of effective chemotherapeutic drugs are available, it is difficult to predict which combination of drugs shows clinical benefit in the individual patient. Clearly, molecular markers are needed that can predict therapy efficacy.
Our goal is to identify protein markers that can predict resistance to anthracycline-based chemotherapy in breast cancer patients using a **comparative proteomics approach**.
Materials and Methods: Snap frozen primary breast tumour tissues (n = 34) were used from patients treated with first line anthracycline-based chemotherapy for recurrent disease. 23 patients responded to treatment (objective response, OR), 15 were resistant (progressive disease, PD). From each tumour tissue, 10 mm cryosections were subjected to laser capture microdissection. Per tissue, ~4,000 epithelial tumour were collected, tryptic digests were prepared, and measured by MALDI-FTICR mass spectrometry (MS). Comparative peptide profiling performed for the identification of differentially abundant proteins, which were subsequently associated with clinical parameters.
Results: A total of 165 differential peptides were identified ($p < 0.05$), of which 16 had a $p < 0.01$. Of the latter peptides, 1 was higher in OR, 11 were higher in PD, and 4 were uniquely present in PD. Through targeted MS/MS, amino acid sequence of 10 peptides was revealed. 10 out of 16 peptides associated with progression free survival upon first line FEC/FAC treatment. Using step-down analysis, a 2 peptide predictor (representing NONO and RPS2A proteins) was built. The predictor had a strong correlation with therapy resistance ($X^2 = 14.8$, $p < 0.0001$), HR = 7.0, [95% CI: 2.4–20.3], $p < 0.0001$.
Conclusion: A 2-peptide predictor was identified for 1st line chemotherapy resistance in breast cancer. Further validation in independent samples is needed to determine clinical relevance.

5083 POSTER

The Predictive and Prognostic Significance of PTEN, P27 and PI3K Expression in HER2 Overexpressing Metastatic Breast Cancer

K. Okutur[1], N. Bassülü[2], K. Aydin[1], M. Bozkurt[1], E. Namal[1], C. Tecimer[3], N.M. Mandel[4], Z. Akçali[1], G.B. Dogusoy[2], G. Demir[1]. *[1]Istanbul Bilim University, Medical Oncology, Istanbul, Turkey; [2]Istanbul Bilim University, Pathology, Istanbul, Turkey; [3]Gayrettepe Florence Nightingale Hospital, Medical Oncology, Istanbul, Turkey; [4]VKV American Hospital, Medical Oncology, Istanbul, Turkey*

Background: In this study we aimed to investigate the predictive and prognostic significance of PTEN, PI3K and p27 expression which are components of the PI3K/Akt signaling pathway in HER2 positive metastatic breast cancer.
Material and Methods: Twenty-five patients who carried a diagnosis of metastatic HER2 positive breast carcinoma and who have received trastuzumab-based therapy as first-line treatment were recruited for the study group. All of the patients breast tissue samples were evaluated for PTEN, PI3K and p27 expression by immunohistochemistry and their correlations with tumour characteristics, response to treatment, time to progression (TTP), time to recurrence (TTR) and overall survival (OS).
Results: In 76% (n = 19) of the patient group PTEN expression and in 80% (n = 20) p27 expression was found to be negative. In 24 subjects (96%) PI3K expression was reported as positive. When the group of patients who respond to trastuzumab treatment was compared to the group who did not respond to treatment with trastuzumab, there was no statistically significant association found for expression of PTEN, p27 and PI3K. When the same comparison was made for tumour characteristics a significant relation was found between tumour size and PTEN, p27 and PI3K expression (p values 0.009, 0.003 and <0.001, respectively); but a statistically significant relation between expression of the above stated expression of the genes and tumour grade, lymphatic invasion, vascular invasion, and presence of distant metastasis was not found to be present. OS and TTP was significantly longer in the patient group who responded to trastuzumab based treatment compared to the group who was unresponsive to the treatment (p values 0.016 and 0.006, respectively). Although the status of PTEN, p27 and PI3K expression was not found to be significantly correlated with response to trastuzumab treatment a trend towards lower OS, TTP and TTR in patients with loss of PTEN expression which did not reach a statistical significance was observed. A similar trend for lower OS and TTR in patients whose tumour tissues did not express p27 was also found. A relation between PI3K expression and tumour characteristics with OS, TTP and TTR was not found.
Conclusions: Loss of expression of PTEN, low expression of p27 and positive PI3K expression was found to be frequent in HER2 positive breast cancer. Their frequency was higher when compared to frequency of expression in sporadic breast cancer reported in the literature. Although the small number of our study group has made statistical interpretation of the results difficult, we propose the results of our study support the view that the presence of PTEN and p27 expression in HER2 positive metastatic breast cancer predicts response to trastuzumab treatment and shortened OS. Our results indicate that PI3K/Akt signaling pathway is active in HER2 overexpressing breast cancer.

