13%), tumor size (T1 59% T2 39% T3 1.6%) and grade (G1 32% G2 39% G3 15%).
Results: Median follow-up was 47 months. Actuarial (OS and DFS) at 4 years were luminal A (99.3% and 95.8%), luminal B (95.6% and 80.4%), Her2 (89.8% and 80.9%) and triple negative (74.6% and 58.7%), p = 0.0001; for N0 (98.8% and 93.9%) N1−3 (90.8% and 83.6%) and N > 4 (87.1% and 71.8%), p = 0.0001. Significant independent prognostic factor for OS were BC subtypes (with relative risk (RR) luminal B 4.7, Her2 3.6 and triple negative 12.08 referent to luminal A, p = 0.0001), and the number of positive nodes (N1−3 RR = 6.6 and N > 4 8.3 referent to N0 category, p = 0.004), respectively. Significant independent prognostic factor for DFS were BC subtypes (with relative risk (RR) luminal B 4.8, Her2 3.9 and triple negative 7.7 referent to luminal A, p = 0.0001), the number of positive nodes >4 (RR 2.01 referent to N0 category, p = 0.049) and tumor size (T2 RR 2.28 referent to T1 category, p = 0.004).
Conclusions: A simple immunopanel can divide breast cancers into biologic subtypes with independent prognostic effects and provides additional information to nodal status. Triple negative status emerged as a strong adverse prognostic factor.

5163 POSTER

Methylation in breast cancer and correlate ER with tumor phenotypes and prognostic factors

J. Martínez-Galán[1], B. Torres[2], V. Gutierrez Calderón[1], G. Durán Ogalla[1], R. Del Moral Avila[3], M. Cobo Dols[1], J. López Peñalver[2], M. Benavides Orgaz[1], M. Ruiz de Almodóvar[2], M.I. Núñez[2]. *[1]Hospital Carlos Haya General, Medical Oncology, Malaga, Spain; [2]Centro de Investigaciones Biomédicas, Radiobiology, Granada, Spain; [3]Hospital Virgen de las Nieves, Radiation Oncology, Granada, Spain*

Background: To investigate the association between ESR1 gene hypermethylation and tumor phenotype including diagnosis and treatment response are the objetive of this studies. Other gene as 14−3-3σ were also analized.
Materials and Methods: Since January 2002 to June 2005, 107 women with breast cancer and 108 control subjects were recruited. Real Time QMS-PCR SYBR green (methylation-specific PCR) was used to analyze the methylation of *ESR1* and *14−3-3σ* gene promoter regions as breast cancer biomarkers. Tumours were classified as phenotype basal, luminal A, Luminal B and phenotype HER2+.
Results: Ours analyses revealed low or absent methylation *ESR1*and *14−3-3σ* in healthy controls and significant differences between breast cancer patients (pts) and healthy controls in relative serum levels of methylated gene promoters *ESR1 (p* = 0.0112) and *14−3-3*s (p = 0.0047). Presence of methylated *ESR1* in serum of breast cancer patients was associated with *ER-negative* phenotype (*p* = 0.0179). Of the available cases, 60 pts (56%) were Luminal A, 10 pts (9.3%) Luminal B, 13 pts (12%) Basal like and 9 pts (8.4%) HER2+. We observed that methylated *ERS1* was preferably associated with phenotype Basal Like and worse interval progression free and survival global though p > 0.05. We observed that hypermethylation of *ERS1* and *14−3-3σ* combined differentiated between breast cancer patients and healthy controls (p = 0.0001) with a sensitivity of 81% (95% CI: 72−88%) and specificity of 88% (95% CI: 78−94%). In addition observed lower methylated *ERS1* and *14−3-3σ* value after surgery, respect pretreatment levels, but without an overall statistically significant difference. With a median follow up of 6 years, we found that patients with a significant decrease of sera methylated levels of both genes after surgery had better time to progression an overall survival respect patients without this observation.
Conclusions: This study identifies the presence of variations in global levels of methylation promoters genes in healthy controls and breast cancer with different phenotype classes and shows that these differences have clinical significance. In the future this panel of genes detected could be useful as markers for early detection of breast carcinoma. These findings cast some doubts on the utility for early cancer diagnosis of highly sensitive techniques to identify hypermethylation of specific gene promoters in DNA extracted from serum. Although numerous issues remain to be resolved, the quantitative measurement of circulating methylated DNA is a promising tool for cancer risk assessment.

