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.

Material and Methods: From January, 2008, to December, 2009, 455 patients from 99 participating sites were randomized to lapatinib, trastuzumab or both drugs for 6 weeks (no chemotherapy). After this biological window, patients continued on the same targeted therapy plus weekly paclitaxel for a further 12 weeks until surgery. After surgery, patients received 3 cycles of adjuvant FEC followed by the same anti-HER2 therapy for a further 34 weeks.
FDG PET/CT was performed in a subset of 85 patients at baseline, week 2 and week 6 for early assessment of response to targeted therapies alone without chemotherapy in patients enrolled in 30 qualified sites. Central validation of acquisition parameters and imaging analysis were performed by two blinded reviewers. Description of the lesions includes localization, metabolic volume, SUVmax and SUVmean. Metabolic parameters at week 2 and week 6 will be compared to baseline. Patients will be classified as responders in case of tumour metabolic complete response (mCR) or partial response (mPR) and non-responders in case of metabolic stable disease (mSD). The aim of this analysis is to test whether metabolic response with anti-HER2 therapies alone predicts pCR at the time of surgery.
Results: The last breast surgery was performed in May 2010. FDG PET/CT data cleaning and analysis will be completed by April 2011 and final results on the predictive value of early FDG PET/CT in this large phase III study will be presented at the meeting.
Conclusions: FDG PET/CT data analysis will be discussed at the meeting according to the results obtained.

5014 ORAL

High Risk of Non-sentinel Node Metastases in a Group of Breast Cancer Patients With Micrometastases in the Sentinel Node

T.F. Tvedskov[1], M.B. Jensen[2], E. Balslev[3], N. Kroman[1]. [1]*Copenhagen University Hospital, Department of Breast Surgery, Copenhagen, Denmark;* [2]*Danish Breast Cancer Cooperative Group, Danish Breast Cancer Cooperative Group, Copenhagen, Denmark;* [3]*Herlev Hospital, Department of Pathology, Copenhagen, Denmark*

Background: Axillary lymph node dissection (ALND) in breast cancer patients with positive sentinel nodes (SN) is under debate. The aim of this study was to identify a group of breast cancer patients with micrometastases or isolated tumour cells (ITC) in the SN where ALND might still be recommended because of a high risk of non-sentinel node (NSN) metastases. Furthermore, the aim was to identify a group of patients with a minimal risk of NSN metastases, justifying omission of ALND in any case.
Materials and Methods: A total number of 1577 breast cancer patients with micrometastases and 304 patients with ITC in the SN, operated on between 2002–2008 with sentinel lymph node dissection and subsequent ALND, was identified retrospectively using the nationwide Danish Breast Cancer Cooperative Group database. Data was validated using original pathology files and specimens. The risk of NSN metastases was calculated according to clinicopathologic variables in univariate and multivariate logistic regression analyses.
Results: 18% of patients with micrometastases and 9% of patients with ITC had NSN metastases. The risk of NSN metastases in patients with micrometastases was significantly related to tumour size, lymphovascular invasion, hormone receptor status, location of tumour in the breast and proportion of positive SN in the multivariate analysis. A model based on these risk factors identified 5% of patients with a risk of NSN metastases as high as 37%. On the other hand, the model was only able to identify less than 10% of patients with a very low risk of NSN metastases.
The risk of NSN metastases in patients with ITC in the SN was significantly related to age, tumour size and proportion of positive SN in the multivariate analysis. No subgroup of patients with ITC had a risk of NSN metastases over 25%. Patients with tumour size <2 cm and one or more negative SN, corresponding to 34% of patients with ITC, had a very low risk of NSN metastases. Omission of ALND in this group would result in a false negative rate of only 7%.
Conclusions: We have identified a group of patients with micrometastases in the SN with high risk of NSN metastases on nearly 40%, comparable to the risk for patients with macrometastases. ALND may still be recommended in these patients despite new evidence indicating omission of ALND to be safe in selected patients with positive SN. In patients with ITC no subgroup had a risk of NSN metastases over 25%, whereas 1/3 of the patients had a very low risk of NSN metastases, justifying omission of ALND.