5084 POSTER

Receptor Conversion in Breast Cancer Brain Metastases (BM)

R. Duchnowska[1], B. Szostakiewicz[2], R. Dziadziuszko[2], T. Mandat[3], W. Olszewski[3], B. Jarosz[4], B. Czartoryska-Arlukowicz[5], B. Radecka[6], W. Biernat[7], J. Jassem[2]. *[1]Military Institute of Medicine, Department of Oncology, Warsaw, Poland; [2]Medical University of Gdansk, Department of Oncology and Radiotherapy, Gdansk, Poland; [3]Maria Sklodowska-Curie Memorial Institute of Oncology Warsaw, Department of Neurosurgery, Warsaw, Poland; [4]Medical University of Lublin, Department of Pathology, Lublin, Poland; [5]Regional Oncology Center Bialystok, Department of Oncology, Lublin, Poland; [6]Regional Oncology Center Opole, Department of Oncology, Opole, Poland; [7]Medical University of Gdansk, Department of Pathology, Gdansk, Poland*

Background: Several studies have indicated that the phenotype of breast cancer metastases may differ from those of primary tumour (PT). However, the data on such conversion for BM is limited. We compared the immunohistochemistry (IHC) expression of ER, PgR and HER2 in BM with that in the matched PT in 96 breast cancer patients (pts) who underwent excision of BM.
Methods: Pt characteristics (after exclusion of missing data): mean age at brain surgery: 52 years (29−83 years); 84% ductal carcinoma, 58% grade 3, 41% ER+, 30% PgR+ (⩾10% tumour cells with nuclear staining), 44% HER2+ (IHC 3+ or FISH+), 32% triple-negative (TN). 95% of pts underwent breast cancer surgery, preceded in 40% by systemic therapy. Prior to brain surgery 96% and 42% of pts, respectively, received chemotherapy or endocrine therapy as (neo)adjuvant or palliative treatment, and 77% of HER2+ pts received trastuzumab. The median time from breast cancer diagnosis to brain surgery was 29 months (range: 0 to 166 months). 67% of pts had single BM and 24% had 1−3 lesions. The most common site of BM was cerebellum and parietal lobe. 64% of pts had controlled extracranial disease at brain surgery and 87% had Karnofsky PS ⩾ 70%. After brain surgery 83% of pts received radiotherapy, 57% chemotherapy, 21% endocrine therapy and 27% anti-HER2 therapy.
Results: ER and PgR conversed to negative in 47% and 59% of pts, respectively, and to positive in 19% and 13% (Table). The respective conversions for HER2 were 8% and 13%. Of the 31 TN cancers 8 (26%) gained ER or PgR and 2 (7%) HER2. The percentage of hormone receptor (HR) positive tumours was lower in BM than in PT (ER: 33% vs 41%; PgR: 22% vs 30%, respectively), whereas it was similar for HER2+ (44% vs 47%) and TN cancers (32% vs 34%). HER2 loss in BM occurred in only 8% of pts who received trastuzumab. The median overall survival from brain surgery in the entire group was 13.4 months (16.1, 12.2 and 15.7 months for ER/PgR+, TN and HER2 in BM, respectively; all HER2+ pts were assigned to HER2+ group, irrespective of HR expression). There was no apparent prognostic impact of any receptor conversion.
Conclusions: Receptor conversion is a common event in breast cancer BM. Predominant conversion includes the loss of HRs, whereas HER2 and TN phenotypes are more stable. Trastuzumab therapy does not impact HER2 expression in BM.

Receptor	Total	pos-neg (%)	neg-pos (%)
ER	95	18/38 (47%)	11/57 (19%)
PgR	94	16/27 (59%)	9/67 (13%)
HER2	92	3/40 (8%)	7/52 (13%)

5085 POSTER

New Curcumin Analogue RL-66 Has Promising Anti-breast Cancer and Antiangiogenic Activity

B. Yadav[1], S. Taurin[1], L. Larsen[2], R. Rosengren[2]. *[1]University of Otago, Department of Pharmacology and Toxicology, Dunedin, New Zealand; [2]University of Otago, New Zealand Institute for Plant and Food Research, Dunedin, New Zealand*