5164 POSTER

Impaired glucose tolerance in non-diabetic women during adjuvant chemotherapy for breast cancer

L. Purandare[1], T. Hickish[1], G. Astras[1], S. Penfold[2], P. Thomas[3], D. Kerr[2]. *[1]Royal Bournemouth Hospital, Medical Oncology, Bournemouth, United Kingdom; [2]Royal Bournemouth Hospital, Endocrinology, Bournemouth, United Kingdom; [3]Bournemouth University, Statistics, Bournemouth, United Kingdom*

Background: Up to 16% of patients with breast cancer have diabetes and diabetic individuals tend to have poorer outcomes following treatment for breast cancer. During chemotherapy, dexamethasone is widely used to prevent side effects. However, glucocorticoid administration is associated with impairment of insulin sensitivity, elevations in peripheral glucose levels as well as suppression of the hypothalamic-pituitary-adrenal axis for up to 3 weeks. We measured blood glucose levels in a group of non-diabetic women receiving adjuvant chemotherapy for breast cancer.
Materials and Methods: 39 women (age 58.6±12.8 years, BMI 27.2 +/-4.9 kg/m^2) participated in this study which was approved by the local ethics committee and all patients gave informed consent. Patients received either 6 cycles of fluorouracil, epirubicin, cyclophosphamide (FEC) (18) or 3 cycles of FEC followed by 3 cycles of docetaxel (21). Before each cycle of FEC, patients received 8 mg of dexamethasone (po). The patients who received docetaxel had 8 mg of dexamethasone (po) 24 hours, 12 hours and immediately before docetaxel. For each cycle of chemotherapy non-fasting glucose was measured before the treatment cycle began, immediately after the pre-chemotherapy Dexamethasone was administered but before chemotherapy and, immediately after chemotherapy and 10 days after each cycle.
Results: There was an increase in blood glucose levels with later cycles among women who received the higher dose of Dexamethasone in combination with docetaxel (cycle 5: P < 0.001; cycle 6: P = 0.002 [paired *t* tests]) (Table). Before the first cycle of chemotherapy, none had blood glucose levels in either the impaired glucose tolerance range (ie, 7.8−11.1 mmol/L) or the diabetic range (ie, >11.1 mmol/L). Increasing number of patients developed glucose intolerance as cycles progressed; 6 had blood glucose levels in the impaired tolerance range and 8 had levels within the diabetic range following the 5th cycle.

Cycle	Before treatment cycle	Immediately after Dexamethasone but before chemotherapy	After Dexamethasone and chemotherapy	10 d after chemotherapy
1	5.8 [1.1]	4.8 [0.7]	5.7 [2.0]	5.7 [0.7]
2	5.5 [0.9]	5.6 [1.0]	5.5 [0.8]	5.5 [1.0]
3	5.3 [0.9]	5.5 [1.3]	5.9 [1.5]	5.8 [1.4]
4	5.6 [0.9]	6.0 [1.6]	6.4 [1.8]	5.6 [0.8]
5	5.3 [1.0]	7.7 [3.0]	7.8 [2.7]	6.0 [2.0]
6	5.5 [0.8]	8.0 [2.7]	8.1 [3.1]	6.0 [1.2]

Conclusions: The implications of transient hyperglycaemia on the efficacy of chemotherapy in this setting is uncertain and further investigation is indicated.

5165 POSTER

Microarray based determination of ER, PR and HER2 receptor status compared to local IHC assessment in 11 hospitals

J. Palacios[1], J.M. Guinebretiere[2], G. Seitz[3], T. Anzeneder[4], P. Roepman[5], L. Stork-Sloots[6], E. van Lienen[6], Y. Tokunaga[7], T. Watanabe[7]. *[1]HHUU Virgen del Rocio, Servicio de Anatomia Patologia, Sevilla, Spain; [2]Centre Rene-Huguenin, Service de Pathologie, Saint Cloud, France; [3]Socialstiftung Bamberg, Institut fur Pathologie, Bamberg, Germany; [4]Stiftung PATH – Patients Tumorbank of Hope, Projektleitung, Augsburg, Germany; [5]Agendia, Bioinformatics, Amsterdam, The Netherlands; [6]Agendia, Clinical Department, Amsterdam, The Netherlands; [7]Hamamatsu Oncology Center, Department of Oncology, Hamamatsu, Japan*

