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.