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Development and validation of an array based Molecular Subtyping Profile for breast cancer

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Background: Classification of breast cancers into molecular subtypes may be important for accurate selection of therapy for patients. Here we report the respective chemotherapy responsiveness of the molecular subtyping profile defined Luminal-, Her2- and Basal-type breast cancer. In addition, we report on conversion of this robust gene expression profile to a high-throughput, extensively validated clinical diagnostic tool.

Methods: A 80-gene subtyping profile was developed based on a series of 200 breast cancer samples with concordant ER, PR and Her2 receptor IHC and single gene read-out status. The profile classification was validated using 784 samples. Here, we report a second (in silico) validation consisting of 133 samples (Hess et al, JCO, 2006) where we tested the profile as a predictor of pathological Complete Response (pCR) in these patients treated with T/FAC neoadjuvant chemotherapy.

Currently, experiments are carried out on custom made diagnostic microarrays to determine the test reproducibility, accuracy and precision.

Results: The overall concordance of the classification by the 80-gene profile with the hierarchical clustering as defined by Perou et al. is 96%. In the validation set (n = 784) the profile classified 66% (517) as Luminal-type, 14% (110) as Basal-type and 20% (157) as HER2-type. Similar proportions were observed by in silico validation on patients treated with neoadjuvant chemotherapy; 62% (82) as Luminal-type, 20% (27) as Basal-type and 18% (24) as HER2-type. Chemotherapy response was reported by pathological Complete Response (pCR) at the time of surgery. In the Luminal-type subgroup 9% (7/82) of patients achieved a pCR, in the HER2-type subgroup 50% (12/24) of patients had a pCR and in the Basal-type subgroup 56% (15/24) of patients had a pCR. To make the test available clinically, the profile was translated into a diagnostic test using the Agilent 8-pack format that supports high throughput, high quality and robustness.

Conclusions: The developed Molecular Subtyping Profile can classify breast cancer tumors into Luminal-, Her2- and Basal-type subgroups. Within the subgroups, a significant difference in chemotherapy response, as measured by pCR, is observed. Implementation of this knowledge may improve the clinical management of breast cancer patients, by enabling the physician to decide who is most likely to benefit from chemotherapy or endocrine therapy prior to surgery.

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Outpatient core needle biopsy to assess a multigene assay for prognostic information in breast cancer

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Background: Multigene assays are used in breast cancer to add prognostic information besides the classical pathological markers. This extra genetic information results in a change in treatment in approximately 30% (mainly avoiding chemotherapy). Normally the pathologist performs a biopsy of resected breast cancer tissue for the multi gene assay post operatively. This study was performed to analyze the feasibility to asses multigene assays (Mammaprint including TargetPrint) on outpatient core needle biopsies in breast cancer patients, moreover the results of the MammaPrint were compared with the classical pathological markers (hormone receptor status and HER2 status).

Material and Methods: In 2009, 85 patients with a high suspicion of breast cancer underwent a diagnostic core needle biopsy in our outpatient breast cancer clinic. The ultra sound guided biopsies were performed by a radiologist using a 14 gauche needle. Per patient, 1 or 2 biopsies were taken for MammaPrint analysis and 2 for classical pathological examination. Feasibility of MammaPrint assessment in core biopsies was analyzed and the results were compared with the classical pathology report.

Results: Of the 85 patients with a high suspicion of breast cancer, 8 patients (9%) were according to local pathology found not to have invasive breast cancer. Of the 77 patients with invasive cancer, a MammaPrint could not be performed in 21 patients (27%) due to insufficient biopsies. Of the 45 patients for which gene expression readout (TargetPrint) and IHC/FISH assessment of ER, PR and HER2 was available, 9 samples (20%) showed discordant results. Three samples were found to be ER negative by IHC, whereas TargetPrint classified these as positive, 5 patients had discordance in PR status and 3 HER2 positive patients by FISH were negative according to TargetPrint. A heterogeneous tumour and small biopsies could be an explanation for this.

Conclusion: MammaPrint can be obtained by outpatient core needle biopsies, although in 27% of the patients insufficient breast cancer tissue was collected to assess the MammaPrint. For these patients the post operative surgical specimen could be used for MammaPrint analysis. In 20% there was a discordance of the hormone receptor or HER2 status, probably due to a heterogeneous tumour and small biopsies. Further research should focus on these items.

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Identification of lymphovascular invasion in breast biopsy specimens

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Introduction: Lymphovascular invasion is a significant predictor for SLN metastases and non sentinel node metastases in breast cancer. It is associated with a worse prognosis. The absence or presence of lymphovascular invasion is usually determined at definitive pathology. The goal of this study was to determine if lymphovascular invasion can also be accurately identified in breast biopsy specimens.

Method: From our pathology laboratory information system 85 patients operated in 2008 with lymphovascular invasion identified on the definitive pathology specimen (lumpectomy or amputation) were selected. Patients were operated in 3 community hospitals for which the pathology department of one of the hospitals reviews all specimens.

Of the selected patients new biopsy specimens slices were cut, measured and stained with HE and CD31 (PECAM-1, platelet endothelial cell adhesion molecule 1). All definitive breast pathology specimen slides were reviewed by one pathologist to confirm the presence of lymphovascular invasion.

Results: Lymphovascular was identified on the HE stained biopsy slides in 11 of 85 patients (12.9%) and in 12/85 CD31 stained slides (14.1%). This difference was not statistically significant.

	Identification of LVI	No identification of LVI	Possible identification of LVI
HE slides	11/85 (12.9%)	73 (85.9%)	1/85 (1.2%)
CD31 slides	12/85 (14.1%)	73 (85.9%)	0

Ductal carcinoma was identified in 96% of cases and lobular carcinoma in 4% of cases. The breast biopsy specimens varied between 1.2 and 2.1 cm. Lymphovascular invasion could not be identified in most specimens smaller than 1.3 cm.

Conclusions: Lymphovascular invasion is only identified in a small percentage of breast biopsy specimens. Staining with the CD31 endothelial marker does not improve identification of lymphovascular invasion significantly. A breast biopsy specimen should measure at least 1.3 cm for lymphovascular invasion to be identified. Lymphovascular invasion is predominantly identified in ductal carcinomas.

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High pathological complete response rate of neo-adjuvant combination of docetaxel, carboplatin and trastuzumab in patients with HER2-overexpressing breast cancer: preliminary results

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Background: Trastuzumab (T) in combination with chemotherapy improves results of treatment in patients with HER2-positive breast cancer. Docetaxel (D) + Carboplatin (C) + T combination has high efficacy in metastatic breast cancer. In our prospective clinical trial we study the efficacy and safety of triple combination (T+D+C) in neoadjuvant setting of locally advanced HER-2 positive breast cancer.

Methods: 15 patients (pts) with clinical stage IIIB/IIIC (8/7) histologically confirmed HER2-positive invasive breast cancer were included in this study. The pts received T 8 mg/kg loading dose then 6 mg/kg q3w and concurrently D 75 mg/m^2 and C AUC 5 q3w for 4−6 cycles followed by surgery.

Results: Median age of 54 years (range 35 to 76), 40% of pts were premenopausal. 9 pts (60%) − estrogen/progesterone receptor positive. TNM stage distribution at time of diagnosis: T2 − 13.3%; T4b − 66.7%; T4d − 20%; N1 − 40%; N2 − 13.3%; N3 − 46.7%.

Clinical response rate was achieved in 85.7% (12/14 pts): 3 − CR, 9 − PR. 2 pt had stabilization of disease during of neo-adjuvant therapy.