is mutated the maintenance of DNA integrity may be reduced, resulting in copy number alterations (CNA) in the tumor. Aneuploidy is an important feature of cancer cells, and specific CNAs have shown to be of both prognostic and predictive value. Most of these alterations can be detected by use of array Comparative Genomic Hybridization (aCGH). The aim of this study was to investigate the CNAs in breast tumors, with a high resolution platform, in relation to *TP53* mutation status.

Tumor tissues from a series of 212 primary breast cancer cases were sequentially collected at Ullevål University Hospital in Oslo between 1990 and 1994. Tissues were sampled at the time of primary surgery and snap frozen. *TP53* mutation data from previous sequence analyses were available for 203 of the samples. We performed aCGH on 167 of these tumors. DNA was isolated using chloroform/phenol extraction, followed by ethanol precipitation. The aCGH-platform was the Agilent Human-Genome-CGH Microarray 244k. For detection of aberrations we used ACE (analysis of copy errors) and PCF (piecewise constant fit), both implemented in the CGH-explorer software. For visualization we used the software Nexus 2.0. Significance Analysis for Microarray (SAM) was performed using the R/BioConductor package "samr".

Many significant genetic alterations were found, with a large heterogeneity between the different tumors. The most frequently observed alterations were amplification of 1q, 8q, 16 p and 17q and deletion of 1p, 8p, 16q and 17p. When grouping the tumors by *TP53* mutation status we found a significant difference in the CNA patterns between *TP53* mutated vs. none-mutated tumors. Overall the mutated group had more aberrations than the wild type group, and interestingly the aberrations were not evenly distributed along the genome, suggesting that some chromosomal areas are more prone to instability or selected for in the presence of a mutated TP53 protein. The most frequent losses associated with *TP53* mutation status were regions on 3p, 4p, 4q, 5q and 8p, whereas significant gains were observed for 8q and 10p. A detailed structure of the CNAs and the involved genes will be presented in relation to type of mutations and to various clinical data.

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Comparison between circulating and disseminated tumor cells in breast cancer

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Background: The presence of disseminated tumor cells in bone marrow (BM) of breast cancer patients is an independent prognostic factor. The role of circulating tumor cells (CTC) in blood is not yet defined. Since BM aspiration is less accepted by patients compared to blood drawing, it would be highly desirable to replace bone marrow aspiration by blood analysis. Therefore, the purpose of the present study was:

1. to examine the presence of tumor cells in peripheral blood,
2. to evaluate how surgery affects the presence of CTC,
3. to assess the correlation between results in blood and in BM.

Materials and Methods: 314 blood samples from breast cancer patients were collected. 130 patients underwent blood sampling both pre- and postoperatively. All aspirates underwent immunomagnetic enrichment using AdnaTest BreastCancerSelect within 4 hours after blood withdrawal followed by RNA isolation and subsequent gene expression analysis by reverse transcription and Multiplex-PCR in separated tumor cells using AdnaTest BreastCancerDetect. Three breast cancer associated tumor markers and one control gene were amplified: GA733−2, Muc-1, Her-2 and β-actin (internal PCR control).

Furthermore bone marrow aspirates from 176 of these patients were analyzed by immunocytochemistry (pancytokeratin antibody A45-B/B3) using ACIS system (Chromavision) according to the ISHAGE evaluation criteria.

Results: 184 patients could be included in to this study. 10% of these patients had detectable tumor cells in the bloodstream. To assess the influence of surgery, 130 blood samples were analyzed both pre- and postoperatively. The positivity rates postoperatively were slightly higher but did not differ significantly (13% preoperatively and 17% postoperatively). It seems therefore that surgery or invasive procedures (core cut biopsy) do not influence positivity rates in the blood. The positivity rate in the bone marrow was 11%. The correlation between both compartments (blood and BM) in these patients was 80%.

Conclusions:

1. Circulating tumor cells can be detected in primary breast cancer.
2. Surgery does not influence significantly the tumor cell load in the blood stream.
3. Positivity rates obtained from both compartments (blood and BM) correlate highly. However, the prognostic significance of CTCs has to be further evaluated.