Previously we reported that the new curcumin analogue RL-66 had promising cytotoxicity in various estrogen receptor (ER) negative breast cancer cells. Therefore, we further explored the anticancer potential of RL-66 in MDA-MB-468 human breast cancer cells. Cell cycle distribution and apoptosis induction were assessed by flow cytometry and protein expression was analyzed by Western blotting. Furthermore, the effect of RL-66 on tumour growth was examined in MDA-MB-468 mouse xenograft

model. We also examined antiangiogenic potential of RL-66 *in vitro* using the endothelial cell tube formation assay and transwell migration assay.
The results showed that RL-66 arrested MDA-MB-468 cells in the G2/M phase of cell cycle. Furthermore, RL-66 increased apoptosis in MDA-MB-468 cells by 4-fold compared to control. Moreover, RL-66 altered the expression and phosphorylation pattern of a variety of proteins which are involved in either cell proliferation or apoptosis. Importantly, treatment of nude mice bearing MDA-MB-468 xenografts with RL-66 (8.5 mg/kg/d, 70d, PO) significantly reduced tumour growth by 50% compared to control. In addition, RL-66 showed antiangiogenic properties by inhibiting endothelial cell invasion and the ability of these cells to form a capillary like tube network.
Thus our findings provide evidence that RL-66 has promising anticancer activity *in vitro and in vivo* in ER negative breast cancer in addition to antiangiogenic activity *in vitro* and thus it has potential clinical application.

5086 POSTER

Quality of Life − Patient-reported Outcomes in Patients With Advanced Hormone Receptor Positive Breast Cancer

Y. Su[1], E. Flood[2], J. Devlen[2], S. Tsay[2], K. Beusterien[2], S. Wagner[1]. *[1]Bristol-Myers Squibb, Global Health Economics and Outcomes Research, Princeton NJ, USA; [2]Oxford Outcomes, Patient-reported Outcomes, Bethesda MD, USA*

Background: Quality of life/patient-reported outcomes (PRO) are an important consideration in the care of patients with advanced breast cancer (BC). Approximately 75% of invasive BCs are hormone receptor positive (HR+). HR+ BC is distinct from HR negative BC in its pathological, clinical, and prognostic features. The aim of this study is to identify PRO instruments that are fit for purpose in these patients and meet regulatory standards for PRO claims of new medicines.
Materials and Methods: Data were obtained from a systematic literature review and interviews with 2 clinical experts (1 US, 1 EU). Literature search was conducted using OVID (EMBASE & Medline) for publications from 2000–2010.
Results: The literature search yielded 636 abstracts; of these, 33 assessed PRO in advanced HR+ BC. Symptoms and functional impacts of the disease and treatments identified through literature and expert input include bone pain due to bone metastasis, weakness, fatigue, abdominal fullness and dyspnea due to liver and lung metastases respectively, and endocrine symptoms related to hormone treatments. The most commonly used PRO instruments included the EORTC and Functional Assessment of Cancer Therapy (FACT) questionnaires core and BC-specific modules (EORTC QLQ-C30, QLQ-BR23, FACT-B). These instruments however do not capture the key issues important to these patients. Symptom-specific instruments such as bone pain or bone metastasis specific instruments EORTC QLQ-BM22, BOMET-QOL10, and FACT-Bone Pain, and endocrine symptom-specific instrument FACT-ES are not widely used and not BC specific. All of the instruments failed to show that input from advanced HR+ BC patients is solicited in the development of the questionnaires. Their validity, sensitivity, and reliability in this patient population are unclear. To address all aspects of PRO in this patient population, it would seem necessary to use multiple instruments with redundant questions of varying validity and reliability. No single instrument is fit for purpose in this patient population by regulatory standards.
Conclusions: Symptoms and functional impacts important to these patients need to be confirmed with patient input. New medicines interested in claims of PRO benefits would need a single instrument that captures all key issues confronting this patient population with sensitivity, validity, and reliability and without undue burden to the patients.

5087 POSTER

An Integrated Approach for Causal Association Among Gene Expression, Genotype Variation and Chronic Fatigue in Breast Cancer

H.S. Solvang[1], H.L.H. Landmark-Høyvik[1], H.K.R. Reinertsen[1], J.H.L. Loge[1], S.D.F. Fosså[1], A.L.B.D. Børresen-Dale[1], V.N.K. Kristensen[1], H.E. Edvardsen[1]. *[1]Norwegian Radium Hospitalet, Genetics, Oslo, Norway*

