which could be used to tailor treatment to individual patients. Most chemotherapeutic agents preferentially target proliferating and cycling cells, which results in mitotic catastrophe and apoptosis. We therefore hypothesized that the level of Bcl2 protein, which is a cell cycle/apoptosis regulator, could predict response and clinical outcome to these agents.
Material and Methods: Bcl2 expression was immunohistochemically evaluated in high risk (Nottingham prognostic index >3.4) luminal "ER+/HER2−" breast cancer from four independent series; (a) 135 BC patients treated with surgery + radiotherapy at Nottingham City hospital before 1986 who did not receive any endocrine therapy, (b) 430 BC patients treated with surgery + radiotherapy followed by Tamoxifen therapies, (c) 179 BC patients treated with surgery + radiotherapy followed by Tamoxifen and anthracycline-based chemotherapy and (d) 70 locally advanced primary BC patients treated with an anthracycline-based combination (FEC) followed by surgery + radiotherapy and Tamoxifen.
Results: Luminal BC patients with low Bcl2 expression had 2 to 4 fold increase risk of death and recurrence compared to those with high Bcl2 irrespective of Tamoxifen treatment (Table 1). After anthracycline-based neo-adjuvant chemotherapy, 33% of low Bcl2 expression luminal BC achieved pCR vs. 7% of high Bcl2 expression luminal BC (p = 0.02). Luminal BC patients with low or high Bcl2 expression who had received anthracycline based combined therapy in addition to Tamoxifen in either neo-adjuvant or adjuvant sittings had similar BC specific survival and progression free survival (p = NS).

Table 1

Variable	Breast cancer specific survival		Progression free survival	
	HR (95% CI)	P	HR (95% CI)	P
High risk luminal breast cancer patients who did not received Tamoxifen (n = 135)				
Bcl2+	1	0.00006	1	0.003
Bcl2−	3.7 (1.9−6.9)		2.3 (1.3−4.0)	
High risk luminal breast cancer patients who received Tamoxifen (n = 430)				
Bcl2+	1	0.00000001	1	0.0000003
Bcl2−	2.6 (1.8−3.7)		2.1 (1.5−2.9)	

Conclusions: Low Bcl2 expression was associated with poor prognosis of high risk luminal BC irrespective of hormone therapy. Bcl2 status could predict the potential benefit of anthracycline based chemotherapy of luminal BC which is resistance to Tamoxifen. Clinical trials based on Bcl2 expression in luminal breast cancer are warranted.

5176 POSTER

Gene Expression Profiles Predict Pathological Complete Response to Standard Neoadjuvant Fluorouracil, Doxorubicin, and Cyclophosphamide and Paclitaxel With or Without Trastuzumab in Early Breast Cancer

K. Tamura[1], F. Koizumi[2], C. Shimizu[1], T. Hojo[3], S. Akashi-Tanaka[3], T. Kinoshita[3], K. Aogi[4], N. Masuda[5], T. Yoshida[2], Y. Fujiwara[1]. *[1]National Cancer Center Hospital, Department of Breast and Medical Oncology, Tokyo, Japan; [2]National Cancer Center Research Institute, Division of Genetics Genomics and Genetics Group, Tokyo, Japan; [3]National Cancer Center Hospital, Department of Breast Surgery, Tokyo, Japan; [4]NHO Shikoku Cancer Center, Department of Surgery, Ehime, Japan; [5]National Hospital Organization Osaka National Hospital, Department of Surgery, Osaka, Japan*