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Concordance between tissue microarray and whole tissue sections for ER expression and PgR and HER-2 status: a pilot study for the Trans-ATAC trial

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Background: The Tissue Microarray (TMA) format allows high-throughput molecular characterisation of tissue specimens with rapid linkage to clinical endpoints. However, assessment of the number of cores needed to represent the biomarker expression in whole tissue sections (WS) is a prerequisite of their valid use. Some studies suggest [1,2] that one core is adequate to represent the expression of ER, PgR and HER-2 in a WS, whilst others recommend at least two cores [3]. Therefore, we investigated whether ER, PgR and HER-2 expression in one TMA core was sufficient to represent the expression in WS in the Trans-ATAC trial population.

Materials and Methods: 4 μm thick sections were cut from 7 of the Trans-ATAC TMAs and stained for ER (6F11, Novocastra 1:40), PgR (Novocastra 312, 1:100) and the HER-2 protein (DAKO Herceptest™ kit), with HER-2 2+ cases being analysed by FISH (PathVysion™, Abbott). A random selection of 30 tissue cores (total 210 cores) from each of the 7 TMAs was evaluated. Expression was considered positive for ER with a H-score >1, for PgR expression with >10% cells showing nuclear staining and for HER-2 protein expression if 3+ according to the DAKO Herceptest, or 2+ with HER-2:Chr17 gene ratio >2.

Results: Positive HER-2 expression (protein and gene amplification) was found in 8.1% of cores and WS. The concordance between the cores and WS HER-2 scores was 97.1%, (95% CI 94.8−99.4, $\kappa = 0.81$, 95% CI 0.75−0.86). For PgR expression, 67.2% of cores were positive vs 78.9% of WS. The concordance between the cores and WS for PgR expression was 82.2% (95% CI 76.9−87.5, $\kappa = 0.56$, 95% CI 0.49−0.63).ER positivity was found in 98.9% and 99.0% of the cores and WS respectively, with a concordance of 99.5% (95% CI 89.7–100). Considering the ER H-score as a continuous variable the correlation coefficient between one core and WS H-scores was r = 0.78, r2 = 0.61. The correlation between the mean H-score for ER (of the 3 TMA cores) versus the H-score of the WS was increased to r = 0.83, r2 = 0.69.

Conclusion: The disconcordance rate between TMAs and WS for HER-2 was only 3%, but this may be significant when considering the low prevalence of HER-2 expression in an ER positive cohort. The disconcordance of >10% for PgR is unacceptable and studies may require multiple cores or WS to assess this marker. There was a high concordance and correlation between the one core and WS for ER status, but quantitative assessment of ER on TMAs is substantially improved if three cores are taken.

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312 Poster Discussion

Molecular qRT-PCR grade index: a new tool for breast cancer (BC) patient grading improvement

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Background: We have recently shown that proliferation captured by the GGI is one of the most important prognostic indicators in BC and may encompass a significant portion of the predictive power of several previously published prognostic signatures in particular for ER+ disease. The aims of this study were 1) to convert this microarray index to an index using qRT-PCR and 2) to assess its prognostic and predictive value for tamoxifen response.

Methods: A qRT-PCR genomic grade index (PCR-GGI) was developed based on the expression of 4 genes selected from the GGI microarray signature and 4 reference genes. The accuracy and concordance with the original microarray-derived GGI was assessed using a BC set from which frozen, FFPE tissues and microarray data were available (N = 19). The evaluation of the prognostic value of the PCR-GGI was assessed using a consecutive series of 212 systemically treated early BC patients according to the standard of care at the time of diagnosis using FFPE material. The predictive performance for tamoxifen response was assessed using an ER+ BC population treated either with adjuvant tamoxifen only ($n = 141$) or first-line tamoxifen for advanced disease ($n = 279$).

