mutations and three variants of indefinite biological effect). Ten frame shift mutations were detected, the result of which was production of truncated protein. They included: 5467insT, 6174delT, 6192delAT, 6675delTA, 8141del5, 9152delT, 9326insA and 9631delC. The 8141del5 mutation was detected in 3 patients. The group of pathogenic mutations was completed with the nonsense change E394X and splice site mutation IVS23−2A>G. The presence of 10 missense type mutations was detected: N289H, N372H, T598A, G602R, N991D, D1420O, K1690N, T1915M, I2627F, N3124I. The frequency of N991D, D1420O and N3124I was compared between breast cancer patients and the control group of healthy subjects.

Conclusions: 1. A high diversity was found of the mutations detected in *BRCA2* gene; their frequency depended on the study population and family history of the patients subjected to genetic tests. 2. The determination of pathogenic status of molecular variants detected in BRCA2 gene, described in the BIC database as "unclassified variants" depends on many parameters. Most important is the assessment of the evolutionary conservation of their protein sequences and studying of the frequency of molecular variants detected in breast cancer and in healthy population.

178 Poster

Phenotypic profile of triple negative and hormonal receptors positive breast cancer cells treated with growth factors for mammospheres formation − preliminary results

M. Carvalho[1], M. Laranjo[2], A. Paiva[3], A. Abrantes[2], F. Falcão[1], M.F. Botelho[2], C.F. Oliveira[4]. *[1]Hospitais da Universidade de Coimbra, Gynaecology Service, Coimbra, Portugal; [2]Biophysics and Biomathematics Institute, IBILI − Medicine Faculty of Coimbra, Coimbra, Portugal; [3]Histocompatibility Center of the Center, Histocompatibility Center of the Center, Coimbra, Portugal; [4]Universitary Clinic of Gynaecology, Medicine Faculty of Coimbra, Coimbra, Portugal*

Backgrounds: Breast cancer patients are stratified into 3 main groups: tumors expressing hormonal receptors (HR), HER2 positive tumors and triple negative tumors, the last one show a more aggressive clinical behavior.

In human breast cancer cell lines exposed to bFGF and EGF is possible to identify groups of spherical colonies in suspension with stem cell properties designated mammospheres. In solid tumors a subpopulation of tumorigenic cells is thought to express CD44+/CD24-, ESA+, Oct4, Musashi1 and CD133+, possibly representing stem cell markers.

The aim of this study is to analyze breast cancer cells exposed to bFGF and EGF considering HR expression, which is positive in MCF7 and triple negative in HCC1806 breast cancer cell lines, particularly CD24/CD44 expression.

Material and Methods: The adherent human breast cancer cell lines MCF7 and HCC1806 were cultured according to recomended procedures. To perform the mammospheres forming protocol, both cell lines were cultured in DMEM-F12 supplemented with bFGF and EGF. After, cell lines were analyzed by flow cytometry with anti-CD45, anti-CD44and anti-CD24 in a FACSCalibur. To access underlying cell death pathways, treated cells were also analyzed with Annexin-V (An).

Results: The cell lines studied showed different phenotypes in culture. MCF7 cells formed several spherical colonies in suspension and HCC1806 cells kept mainly the adherent phenotype with a few groups of spherical colonies in suspension.

The characterization of the adherent population of MCF7 identified 2 subpopulations, one representing 14−22%, with CD44 expression higher than the main population, which was CD44 negative. In contrast, suspended population presented a prevalent subpopulation (83−86%) expressing CD44. Regarding HCC1806 cells we found similar behavior, the adherent subpopulation expressed CD44 less frequently (15−25%) than suspended subpopulation (91−95%).

The An profile was positive in 11−17% of HCC1806 suspended subpopulation, with insignificant marks in the other cells studied.

Conclusions: The mammospheres forming protocol developed more suspended cell colonies in HR positive cells than in triple negative. The exposure of both cell lines to growth factors separated suspension population expressing CD44 in a higher degree than the adherent population, similar to cells in standard conditions. The former is thought to harbor stem cells properties, emphasized by CD44 positivity. Apoptosis marker (An) had an irrelevant expression.