Background: To examine the feasibility of gene expression signature as a predictor of pathological complete response (pCR) to sequential fluorouracil, doxorubicin, and cyclophosphamide (FEC) and weekly paclitaxel (P) with or without trastuzumab (T) neoadjuvant chemotherapy.
Materials and Methods: We have conducted consecutive two prospective phase II, establishing training and validation sets, with similar eligible criteria include, stage IIA-IIIC, chemotherapy-naïve, measurable disease, age ⩾20, PS 0/1, and adequate organ function. Patients were treated preoperatively with 4 cycles of FEC (500/100/500 mg/m^2) followed by 12 cycles of weekly P (80 mg/m^2) with or without T (2 mg/kg). Patients underwent pretreatment fine-needle biopsy for cDNA microarray using Affimetrix Gene Chip U133 plus 2.0 arrays with 30,000 differential expressions of various genes. We ranked gene probes from training sets according to a predictive power concerning pCR by Wilcoxon, and validated them using validation sets by SVM.
Results: Between July 2007 and December 2010, 122 patients were enrolled in the two consecutive prospective studies (training: 89 pts, validation: 33 pts). Median age was 51. PS 0/1: 115/7; Stage IIA/IIB/IIIA/IIIB/IIIC: 30/57/20/14/1; Histological subtype: ER+HER2- (LA)/ER+HER2+ (LB)/ER-HER2- (TN)/ER-HER2+ (enrich-HER): 51/18/24/29. All patients have received curable operations. pCR rate was 31.1% (LA; 2.0%, LB; 44.4%, TN; 37.5%, enrich-HER; 69.0%). 104 (85.2%) sufficient mRNA for cDNA microarray from individual primary breast cancer tissues fine-needle biopsy are available. As reported previously, the breast cancers were classified into a Luminal A/B, Basal-like, HER2-enriched, Claudin-low intrinsic subtypes, indicating a high quality of the representative method. In HER2 positive breast cancer, HER2-enriched subtype was a reproductive predictive marker. In contrast, In HER2 negative breast cancer, three genes (N-myc and STAT interacter, Tryptophanyl-tRNA synthetase, and IQCE) and basal-like subtype were validated as the predictors of pCR. The three genes were also identified as predictors of pCR in the triple negative population.
Conclusions: Specific gene expression profiles predict pCR to standard neoadjuvant regimen, especially in triple negative breast cancer.

5177 POSTER

Correlation Between PARP-1 Expression and In-vitro Chemotherapy Sensitivity in Patients With Breast Cancer

C. Liedtke[1], J. Packeisen[2], C. Denkert[3], O. Gluz[4], J. Tio[1], L. Kiesel[1], U. Vogt[5], B. Müller[3], K.R. Hess[6], H. Bürger[7]. *[1]University of Münster, Gynecology and Obstetrics, Münster, Germany; [2]Klinikum Osnabrück, Pathology, Osnabrück, Germany; [3]Charité, Pathology, Berlin, Germany; [4]WSG, Clinical Trials, Mönchengladbach, Germany; [5]European Laboratory Association, Pathology, Ibbenbüren, Germany; [6]MD Anderson Cancer Center, Biostatistics, Houston Texas, USA; [7]Klinikum Paderborn, Pathology, Paderborn, Germany*

Background: Expression of Poly-A-Ribose-Polymerase-1 (PARP-1) has come into scientific focus based on its potential exploitation as a therapeutic target through PARP inhibitors. Furthermore, it could recently be demonstrated that cytoplasmic expression of PARP-1 varies depending on molecular breast cancer subtypes and is correlated with an increased response to neoadjuvant taxane-anthrazykline containing chemotherapy (von Minckwitz et al., J Clin Oncol (in press)). *In-vitro*-chemotherapy sensitivity and resistance assays (CSRAs) allow for the direct measurement of chemotherapy sensitivity in a given tumour independent of host factors.
Methods: We conducted an immunohistochemical tissue-microarray (TMA) analysis of 550 samples of invasive breast cancers with regard to expression of a set of molecular markers including estrogen receptor (ER), progesterone receptor (PR) and HER2 as well as PARP-1. Triple negative breast cancers (TNBC) were identified through lack of expression of ER, PR and HER2. All cancers were analyzed in an *in vitro* CSRA analysis for epirubicin/docetaxel (ED) and epirubicin/cyclophosphamide (EC). *In-vitro*-chemotherapy sensitivity was analyzed using an adenosine triphosphate (ATP) bioluminescence assay.
Results: A moderate/high PARP-1 expression was found in 48 and 33% of cases with TNBC and non-TNBC, respectively (p = 0.015). A correlation between TNBC phenotype and cytoplasmic expression was not observed. Instead, an increased both cytoplasmic and nuclear expression of PARP-1 was correlated with an increased *in-vitro* sensitivity against ED (p = 0.012 and 0.025, respectively) but not EC (p = 0.27 and 0.62, respectively).
Conclusion: Our results support previous observations in that expression of PARP-1 is correlated with an increased sensitivity against taxane-anthracycline chemotherapy independent of tumour phenotype.