Results: A statistically significant correlation was observed between GGI derived from microarray and qRT-PCR assay using frozen ($\rho = 0.95$, $p < 10^{-6}$) as well as FFPE material ($\rho = 0.89$, $p < 10^{-6}$). Similarly to our previous microarray results, PCR-GGI redistributed histological grade 2 (HG2) tumors into two subgroups with statistically distinct clinical outcome similar to those of HG1 and HG3 tumors, respectively (HR = 2.27; 95CI: 0.94−5.48, $p = 0.068$). Of notice, PCR-GGI was more informative than Ki67 (IHC) in discriminating HG2 patients into good and bad prognosis group. Additionally, PCR-GGI identified two distinct ER+ subgroups with statistically different DMFS and response to tamoxifen treatment (PFS) in both early (HR = 2.26; 95CI: 1.075−4.751, $p = 0.03$) and advanced setting (HR = 1.95; 95CI: 1.49−2.544, $p < 10^{-6}$) respectively. Interestingly, among the 66% of ER+ node-negative early BC patients assigned as low risk, only 7% exhibited distant recurrence compared to 47% for the high-risk patients at 10 years follow-up.

Conclusions: GGI using a qRT-PCR assay based on a limited number of genes recapitulated in an accurate and reproducible manner the performances of the GGI developed by microarray using both frozen and FFPE tumor samples. PCR-GGI can be used as a powerful tool for BC management.

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PTEN Immunohistochemical detection in breast cancer

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Background: Increasing evidence points to major role of PTEN in breast cancer. Can immunohistochemistry (IHC) correctly detect PTEN alterations in breast cancer and is IHC PTEN of prognostic significance?

Materials and Methods: IHC PTEN using 6H2.1 antibody was validated on tumor cell lines (SKBR3, BT-474, MDA MB 468, ZR75−1) with known *PTEN* status. IHC PTEN was then performed on a TMA of 1046 invasive ductal carcinomas (IDC) operated on between 1989 and 1994 [median follow-up 167 months, 284 (27.2%) metastatic events]. Percentage of invasive tumor cells and staining intensity (0, 1+, 2+, 3+) were used to score IHC PTEN status. Array CGH was also performed in a subgroup of 135 cases in the series.

Results: Negative IHC PTEN was significantly correlated to array CGH 10q23 (*PTEN* locus) copy number loss (CNL) ($p = 3{\cdot}10^{-6}$)with a sensitivity of 33% and a specificity of 96%. Negative IHC PTEN was observed in 7% of the 1046 IDCs and was correlated to younger age, SBR grade III, negative ER and PR status, negative Her2 status, high Mib1 index and basal phenotype ($p = 0.05$, $p = 9{\cdot}10^{-5}$, $p = 4{\cdot}10^{-6}$, $p = 4{\cdot}10^{-5}$, $p = 1{\cdot}10^{-5}$, $p = 2{\cdot}10^{-3}$ and $p = 3{\cdot}10^{-17}$, respectively). Negative IHC PTEN was not correlated to metastasis free survival contrarily to pN status, SBR grade, ER, PR, Her2 and Mib1 ($p = 7{\cdot}10^{-17}$, $p = 2{\cdot}10^{-12}$, $p = 2{\cdot}10^{-3}$, $p = 1{\cdot}10^{-3}$, $p = 8{\cdot}10^{-5}$ and $p = 3{\cdot}10^{-10}$, respectively).

Conclusions: IHC PTEN using 6H2.1 can detect 10q23 CNL in one third of the cases with a high specificity. Negative IHC PTEN status is mainly, but not exclusively, observed in invasive ductal carcinomas with basal phenotype. No association between IHC PTEN and metastatic relapse was observed in this large series of invasive breast cancers with long follow up.

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Circulating tumor cells (CTCs) in peripheral blood of primary breast cancer patients – translational research program of the German SUCCESS-Trial

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Background: The presence of CTCs has been shown to be associated with worse outcome in metastastic breast cancer patients. The German SUCCESS-Trial is the first trial to evaluate the role of CTCs in a large number of breast cancer patients receiving adjuvant chemotherapy as well as endocrine and bisphosphonate treatment.