179 Poster

Early breast cancer detection: validation of a commercially available blood-based gene expression test

D. Tobin[1], N. Hagen[1], A.L. Børresen-Dale[2], A. Hollingsworth[3], E. Schlicting[4], P. Skaane[5], F. Wärnberg[6], L. Bergkvist[7], C. Ingvar[8], P.E. Jönsson[9]. *[1]DiaGenic ASA, Research and Development, Oslo, Norway; [2]Rikshospitalet-Radiumhospitalet, Department of Genetics, Oslo, Norway; [3]Mercy Health Center, Breast MRI of Oklahoma, Oklahoma, USA; [4]Ullevaal University Hospital University of Oslo, Breast and Endocrinology Surgical Department, Oslo, Norway; [5]Ullevaal University Hospital University of Oslo, Department of Radiology, Oslo, Norway; [6]Uppsala University Hospital, Department of Surgery, Uppsala, Sweden; [7]Centrallasarettet, Department of Surgery, Västerås, Sweden; [8]University hospital of Lund, Department of Surgery, Lund, Sweden; [9]Helsingborgs Lasarett, Department of Surgery, Helsingborg, Sweden*

Background: Despite screening programmes, breast cancer continues to be the second most common form of cancer death in women. Better diagnostic tools for the detection of cancer at early stages would contribute to increased survival. We have previously reported the development of a blood-based gene expression test for the detection of breast cancer. The test was developed from an initial whole genome array analysis for selection of informative assays. Further assay selection was performed that resulted in the present day, commercially available, 96-assay set. Gene expression is measured using reverse-transcriptase real-time PCR to determine mRNA levels. An algorithm was developed in a so-called calibration study to distinguish between BC and non-BC patients. The current study reports the calibration and validation results obtained with the gene expression test (BCtect®).

Materials and Methods: In a multicentre-study, blood samples were collected from women in 3 groups (1) Stage 0−III BC, (2) benign breast lesions, or (3) negative mammograms. Blood samples (2.5 mL per sample) were collected in PAXgene™ tubes and shipped on dry ice to a central laboratory for RNA extraction according to the manufacturer's instructions. Quality control of RNA was performed using the Agilent 2100 BioAnalyzer and Nanodrop ND-1000. Gene expression analysis was performed using real time RT-PCR (AB7900 HT) with a microfluidic card containing the BC-specific gene signature in a 96-gene assay format. Modelling was performed using Partial Least Square Regression providing an algorithm for application to gene expression data. Leave-one-out cross validation was used to obtain performance characterstics from the calibration study. The final algorithm was used with the test software to provide a test score for each subject in the independent validation cohort. A positive test score classified a subject as positive for BC, whilst a negative score classified a subject as negative for BC. Overall, 332 samples were included, 223 samples were used to develop the algorithm and 109 samples were used as an independent validation set to describe its performance.

Results: The model correctly predicted the class of 78 of the 109 validation samples and 162 of the 223 calibration samples (overall accuracy of 72%). Performance was similar for early and late stage cancer with a sensitivity of 74% for stage 0/1 breast cancer (stage 0 = in situ cancer, and stage 1 = T1N0M0; staging defined by AJCC 2002). The test performed equally well in pre- and post-menopausal women. Use of hormonal based contraceptives, hormone replacement therapy and common medications did not appear to affect the accuracy of the blood test.

Conclusions: The blood-based gene expression test showed efficacy for the detection of early breast cancer in both pre- and post-menopausal women. The test may be of clinical benefit as a complement to mammography for all women, and particularly for pre-menopausal women for whom mammography is known to have limitations.

180 Poster

Knock-down of the estrogen receptor GPR30 in triple-negative breast cancer reduces cell proliferation

E. Peralta[1], D. Farr[1], M. Rutz[1], J. Steiman[1], S. Louis[1]. *[1]Southern Illinois University School of Medicine, Surgery, Springfield IL, USA*

Background: The G protein coupled receptors (GPCR) are a large family of membrane-bound receptors characterized by a 7-mer alpha helical structure. They mediate rapid response to a wide range of ligands including opiates, dopamine, chemokines and steroids. GPR30 is a G protein-coupled receptor that is activated not only by estrogen but also tamoxifen and other selective estrogen receptor modulating drugs. We have previously reported that GPR30 is frequently expressed in triple negative (ER−, PR, HER2 neu−) breast cancers, but its influence on breast cancer biology is unknown.

Hypothesis: GPR30 functions to stimulate cancer cell growth and increases resistance to tamoxifen (TAM).

