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.

310 Poster Discussion

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: 4μm 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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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