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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.

Pathological response was evaluated in 13 pts, 1 pt was additionally treated by preoperative locoregional radiotherapy, 1 pt continue the treatment.

pCR = 9/13 pts (69.2%) − complete disappearance tumor in the breast and lymph nodes occurred in 8 pts (61.5%) and 1 pt (7.6%) had pCR + in situ (in situ lesions only in breast tissue).

Toxicity was assessed for 75 treatment cycles. Grade III–IV neutropenia was observed in 76% of cycles. Febrile neutropenia was observed in 14.7% of cycles, no intravenous antibacterial therapy was required. Grade I−II transaminase increase and/or bilirubin was recorded in 29.3% of cycles; grade II mucositis − 10.7%. Only 1 pt had asymptomatic LVEF decrease on 10%.

Conclusion: the combination of Docetaxel 75 mg/m^2 + Carboplatin AUC 5 + Trastuzumab every 3 weeks is promissing regimen (pCR − 69.2%) with manageable toxicity for treatment of locally advanced HER-2 overexpression breast cancer.

Wednesday, 24 March 2010 18:15–19:15

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Predictive and prognostic factors

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A prognostic model for breast cancer-related events in primary operated invasive lobular breast cancers from one centre

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Background: Invasive lobular breast cancers (ILA) differ from non-ILA in many perspectives. ILA are a heterogeneous group with a large variety in histologic subtypes and disease free survival (DFS); a prognostic model for ILA is not available. We propose a model based on demographic and clinicopathological features.

Material and Methods: A retrospective cohort study of 380 consecutive patients treated between Jan 2000 and Dec 2006 for primary operable ILA, none E-cadherin positive, and all receiving local and systemic adjuvant therapy (108/380 or 28.4% had chemotherapy). None received neo-adjuvant therapy and those with a bilateral or multifocal disease with the non-ILA having a higher NPI than the ILA were not in this cohort. We investigated independent demographic and clinic-pathological variables for relapse.

Results: After a mean follow-up of 5.3 yrs, 37 patients (9.7%) experienced a breast cancer-related event. In a univariate setting, variables considered as significant ($p < 0.05$) were: node positivity (np), tumor size, grade (1−2 vs 3), mitotic count (1 vs 2−3, mito), the amount of nuclear pleomorfism (1−2 vs 3, pleo) and subtype, classical ILA or not. The tubule formation was not considered as a variable since 98.4% of the patients had less than 10% of the tumor forming tubules. A multivariate Cox model revealed that np, nuclear atypia and mitotic count are independent prognostic factors. We propose to divide patients into risk groups as illustrated in Table 1. Patients in group 1 (node negative) are considered as low risk, patients in group 3 are high risk. Table 1 also describes the predicted (S_{COX}) and observed survival (S_{KM}).

Conclusions: Our prognostic model of operable ILA showed that nor histological grade or subtype nor nuclear atypia nor mitotic activity are prognostic in node negative ILA. In node positive ILA, the combination of nuclear atypia and mitotic index distinguished a medium and high risk group. Risk groups can be defined without the complex definition of classical ILA.

Table 1: Categorizing patients into risk groups

Nuclear atypia	Mitotic count	Node positive	Risk group	N$^+$	# events	Event rate*	S_{KM} (5-yr)	S_{COX} (5-yr)
1 of 2	1, 2 of 3	no	1					
3	1	no	1					
3	2 of 3	no	1	211	8	0.01	0.95	0.95
1 of 2	1, 2 of 3	yes	2	136	21	0.03	0.84	0.86
3	1	yes	2					
3	2 of 3	yes	3	29	8	0.07	0.68	0.62

[a]4 patients had no value for one or more model variables.
[b]Expected percentage of events per year of follow-up.