5015 ORAL

Age Specific Competing Mortality in Breast Cancer Patients – a TEAM Study Analysis

C. Markopoulos[1], W. van de Water[2], H. Putter[3], C. Seynaeve[4], A. Hasenburg[5], D. Rea[6], J.M. Vannetzel[7], R. Paridaens[8], C.J.H. van de Velde[2], S. Jones[9]. [1]*Athens University Medical School, Surgery, Athens, Greece;* [2]*Leiden University Medical Center, Surgery, Leiden, The Netherlands;* [3]*Leiden University Medical Center, Biostatistics, Leiden, The Netherlands;* [4]*Erasmus MC Daniel Den Hoed, Medical Oncology, Rotterdam, The Netherlands;* [5]*University Hospital Freiburg, Gynecological Oncology, Freiburg, Germany;* [6]*University of Birmingham, Medical Oncology, Birmingham, United Kingdom;* [7]*Institut du Sein Henri Hartmann (ISHH), Oncology Radiotherapy, Neuilly sur Seine, France;* [8]*U. Z. Gasthuisberg(ISHH), Medical Oncology, Leuven, Belgium;* [9]*US Oncology Research LLC., Medical Oncology, The Woodlands Texas., USA*

Background: In addition to tumour related prognostic factors, characteristics of breast cancer patients may affect outcome. The aim of this study was to assess competing mortality in postmenopausal women with early breast cancer of the TEAM (Tamoxifen Exemestane Adjuvant Multinational) trial.
Material and Methods: 9,766 women were enrolled in the TEAM trial, randomized to either Exemestane 25 mg daily for 5 years or Tamoxifen 20 mg daily for 2.5−3 years, followed by Exemestane 25 mg daily for 2−2.5 years. Five-year results showed no difference in outcome between both arms (*Lancet* 22;377:321, 2011) enabling to analyze causes of mortality in the entire population. Patients were stratified by age at diagnosis (<65, 65−74, ⩾75 years) and survival was calculated by a Cox Proportional Hazard Model (A) as well as a Fine and Gray Model for competing mortality (B).
Results: All patients had hormone receptor positive tumours, 50% had node negative disease, 68% received radiotherapy, and 36% received chemotherapy. After a median follow up of 5.1 years, multivariable analysis using model A showed a higher proportion of breast cancer specific and non breast cancer related mortality with increasing age ($p < 0.001$). Using model B, which takes into account the risk of competing mortality, a higher breast cancer mortality over age was confirmed ($p < 0.001$) (Table 1).
Conclusions: Though the chance of dying from other causes than breast cancer was much higher in elderly patients, breast cancer mortality increased with higher age as well. Additionally, survival analyses evaluating the risk of competing mortality confirmed a higher absolute breast cancer mortality in the elderly, suggesting the possibility of suboptimal treatment. These data underline the need for optimal, individualized treatment of the elderly breast cancer patient, taking into account biological age and life expectancy, in order to improve breast cancer outcome in all age groups.

Table 1. Mortality analyses

	Breast cancer mortality			Non breast cancer mortality		
	5 yrs %	HR* (95% CI)	p value	5 yrs %	HR (95% CI)	p value
Cox Regression			**<0.001**			**<0.001**
<65 years	5	1 (reference)		1	1 (reference)	
65−75 years	6	1.23 (1.09–1.61)		5	2.56 (1.94–3.37)	
⩾75 years	8	1.86 (1.44–2.40)		14	7.08 (5.32–9.41)	
Fine and Gray			**<0.001**			**<0.001**
<65 years	5	1 (reference)		1	1 (reference)	
65−75 years	6	1.22 (1.00–3.26)		5	2.46 (1.86–3.25)	
⩾75 years	8	1.50 (1.16–1.94)		14	6.57 (4.90–8.80)	

*HR (hazard ratios) adjusted for country, histological grade, tumour size, nodal staus, ER, PgR, type of surgery, radiotherapy and chemotherapy.

Poster Discussion Presentations (Mon, 26 Sep, 11:00−12:00)

Breast Cancer

5016 POSTER DISCUSSION

BIG 1-98 Update: Evaluating Letrozole and Tamoxifen Alone and in Sequence at 8 Years Median Follow-up for Postmenopausal Women With Steroid Hormone Receptor-Positive Breast Cancer

R.D. Gelber[1], B. Thürlimann[1], A. Giobbie-Hurder[1], L. Mauriac[1], B. Ejlertsen[1], R. Paridaens[1], K.N. Price[1], M.M. Regan[1], A.S. Coates[1], A. Goldhirsch[1]. [1]*International Breast Cancer Study Group, BIG 1-98 Collaborative Group, Bern, Switzerland*

Background: BIG 1-98 is a Phase III randomized, double-blind trial comparing letrozole (Let), tamoxifen (Tam) and sequences of Let and Tam as adjuvant endocrine therapy for postmenopausal women with endocrine-responsive early breast cancer. In 2005 the superiority of Let over Tam for