Background: Fatigue is the most common late effect of cancer therapy. The etiology of fatigue is still unclear. To elucidate the mechanisms behind fatigue, we have so far applied basic statistical approaches to two data sets including mRNA expression, SNPs, cancer-related information and the fatigue questionnaire (FQ) scored to identify chronic fatigue (CF). Incorporating information of genotype, expression and disease may improve understanding of disease etiologies, we focus on developing an integrated approach. The method of choice is model-based statistical tests [1] that identified causality among specific genotype variation, mRNA expression levels and longitudinal clinical data.
Material and Methods: Women treated for BC stage II/III at the Norwegian Radiumhospitalet were in 2004/2005 invited to attend a primary follow-up study on late effects. 76% of the invited women eligible subjects completed the FQ. RNA and DNA were isolated from peripheral blood and mRNA and SNP were obtained by Illumina platform. Using SNP, mRNA and CF data, we consider possible relationships among them by following three models, causal, reactive and independent models defined in [1]. Each model is represented by a Bayesian model and likelihood-based model selection is applied to select the best-fit-model to each genomic location. After matching these locations to genes, pathway analysis (IPA http://www.ingenuity.com) is performed for the gene lists obtained by the best-fit-model to investigate the biological functional mechanisms.
Results: We applied three models to in-*cis* relationships of mRNA and SNP for ~7,500 genes and 177 samples. The causal model identified ~2,400 genes and the reactive model identified ~5,000 genes. The independent model identified 29 genes. By the gene lists for the causal model, IPA estimated the biological functions related to inflammatory response, infection and inflammatory diseases, hematological system development, cell-mediated immune response and immune cell trafficking, and regulation of the immune response for the canonical pathways. For the reactive model, the biological functions significantly indicated inflammatory mechanisms, B cell receptor signaling and CD40 signaling in the canonical pathways.
Conclusions: The causality and reactive models involving genotype variation, mRNA expression and CF indicated more comprehensive information than only applying statistical procedure to two data combinations. To identify more specific biological characteristics, we plan to look into the genomic region related to immune system and apply specific statistical methodology. We could also involve other clinical variables related to CF such as BMI to these models.

References

[1] Lee et al. Genomics, 2009.

5088 POSTER

Assessment of Burden of Illness in Women With HER2+ Metastatic Breast Cancer: Findings From a Community Web-based Survey

M. Mayer[1], J. Doan[2], K. Lang[3], S. Hurvitz[4], D. Lalla[2], R. Woodward[3], M. Brammer[5], J. Menzin[3], D. Tripathy[6]. *[1]AdvancedBC.org, Advocacy, New York, USA; [2]Genentech Inc., Biometrics Health Outcomes and Payer Support, South San Francisco, USA; [3]Boston Health Economics, Health Economics and Outcomes Research, Boston, USA; [4]UCLA Jonsson Comprehensive Cancer Center, Hematology/Oncology, Los Angeles, USA; [5]Genentech Inc., Medical Affairs, South San Francisco, USA; [6]University of Southern California, USC/Norris Comprehensive Cancer Center, Los Angeles, USA*

Background: To better understand burden of illness in women with HER2+ metastatic breast cancer (MBC), we conducted a survey to evaluate their treatment experiences.
Materials and Methods: This one-time, web-based survey was conducted with the help of 4 independent U.S. breast cancer support groups. Respondents were invited to participate via email, and were required to be female, aged 18+ with HER2+ MBC, and to have received active treatment in the past month. Approximately 100 demographic, clinical, employment, quality of life, social and resource use data items were collected.
Results: 130 women with HER2+ MBC completed the survey. The majority of respondents were 45−59 years old (54.6%), white (93.9%), living with a spouse or partner (72.3%) and had at least a college education (70.8%). The most common comorbid conditions were high blood pressure (10.0%), thyroid disease (4.6%), diabetes without complications (4.6%), congestive heart failure (3.9%) and rheumatologic conditions (3.9%). While over 65% were full time employed at the time of MBC diagnosis, only 26% were at the time they completed the survey. 69% were currently taking a trastuzumab containing regimen, commonly with another medication. Frequently used medications included lapatinib and paclitaxel. Symptoms reported as frequently bothersome by at least 20% of women were: tiredness (52%), decreased sexual interest (50%), difficulty sleeping (39%), worry (39%), joint/ muscle pain (34%), difficulty concentrating (27%), alopecia (24%), low back pain (23%), depressed mood (22%), constipation (20%), and tingling of hands or feet (20%). Despite their disease, these women expressed high levels of satisfaction with their lives and relationships (with family, friends, and other women with MBC), but were less satisfied with their employment and their feeling about the future. They expressed higher levels of burden due to pain/discomfort and anxiety/depression than due to usual activities, self-care, and mobility. Fewer than 10% had discussed palliative care options with their doctor.
Conclusions: This community survey of women with HER2+ MBC provides valuable insights into their demographics, work status, treatment, and symptom burden. There were numerous symptoms that were