5178 POSTER

Response of Immunohistochemically (IHC) Defined Breast Cancer Sub-types to Dose-dense Sequential Adjuvant Chemotherapy. Pooled Analysis of Two Randomized Hellenic Cooperative Oncology Group (HeCOG) Phase III Trials

G. Fountzilas[1], M. Bobos[1], C. Papadimitriou[1], H. Gogas[1], H. Linardou[1], K.T. Kalogeras[1], D. Pectasides[1], P. Skarlos[1], A. Koutras[1], U. Dafni[1]. *[1]Hellenic Cooperative Oncology Group (HeCOG), Greece*

Background: To investigate the efficacy of adjuvant dose-dense sequential chemotherapy with epirubicin, paclitaxel and CMF in sub-groups of patients with high-risk operable breast cancer, according to immunohistochemically (IHC) defined tumour sub-types.
Materials and Methods: Formalin-fixed paraffin-embedded (FFPE) tumour tissue blocks from 1030 patients (72% of the eligible patients) participating in two adjuvant dose-dense sequential chemotherapy phase III trials (HE 10/97 and HE 10/00) were centrally assessed in TMAs by IHC for 6 biological markers, i.e. estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), Ki67, cytokeratin 5 (CK5), and epidermal growth factor receptor 1 (EGFR). Cases with HER2 IHC 2+ were further evaluated by CISH or FISH. Patients were classified as Luminal A (ER-positive and/or PgR-positive, HER2-negative); Luminal B (ER-positive and/or PgR-positive, Ki67⩾14); Luminal-HER2 (ER-positive

and/or PgR-positive, HER2-positive); HER2-enriched (ER-negative, PgR-negative, HER2-positive); triple negative (TN) (ER-negative, PgR-negative, HER2-negative); and basal core phenotype (BCP) (TN, CK5-positive and/or EGFR-positive).
Results: There were no significant differences in important patient characteristics between those with available FFPE tissue or not. DFS or OS did not differ significantly between patients treated with different dose-dense regimens.
Survival data according to immunophenotypical sub-types are shown in the table.

	N (%)	5-yr DFS (%)	5-yr OS (%)
Luminal A	268 (26)	81.4	89.9
Luminal B	417 (40.5)	75.8	88.7
Luminal-HER2	122 (12)	65.3	84.2
HER2-enriched	87 (8)	68.6	84.8
TN	136 (13)	62.1	73.0
BCP	92 (9)	62.9	73.7

DFS and OS were significantly different across sub-types (both $p < 0.001$). Luminal A and Luminal B demonstrated higher DFS (Luminal A vs B, $p = 0.072$), followed by HER2-enriched, Luminal-HER2 and TN. No differences were found in DFS between HER2-enriched, Luminal-HER2 and TN (all p=NS). Patients with TN phenotype demonstrated worse OS compared to other sub-types ($p < 0.001$, $p < 0.001$, $p = 0.018$, $p = 0.043$). Locoregional relapse was more frequent in patients with TN tumours ($p = 0.008$). Further, Luminal-HER2 phenotype was more frequently associated with the development of distant metastases.
Conclusions: TN phenotype is an adverse prognostic parameter for OS in patients treated with adjuvant dose-dense sequential chemotherapy. In addition TN phenotype has significantly worst DFS than Luminal A and B.

5179 POSTER

Elevated Pre-treatment Levels of Plasma C-reactive Protein Are Associated With Poor Prognosis After Breast Cancer

S. Bojesen[1], K.H. Allin[1], B.G. Nordestgaard[1], H. Flyger[2]. [1]*Copenhagen University Hospital, Clinical Biochemistry Herlev Hospital, Copenhagen, Denmark;* [2]*Copenhagen University Hospital, Breast Surgery Herlev Hospital, Copenhagen, Denmark*

Background: Previous epidemiologic studies have reported that elevated CRP levels may be associated with poor prognosis of several other types of solid cancers. Although breast cancers rarely are characterized by significant histological inflammation, emerging evidence nevertheless suggests that inflammatory pathways also play an important role in breast cancer progression. We examined whether plasma C-reactive protein (CRP) levels at the time of diagnosis of breast cancer are associated with overall survival, disease-free survival, death from breast cancer, and recurrence of breast cancer.
Methods: We observed 2910 women for up to seven years after they were diagnosed with invasive breast cancer. Plasma levels of CRP were measured at time of diagnosis and we assessed association between CRP levels and risk of reduced overall and disease-free survival, death from breast cancer, and recurrence of breast cancer by using the Kaplan–Meier method and Cox proportional hazards regression. During follow-up, 383 women died, 225 women died from breast cancer, and 118 women experienced recurrence of breast cancer.
Results: Elevated CRP levels across tertiles at the time of diagnosis were associated with reduced overall and disease-free survival and with increased risk of death from breast cancer (log-rank trend for all, $P < 0.001$). The multifactor-adjusted hazard ratio (HR) of reduced overall survival among women in the middle and highest versus lowest tertile of CRP were 1.30 (95% CI, 0.97–1.73) and 1.94 (1.48–2.55), respectively. Corresponding HRs of reduced disease-free survival were 1.16 (0.89–1.50) and 1.76 (1.38–2.25) and of death from breast cancer 1.22 (0.84–1.78) and 1.66 (1.15–2.41). For CRP levels in octiles there was a stepwise increased risk of reduced overall survival (P for trend <0.001) and the multifactor-adjusted HR among women in the highest versus the lowest octile of CRP was 2.51 (1.53–4.12). Compared to women with CRP levels in the 0−25% percentile (<0.78 mg/L), the multifactor-adjusted HR of reduced overall survival among women with CRP levels $\geqslant$95% percentile ($\geqslant$16.4 mg/L) was 3.58 (2.36–5.42). Among women with HER2 positive tumours, the multifactor-adjusted HR of reduced overall survival for the highest versus the lowest tertile of CRP was 8.63 (2.04–36.4).
Conclusions: Elevated CRP levels at the time of diagnosis of breast cancer are associated with reduced overall, and disease-free survival and with increased risk of death from breast cancer.

