2/24) of those with positive but inhibited results and 39% (30/78) of those with macro-metastases had positive NSLN.
Conclusion: In our series, over a third of patients had OSNA positive SLN and underwent axillary surgery at the same operation. OSNA may potentially upstage patients with micro-metastases and long term studies are needed to determine the clinical relevance of molecular micro-metastatic disease.

5011 ORAL

Assessments of Proliferation in Breast Cancer

M. Sundquist[1], E. Holmberg[2], S. Holmberg[3], A. Kovacs[4], G. Mathe[4], O. Stål[5], G. Tejler[6], S. Thorstenson[7]. [1]*County Hospital Kalmar, Surgery, Kalmar, Sweden;* [2]*ROC, Oncology, Gothenburg, Sweden;* [3]*Sahlgrenska University Hospital, Surgery, Gothenburg, Sweden;* [4]*Sahlgrenska University Hospital, Pathology, Gothenburg, Sweden;* [5]*Linköping University Hospital, Laboratory, Linköping, Sweden;* [6]*Hospital, Surgery, Västervik, Sweden;* [7]*Linköping University Hospital, Pathology, Linköping, Sweden*

Background: Proliferation rates of tumour cells provide prognostic and therapy predictive information. Mitotic index (MI), S-phase fraction (SPF) and ploidy assessed by flow cytometry and Ki-67/MIB-1 are commonly used assays of proliferation. Comparative studies of the assays are rare. Also, consensus of cut off values of Ki-67 in breast cancer is lacking.
Patients and Methods: Two different cohorts of patients were used to compare the correlation between assays. MI, SPF and 5-year follow-up data were explored for 1130 patients from the hospitals of Kalmar County (KC). In the second cohort MI, Ki-67 /MIB-1 and 3-year follow up data for 403 patients from the Sahlgrenska University Hospital (SU) were investigated. In further approximately 2000 cases the correlation between Ki-67 and MI is explored.
Results: In the KC cohort, tumours with MI 1 identified pts with the lowest 5 year distant recurrence rate, 4.3% and MI 3 those with the highest proportion of distant recurrence, 17%. Low SPF, diploid tumours had a similarly low 5 year distant recurrence rate as MI 1 tumours, 6%.
In the SU cohort MI and Ki-67 were both significantly correlated to early recurrence, $p < 0.001$. The optimal correlation between MI and Ki-67 was achieved when both were separated in 3 groups with cut off values for Ki-67 of 10 and 30%. Spearman $r = 0.69$, $p < 0.0001$.
Tumours with Ki-67 >30% had 22% distant recurrences within 3 years, those with Ki-67 10−30% recurred in 12% and tumours with Ki-67 <10% in only 1.4%. The proportion of early distant recurrences was almost identically distributed by MI score. Tumours with MI 3 had 21% recurrences, MI 2 11% and MI 1 only 1.6%.
Conclusion: Mitotic index is a solid instrument to identify tumours with inferior prognosis. When Ki-67 is stratified in 3 groups the assay performs equally well as the mitotic index. Low SPF, diploid tumours have approximately the same prognosis as tumours with low MI.

5012 ORAL

Prognostic Value of TWIST1 Expression in Breast Cancer Patients

M. Riaz[1], A. Sieuwerts[1], M. Look[1], M. Smid[1], J. Foekens[1], J. Martens[1]. [1]*ErasmusMC JNI, Medical Oncology, Rotterdam, The Netherlands*