Background: The level of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression is predictive for prognosis and/or treatment response in breast cancer patients. However, differences in immunohistochemistry (IHC) methods and interpretation can substantially affect the accuracy and reproducibility of the results. The recently developed TargetPrint test measures the mRNA expression level of ER, PR and HER2 and provides an objective alternative to IHC. This study describes a

comparison of the microarray based TargetPrint with IHC and fluorescent in situ hybridization (FISH) assessments generated by local standards in 11 hospitals.
Material and Methods: The mRNA level of ER, PR and HER2 was assessed retrospectively on 144 breast tumor samples containing sufficient tumor cells, collected by a German tumor bank. The patients were diagnosed in 7 different hospitals. Prospective tumor samples with sufficient tumor cells were collected for 27 patients presenting to 4 different hospitals between November 2008 up to present. The results of the IHC/FISH assessments performed according to the local standards of the hospitals were compared to the quantitative gene expression readouts.
Results: Sufficient RNA for microarray analysis was obtained from 140 (97%) retrospective samples and from 26 (96%) prospective samples. Comparison of IHC and microarray readout indicated a very high concordance of 97% for ER, 86% for PR and 94% for HER2 on the retrospectively analyzed samples (Table 1). The prospectively collected samples indicated a 100% concordance for ER and HER2 and 77% for PR (Table 1). All PR discordant cases (n = 6) originated from a single centre. Three samples (2 retrospective, 1 prospective) were excluded from concordance analysis as they were scored HER2IHC 2+ without additional FISH analysis. All three HER2 IHC 2+ samples were classified HER2 negative by TargetPrint. Prospective data collection is ongoing and more data will be presented at the meeting.

Table 1.

	ER	PR	HER2
7 centers retrospective	97% (n = 140)	86% (n = 140)	94% (n = 138)
4 centers prospective	100% (n = 26)	77% (n = 26)	100% (n = 25)

Conclusion: Microarray based readout of ER, PR and HER2 status using TargetPrint is highly comparable to local IHC and FISH analysis on retrospectively and prospectively analyzed samples in various hospitals. Using TargetPrint microarray readouts for hormone and HER2 receptor status in addition to standard IHC will improve the molecular characterization of breast cancer tissue.

5166 POSTER

The prognostic significance of age at diagnosis in patients with breast cancer younger than 35 years

W. Bal[1], M. Jarzab[1], E. Nowara[1]. [1]*M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Clinical Oncology, Gliwice, Poland*

In breast cancer patients, age below 35 years (yr) is an independent risk factor of recurrence and death, even after correction for disease stage. However, as breast cancer (ca) occurs rarely in the mentioned age group (2–4% of all patients), prognostic factors for this population are not well understood.
It has been estimated that in general breast ca population the hazard of recurrence decreases with age by 4% per year of life. The aim of our study was to assess the association of age at diagnosis with disease outcome in women with breast cancer, aged 35 years or less.
Methods: The analysis was carried out retrospectively in 190 patients (pts) with breast cancer aged 35 years or less, referred to our Clinic between 1997–2006 (after exclusion of 10 patients with stage IV, 8 patients not treated surgically and 9 patients with incomplete clinical data). For all 190 patients the time to relapse (DFS, disease-free survival) was assessed. The median follow-up time was 47.7 months. Among this group there were 21.6% patients aged 34–35 years, 44.2% 30–33 yr, 25.2% 26–29 yr and 6.4% 25 yr and less.
Results: Relapse occurred in 36.6% of pts (20.5% distant metastases and 16.1% local recurrence only). 5-year recurrence-free survival was 57.2%, with estimated median survival time 10.9 yr. In univariate Cox analysis the most notable prognostic factors were nodal status (the most significant, both by clinical assessment and pathological analysis, $p < 0.005$), positive HER2 and negative ER/PR ($p < 0.05$). In the analyzed cohort of patients below age of 35 years, older patients showed poorer prognosis compared to very young women: patients aged 34–35 showed significantly worse 5-yr survival (41.9%), compared to younger patients (78.4%, $p = 0.004$). In Cox regression modelling, age at diagnosis increased the relapse risk by 7.6% per each year ($p = 0.07$), within the moderately narrow age frame assessed in our study. The effect of age group (34–35 vs younger) was significant also in multivariate analysis, in the context of nodal and hormone receptor status, with hazard ratio of 1.93 ($p = 0.016$).
Conclusions: In women below the age of 35 years, the increase of age seems to elevate the risk of disease relapse. This finding, contradictory to the generally observed poor risk in patients below 35 years and age-related decrease of risk in the whole population, warrants further investigation.