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Bevacizumab combined with chemotherapy as first-line treatment of metastatic breast cancer patients: a meta-analysis based on studies having randomized 2,695 patients

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Background: Bevacizumab, a monoclonal antibody against VEGF, has been shown to improve the outcome of patients with metastatic breast cancer. We conducted a meta-analysis of randomized trials to assess the magnitude of the benefit of adding bevacizumab to chemotherapy in the first-line treatment of metastatic/recurrent breast cancer (MBC) in terms of progression-free and overall survival as well as tumour response rate. The commonest side-effects of bevacizumab were also evaluated.

Methods: Randomized phase III trials evaluating the addition of bevacizumab to chemotherapy for the first-line treatment of MBC were identified using PubMed and/or abstracts presented at major oncology meetings. Hazard Ratios (HR) for time-to event endpoints and odds ratios (OR) for binary endpoints were calculated or retrieved from each study and combined using the fixed-effects or random-effects whenever indicated.

Results: Three studies were selected with a total of 2,695 randomized patients; only one study was published in a peer review journal at the moment this meta-analysis was performed. The addition of bevacizumab to chemotherapy improved progression-free survival (PFS) (HR 0.69; 95% CI 0.63–0.76) and response rates (OR 1.84; 95% CI 1.56–2.18) in patients receiving the combination compared to chemotherapy alone. A trend towards better overall survival was also observed (HR 0.88; 95% CI 0.78–1.00). The benefit of adding bevacizumab to chemotherapy was observed in all subgroups (ER positive or negative, age <65 or $\geqslant$65, short or long disease-free interval, prior adjuvant chemotherapy, and prior taxanes). As expected, toxicity profile included hypertension, proteinuria, sensory neuropathy and left ventricular dysfunction, and was significantly more pronounced in patients receiving bevacizumab.

Conclusion: In our meta-analysis the addition of bevacizumab to chemotherapy in the first-line treatment of patients with MBC significantly improves PFS and response rates in all patient population and across different subgroups. A trend towards better overall survival was also observed. Side effects were more often observed in patients receiving bevacizumab.

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Preoperative capecitabine and docetaxel followed by 5-FU/epirubicin/cyclophosphamide (FEC) and predictive value of protein biomarkers

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Background: Capecitabine (X) and docetaxel (T) have demonstrated synergistic effect in preclinical models and survival benefit in metastatic breast cancer. Useful predictive marker is necessary for breast cancer patients treated with preoperative chemotherapy. This study's purpose was to determine the efficacy of X and T followed by 5-FU/epirubicin/cyclophosphamide (FEC) in the preoperative setting and to evaluate the correlation between protein biomarker expression and pathological complete response (pCR).

Patients and Methods: Patients with stage II/III breast cancer received 4 cycles of XT (capecitabine 1650 mg/m^2 on days 1−14 and docetaxel 60 mg/m^2 on day 8 every 3 weeks), followed by 4 cycles of FEC (fluorouracil 500 mg/m^2, epirubicin 90 mg/m^2, cyclophosphamide 500 mg/m^2 on day 1 every 3 weeks). Primary endpoints were the pathological complete response (pCR) rate and adverse drug reactions. pCR was defined as no microscopic evidence of residual viable tumor cells, invasive or noninvasive, in all resected specimens of the breast. Expression analysis using immunohistochemistry was performed in core needle biopsy samples at baseline.

Results: Seventy-two patients were enrolled and 71 patients were assessable for clinical and pathologic responses. The median age was 51 years (range, 27−69 years). The median tumor size was 3.5 cm (range, 2−8.3 cm). Forty-six (64.8%) patients were clinically node-positive. Overall, 50 (50.1%) patients had hormonal receptor (HR)-positive tumors, and 21 (29.6%) had HR-negative tumors. A HER2 overexpression was detected in 19 cases (26.8%). Ki67 expression ranged from 0 to 92.3% and 34 cases showed 20% and higher of positive nuclei. The overall response rate was 91.5%, including a complete response in 29 patients and a partial response in 36 patients. No patients showed clinical progression of disease. The pCR rate was 14.1% (10/71). Grade 3/4 neutropenia was observed in 32.4%