Background: Twist homolog 1, encoded by the *TWIST1* gene, is a transcription factor that promotes cancer development by inducing epithelial to mesenchymal transition (EMT), a key process of invasion and metastasis, in cancer cells through inhibition of E-cadherin expression amongst other genes. The purpose of the current study was to investigate whether *TWIST1* expression predicts the progression of disease in a large series of well-documented breast cancer patients with long term follow-up and to identify breast cancer genes and associated pathways co-expressed with *TWIST1*.
Materials and Methods: The mRNA expression level of *TWIST1* was analyzed by quantitative RT-PCR in 1,476 primary breast cancers. The expression level was dichotomized using the median value. Metastasis-free survival (MFS) was evaluated in all patients and, separately, in lymph node-negative patients (n = 802) who did not receive adjuvant systemic therapy, and were stratified into estrogen receptor (ER)-positive (n = 566) and ER-negative (n = 236) cohorts. MFS was evaluated using the Kaplan–Meier method and uni- and multivariate analysis was performed using the Cox proportional hazards method. Spearman correlation of *TWIST1* expression with other genes measured on Affymetrix chip was also analyzed. Plausible pathways containing genes showing significant positive and negative correlation with *TWIST1* expression were predicted using BioCarta and KEGG data basis.
Results: In all patients, a high expression level of *TWIST1* was associated with shorter MFS in both uni- and multivariate analysis (HR: 1.29, 95% CI: 1.12–1.49, $p = 0.001$ and HR: 1.33, 95% CI: 1.14–1.54, $p < 0.0001$, respectively). In lymph node-negative high *TWIST1* expression was associated with the luminal A breast cancer subtype and, both in uni- and multivariate analysis, with shorter MFS only in the ER-positive subgroup (HR: 1.37, 95% CI: 1.09–1.73, $p = 0.007$ and HR: 1.35, 95% CI: 1.07–1.71, $p = 0.012$, respectively). Pathway analysis indicated that *TWIST1* expression was correlated positively with genes involved in signal transduction and extracellular matrix and negatively with genes associated with cellular transport processes.
Conclusion: *TWIST1* is an independent prognostic factor for poor prognosis in breast cancer, particularly in lymph node-negative patients with ER-positive disease. Analysis of co-expressed genes suggests an involvement of the microenvironment in *TWIST's* adverse role during breast cancer progression.

5013 ORAL

FDG-PET/CT for Early Prediction of Response to Neoadjuvant Lapatinib, Trastuzumab, and Their Combination in HER2-positive Breast Cancer Patients: the Neo-ALTTO Study Results

C. Gamez[1], P. Flamen[2], E. Holmes[3], J. Robles[4], G. Gebhart[5], S. Di Cosimo[6], H. Eidtmann[7], M. Piccart-Gebhart[8], J. Baselga[9], E. De Azambuja[8]. [1]*University Hospital of Bellvitge, PET Unit-IDI IDIBELL, Barcelona, Spain;* [2]*Institut Jules Bordet Université Libre de Bruxelles, Nuclear Medicine Department, Brussels, Belgium;* [3]*Frontier Science, Inverness-shire, United Kingdom;* [4]*University Hospital of Bellvitge, PET Unit-IDI.IDIBELL, Barcelona, Spain;* [5]*Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium;* [6]*Breast Cancer Center Vall d'Hebron University Hospital and SOLTI, Medical Oncology Service, Barcelona, Spain;* [7]*University Hospital Kiel Universitaetsklinikum Schleswig-Holstein, Department of Gynecology and Obstetrics, Kiel, Germany;* [8]*Institut Jules Bordet Université Libre de Bruxelles, Department of Medicine, Brussels, Belgium;* [9]*Massachusetts General Hospital Cancer Center, Division of Hematology/Oncology, Boston, USA*

Background and Aim: The NeoALTTO study tested the efficacy of neoadjuvant lapatinib, trastuzumab, and their combination plus paclitaxel in women with HER2-positive primary breast cancer (BC). The primary objective of Neo-ALTTO − the rate of pathological complete response (pCR) − was achieved for the combination compared with either lapatinib or trastuzumab alone (51.3% vs. 29.5% vs. 24.7%, respectively; $p < 0.01$ for both) (Baselga et al. SABCS 2010). Positron emission tomography /computed tomography with F-18 fluordeoxiglucose (FDG-PET/CT) was performed in a subset of patients to assess the predictive value of FDG-PET/CT for pCR as a preplanned secondary endpoint.