Materials and Methods: A triple-negative breast cancer cell line (GPR30−1) was established from a clinical specimen under an IRB-approved tumor banking protocol. GPR30 expression in this cell line was demonstrated by immunohistochemical staining and RTPCR. The effect of GPR30 knock-down was assayed on GPR30−1 cells that were transfected with a 29mer short hairpin RNA (shRNA) constructed against GPR30 (Origene Technologies, Inc, Rockville, MD). Control cells were transfected with a non-effective 29mer shRNA cassette. Transient expression of green fluorescence protein (GFP) allowed selection of transfected cells by fluorescence-activated cell sorting (FACS). Proliferation of untransfected GPR30−1, control, and GPR30 knock-down cells was tested in normal medium, 20 and 100 micromolar TAM using the MTS assay.

Results: Control-transfected GPR30−1 cells had an equal proliferation rate to untransfected cells in normal medium (ratio 1.04:1.0). In normal medium, GPR30-knockdown cells had a reduced proliferation rate (ratio 0.34:1.0) compared to negative control or untransfected cells. Untransfected and control-transfected GPR30−1 cells showed a 53% and 43% decrease in proliferation after 24 hours in low-dose (20 micromolar) TAM compared to a 92% reduction in GPR30-knockdown cells. All cells showed a greater than 90% decrease in proliferation in high-dose (100 micromolar) TAM.

Conclusions: Knock-down of GPR30 in GPR30−1 cells lowers the proliferation of cells in normal medium and when exposed to TAM compared to normal and control-transfected cells. These results are consistent with a tamoxifen resistance effect of GPR30. GPR30 may represent a therapeutic target in breast cancer.

181 Poster

Cytotoxicity of docetaxel, epirubicin and carboplatin on hormonal receptors positive and triple negative breast cancer cell lines

M. Laranjo[1], M. Carvalho[2], A. Brito[1], A. Abrantes[1], M.F. Botelho[1], C.F. Oliveira[3]. *[1]Instituto Biofisica/Biomatematica, IBILI − Medicine Faculty of Coimbra, Coimbra, Portugal; [2]Hospitais da Universidade de Coimbra, Gynaecology Service, Coimbra, Portugal; [3]Universitary Clinic of Gynaecology, Medicine Faculty of Coimbra, Coimbra, Portugal*

Background: Breast cancer patients are stratified into three main groups: those expressing hormonal receptors (HR), which respond to therapies targeting estrogen receptors; HER2 positive tumors that are candidate for targeting therapy with trastuzumab or lapatinib; triple negative (TN) tumors, for which the only systemic therapy available is standard chemotherapy. Some studies suggest an increase susceptibility of TN to platinum-derived chemotherapy, with a pathological remission rate of 21%.

The purpose of this study was to evaluate cytotoxic capacity of docetaxel, epirubicin and carboplatin in MCF7 (HR positive) and HCC 1806 (TN) breast cancer cell lines.

Material and Methods: Human breast cancer cell lines MCF7 and HCC1806 were purchased to ATCC and cultured according to recomended procedures. Both cell lines were incubated in absence and presence of the docetaxel, epirubicin and carboplatin in several concentrations ranging from 50nM to 150μM. The sensitivity of the cell lines to the drugs studied was analyzed using the MTT colorimetric assay, performed 24, 48 and 72 hours after incubation. Cytotoxicity was expressed as the percentage of inhibition of cell proliferation correlated with untreated cultures. Dose-response curves were established and the half maximal inhibitory concentration (IC50) was calculated in Origin7 software.

Results: Epirubicin on HCC 1806 at 24h had a higher IC50 than on MCF7 (2.3 *vs.* 1.8μM), but this performance reached a similar level at 72h. Focusing on docetaxel, IC50 was higher on MCF7 than on HCC 1806, showing a better performance considering cell death for HCC 1806. Considering carboplatin, the IC50 was considerably elevated on MCF7. In HCC 1806, carboplatin proved IC 50 of 44.8 μM and 8.6 μM at 48 and 72h, respectively.

Comparing epirubicin *vs.* carboplatin cytotoxicity on MCF7, IC50 was always high in carboplatin studies, with IC50 of 53.5 μM at 72h for these particular cells. Also on HCC 1806, carboplatin showed a worse activity than epirubin, emphasized by higher IC50 for carboplatin at 48h (44.9 μM) and 72h (8.6 μM) than epirubicin.

Conclusions: Epirubicin had a similar effect on HR positive and TN cell lines. On the contrary, docetaxel proved a better performance on TN than on HR positive cells, the last showing elevated IC50. Carboplatin reached less cytotoxicity than epirubicin either in HR positive and TN breast cancer cell lines.