5167 POSTER

French cost effectiveness study of the MammaPrint 70-gene signature in early stage breast cancer patients

D. Zarca[1], L. Stork-Sloots[2], F. de Snoo[3], G. Brink[4], Y. Dervaux[5], L. Boubli[6]. [1]*Clinique du Trocadero, Surgical Oncology, Paris, France;* [2]*Agendia BV, Project Management, Amsterdam, The Netherlands;* [3]*Agendia BV, Medical Affairs, Amsterdam, The Netherlands;* [4]*Agendia BV, Regulatory Affairs and Quality Assurance, Amsterdam, The Netherlands;* [5]*Agendia BV, Marketing and Sales Manager France, Paris, France;* [6]*Service Gynécologie-Obstétrique, Ap-HM Hôpital Nord, Marseille; , France*

Background: In early breast cancer, adjuvant chemotherapy increases the life expectancy of patients with high risk of developing metastases. However, for the other patients, chemotherapy-associated adverse effects outweigh the benefits.
Compared to clinicopathologic risk assessment, the MammaPrint 70-gene test has been shown to provide additional prognostic information for early stage breast cancer patients. However, the cost-effectiveness of this strategy is not well understood.
Materials and Methods: The budgetary impact of Mammaprint was studied using a Markov model. In France the initial target population for Mammaprint are stage I and II node negative breast cancer patients. Every year approximately 37,000 patients meet these criteria.
It has been demonstrated that Mammaprint can reduce the amount of unnecessary chemotherapy by 11% compared to Adjuvant!Online and by 27% compared to the St-Gallen criteria.
Results: In economic terms, we now show that the cost of MammaPrint was offset by the savings, due to a lower number of administered chemotherapies. The model estimates mean savings to be € 9,043 per 100 patients per year in the base case scenario. These results are sensitive to chemotherapy price, to relative usage of St-Gallen and Adjuvant!Online and to risk reduction associated with chemotherapy.
Conclusions: In summary, MammaPrint is a gene expression profiling test that has proved to be more accurate than current risk assessment tools. It helps oncologists to identify patients who may forgo unnecessary adjuvant chemotherapy in comparison to Adjuvant!Online or stGallen criteria. As patient's quality of life and rational use of resources are key factors in decision-making process, Mammaprint can be considered to be an efficient tool. As more costly systemic therapies are likely to become standard in the future, the economic advantages of Mammaprint might become even more apparent.

5168 POSTER

Circulating tumor cells (CTCs) in peripheral blood of breast cancer (BC) patients two years after primary diagnosis – Results from the German SUCCESS trial

B. Rack[1], C. Schindlbeck[1], A. Schneeweiss[2], I. Schrader[3], K. Friese[1], M.W. Beckmann[4], K. Pantel[5], W. Lichtenegger[6], H. Sommer[1], W. Janni[7]. [1]*University of Munich, Department of Gynecology – Innenstadt, Muenchen, Germany;* [2]*University of Heidelberg, Department of Gynecological Oncology, Heidelberg, Germany;* [3]*Henriettenstiftung, Department of Gynecological Oncology, Hannover, Germany;* [4]*University of Erlangen, Department of Gynecology, Erlangen, Germany;* [5]*University Hamburg-Eppendorf, Institute for Tumor Biology, Hamburg, Germany;* [6]*Charité University Hospital, Department of Gynecology, Berlin, Germany;* [7]*Heinrich-Heine-University, Department of Gynecology, Duesseldorf, Germany*

Background: While CTCs have shown promising results as marker of treatment efficacy and early recurrence in MBC, there is a lack of data in the adjuvant setting. The SUCCESS trial evaluates the role of persisting CTCs at primary diagnosis and after chemotherapy as well as two years after diagnosis in primary BC patients treated with zoledronate.
Methods: We analyzed 23 ml of peripheral blood in N+ and high risk N− primary BC pts receiving 3×FEC(500/100/500)-3×Doc100 q3w vs. 3×FEC(500/100/500)-3×DocGemcitabine(75/1000 d1+8) chemotherapy followed by 2 yrs (4 mg q3m×24 m) vs. 5 yrs (4 mg q3m×24 m followed by q6mx36 m) of zoledronate. CTC results after two years are shown. CTCs were assessed with the CellSearchSystem (Veridex, Warren, USA). After immunomagnetic enrichment with an anti-Epcam-antibody, cells were labelled with anti-cytokeratin (8, 18, 19) and anti-CD45 antibodies.
Results: The data of 579 pts at the mean of 29 months (range 20–43) after diagnosis are available. 4.3% of pts (n = 25) presented with >1CTC in peripheral blood. In pts with the detection of CTCs, the mean number of cells was 1 (range 1–29). While we found 1 CTC in 5.9% and 2 CTCs in 1.6% of pts, 1.5% had 3–5 CTCs, 1.2% >5 CTCs. We found no correlation between the presence of >1CTC with tumor size ($p = 0.41$), nodal status