5180 POSTER

Calpain Expression and Survival of Patients With Basal Phenotype Breast Cancer

S. Storr[1], C.M. Woolston[1], A.R. Green[2], I.O. Ellis[2], S.G. Martin[1]. [1]*University of Nottingham, Academic Oncology, Nottingham, United Kingdom;* [2]*University of Nottingham, Histopathology, Nottingham, United Kingdom*

Background: The calpains are intracellular cysteine proteases responsible for the controlled proteolysis of a number of important cellular substrates involved in cytoskeletal remodelling, apoptosis and survival. In cancer, the expression levels of both milli (m)-calpain, micro (μ)-calpain and their endogenous inhibitor calpastatin are altered. We have investigated the altered expression of m-calpain, μ-calpain and calpastatin in breast cancer. We have previously shown in HER2 positive breast cancer patients treated with adjuvant chemotherapy and trastuzumab that low expression of μ-calpain (calpain-1) is a marker of relapse-free survival.
Materials and Methods: In our current study we have analysed the expression of m-calpain, μ-calpain and calpastatin in 1371 breast cancer patients using immunohistochemistry.
Results: m-Calpain is associated with relapse-free survival ($p = 0.022$) and remains so under multivariate analysis ($p = 0.045$). Interestingly high m-calpain expression is important in patients with basal phenotype (CK5/6 and/or CK14 positive), and is able to stratify such patients into those that had worse breast cancer-specific survival and those that had survival rates similar to patients with non-basal phenotype ($p = 0.0004$). A similar observation was made in patients with triple negative disease (ER, PgR, and HER2 receptor negative) whereby high m-calpain could stratify this subset of patients into those with worse breast cancer-specific survival and those with survival similar to patients with receptor positive disease ($p = 0.0003$).
Conclusions: The expression of the calpain system is important in breast cancer; in particular, m-calpain expression is of importance in patients with basal phenotype and triple negative disease. The expression of m-calpain in these subsets of patients could be used to determine those with a poor prognosis.

5181 POSTER

The Risk of Breast Cancer Among Women Who Start Smoking as Teenagers

E. Odiase[1]. [1]*SmokeFree Foundation, Tobacco/Cancer Control, Abuja, Nigeria*

Background: There are several causes of breast cancer in the world today. Recent research has proven that tobacco use also causes breast cancer.
Goals: To examine the effect of smoking on breast cancer risk in a large population-based cohort of women, many of whom started smoking as teenagers.
Methods: We followed 102,098 women, ages 30 to 50 years, completing a mailed questionnaire at recruitment to the Nigerian-Kenyan Cohort Study in 1995/1996, through December 2004. We used Cox proportional hazard regression models to estimate relative risk (RR) of breast cancer associated with different measures of smoking initiation, duration, and intensity adjusting for confounding variables. We conducted analyses on the entire study population, among women who had smoked for at least 20 years, among non drinkers, and separately for each country.
Results: Altogether, 1,240 women were diagnosed with incident, invasive breast cancer. Compared with never smokers, women who smoked for at least 20 years and who smoked 10 cigarettes or more daily had a RR of 1.34 (95% CI, 1.06–1.70). Likewise, those who initiated smoking prior to their first birth (1.27, 1.00–1.62), before menarche (1.39, 1.03–1.87), or before age 15 (1.48, 1.03–2.13) had an increased risk. In contrast, women who had smoked for at least 20 years, but started after their first birth, did not experience an increased breast cancer risk. The increased RR associated with smoking was observed among nondrinkers of alcohol, women with and without a family history of breast cancer, pre-menopausal and post-menopausal women, and in both countries.
Conclusion: Our results support the notion that women who start smoking as teenagers and continue to smoke for at least 20 years may increase their breast cancer risk.