182 Poster

Long-term effect of fulvestrant on hormone receptors and proliferation marker in breast cancer

A. Agrawal[1], J.F.R. Robertson[1], E. Gutteridge[1], K.L. Cheung[1], I.O. Ellis[2], R.I. Nicholson[3], J.M.W. Gee[3]. *[1]University of Nottingham, Professorial Unit of Surgery, Nottingham, United Kingdom; [2]University of Nottingham, Molecular Medical Sciences, Nottingham, United Kingdom; [3]University of Cardiff, Welsh School of Pharmacy, Nottingham, United Kingdom*

Background: Fulvestrant has been shown in short-term (maximum of 4 months) pre-surgical studies of breast cancer to decrease expression of estrogen receptor (ER), progesterone receptor (PgR) and proliferation marker, Ki67. We present changes in these markers from 6 months and beyond (median time to progression of 25.8 months) in breast cancer patients treated with fulvestrant.

Materials and Methods: 32 post-menopausal women with locally advanced (n = 22) or metastatic breast cancer (n = 10) with measurable breast lesions had fulvestrant (250 mg. intra-muscularly monthly) as first-line primary endocrine therapy. Immunohistochemistry was performed on sequential breast tumour biopsies taken at diagnosis (before commencing fulvestrant, T1), 6 weeks (T2), 6 months (T3) and at progression (T4) of disease.

Results: Wilcoxon signed rank sum analysis revealed decrease in the levels of all 3 markers at all subsequent time-points from pre-treatment level (significance at $p < 0.05$) (table).

Marker	Median level at T1 (range)	p-value for change		
		T1−T2	T1−T3	T1−T4
ER H score	130 (60–190)	<0.001	<0.001	0.001
PgR H score	30 (0–270)	Non-significant	0.001	0.012
Ki67% stain	18 (1–60)	0.001	<0.001	0.028

There was non-significant recovery of ER and Ki67 at progression (T4) compared with 6 months level (T3). Kaplan-Meier analysis revealed lower pre-treatment (T1) Ki67 predictive of longer TTP but no similar relation was noted with ER and PgR. While not apparent at T1, higher PgR at 6 weeks (T2) predicted for longer TTP ($p = 0.008$).

Conclusions: The remaining expression of ER (partly due to some recovery) may contribute to acquired resistance as ER is still available for cross-talk with growth factors. However, lack of total depletion of ER at progression on fulvestrant may also explain known clinical response to further endocrine therapies. Mix of low and high PgR cases within the responders in this series (median PgR of 30) suggests fulvestrant activity being largely dependent on ER irrespective of PgR. This study confirms decrease in Ki67 (and ER and PgR) expression seen earlier in literature but is the first study which shows statistically significant decrease beyond a median of 2 years.

183 Poster

A DNA signature to identify high-risk small node-negative breast cancer patients

E. Gravier[1,2,3,4], G. Pierron[5], A. Vincent-Salomon[5,6], N. Gruel[1,6], V. Raynal[6], A. Savignoni[2], Y. De Rycke[2], J.P. Pierga[7], C. Lucchesi[6], F. Reyal[8], A. Fourquet[9], S. Roman-Roman[1], F. Radvanyi[10], X. Sastre-Garau[5], B. Asselain[2,3,4], O. Delattre[6]. *[1]Institut Curie, Department of Translational Research, Paris, France; [2]Institut Curie, Department of Biostatistics, Paris, France; [3]INSERM U900, France; [4]Ecole des Mines de Paris, Fontainebleau, France; [5]Institut Curie, Department of Tumor Biology, Paris, France; [6]INSERM U830, Institut Curie, Paris, France; [7]Institut Curie, Department of Medical Oncology, Paris V Université Paris Descartes, France; [8]Institut Curie, Department of Surgical Oncology, Paris, France; [9]Institut Curie, Department of Radiation Oncology, Paris, France; [10]UMR CNRS/IC 144, France*

Purpose: To identify a DNA signature to predict metastasis of small node-negative breast carcinoma

Experimental Design: The authors used Comparative Genomic Hybridization (CGH) array to analyze 168 pT1T2pN0 invasive ductal carcinoma patients with either good (no event 5 years after diagnosis: 111 patients) or poor (57 patients with early onset metastasis) outcome. A CGH classifier, identifying low and high-risk groups of metastatic recurrence, was established in a training set of 78 patients. This classifier was based on both genomic regions with statistically different alterations between the two groups of clinical outcome and the number of alterations. It was then tested on a validation set of 90 patients and compared to clinicopathological parameters.