Material and Methods: We analyzed 23 ml of peripheral blood from 1500 N+ and high risk N− breast cancer patients before and after adjuvant taxane based chemotherapy. The presence of CTCs was assessed with the CellSearchSystem (Veridex, USA). After immunomagnetic enrichment with an anti-Epcam-antibody, cells were labelled with anti-cytokeratin (8,18,19) and anti-CD45 antibodies to distinguish between epithelial cells and leukocytes.

Results: In 10% of patients ($n = 143$) >1 CTC was detected before the start of systemic treatment (mean 14, range 2–827). While we found 2 CTCs in 4% of patients, 3% had 3−5 CTCs and 1% 6−10 and >10 CTCs respectively. The presence of CTCs did not correlate with tumor size ($p = 0.32$), grading ($p = 0.36$), hormonal status ($p = 0.28$) or Her2/neu status of the primary tumor ($p = 0.82$), but with the presence of lymph node metastases ($p = 0.003$). Three of 74 individuals without malignant disease (4%) showed more than 1 CTC.

After completion of chemotherapy, 9% of patients ($n = 130$) presented with >1 CTC (mean 6, range 2–124). Of those initially CTC positive, 10% remained positive ($n = 15$), whereas of those initially CTC negative, 8% returned with a positive test ($n = 115$, $p = 0.42$).

Outcome data was available for 1438 patients. 21 recurrences occurred during a median follow-up of 12 months and 7 patients died of their disease. While the presence of CTCs before systemic treatment did not show prognostic relevance for DFS ($p = 0.89$) and OAS ($p = 0.71$), persistence of CTCs after chemotherapy was a significant predictor for both reduced DFS ($p = 0.04$) and OAS ($p = 0.03$).

Conclusions: In a considerable number of patients CTCs in peripheral blood can be detected after the completion of chemotherapy. Preliminary results indicate a prognostic relevance of persisting CTCs after chemotherapy. Further follow-up will show whether CTCs can be used as a useful tool for treatment monitoring.

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Factors associated to upstaging at surgery of atypical ductal hyperplasia diagnosed at percutaneous breast biopsy

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Background: A substantial number of patients with atypical ductal hyperplasia diagnosed at percutaneous breast biopsy will be upstaged to carcinoma at surgery. The aim of this study is to evaluate potential variables that could be predictive of malignancy in a large series of patients with atypical ductal hyperplasia diagnosed by percutaneous techniques (core needle biopsy or vacuum-assisted biopsy).

Methods: We retrospectively reviewed 13488 consecutive percutaneous breast biopsies conducted in one institution (Centre des Maladies du Sein Deschênes-Fabia, Quebec City) over a nine years period. Atypical ductal hyperplasia was diagnosed in 511 cases. A total number of 422 biopsies in 415 patients with surgical follow-up were included in the final analysis. Upstaging rate to ductal carcinoma in situ or invasive carcinoma at surgery was determined. Eleven variables were studied for potential association with upstaging rate by univariate and multivariate analysis.

Results: Carcinoma (in-situ or invasive) was found at surgery in 132 cases leading to an upstaging rate of 31.5%. The statistically significant variables in multivariate model were: ipsilateral breast symptoms (OR: 3.78), mammographic lesion other than microcalcifications alone (OR: 2.31), biopsy by 14 G core needle instead of 11G vacuum-assisted biopsies (OR: 1.64), co-diagnosis of papilloma on core biopsy (OR: 2.32), high grade atypia (OR: 4.53), diagnosis made by a pathologist with higher workload volume (OR: 0.55). Age at biopsy, personal history of breast cancer or ADH, 1st degree familial history of cancer, co-diagnosis of lobular neoplasia on core biopsy and absence of microcalcifications when appropriated on core biopsy were not associated to upstaging. One hundred and twenty-eight (128) cases did not have any of the six known variables associated with upstaging. Surgical excision leads to a diagnosis of carcinoma in 22 cases in this subgroup.

Conclusion: In this largest series of percutaneous biopsies realised in one institution, six of the eleven variables analysed were found to be associated to upstaging of ADH to breast carcinoma at surgery. We did not identify any subgroup to which we could offer a safe clinical-only follow-up. We recommend to routinely offer surgery to women with a diagnosis of ADH found at percutaneous breast biopsy.