comparison of the microarray based TargetPrint with IHC and fluorescent in situ hybridization (FISH) assessments generated by local standards in 11 hospitals.
Material and Methods: The mRNA level of ER, PR and HER2 was assessed retrospectively on 144 breast tumor samples containing sufficient tumor cells, collected by a German tumor bank. The patients were diagnosed in 7 different hospitals. Prospective tumor samples with sufficient tumor cells were collected for 27 patients presenting to 4 different hospitals between November 2008 up to present. The results of the IHC/FISH assessments performed according to the local standards of the hospitals were compared to the quantitative gene expression readouts.
Results: Sufficient RNA for microarray analysis was obtained from 140 (97%) retrospective samples and from 26 (96%) prospective samples. Comparison of IHC and microarray readout indicated a very high concordance of 97% for ER, 86% for PR and 94% for HER2 on the retrospectively analyzed samples (Table 1). The prospectively collected samples indicated a 100% concordance for ER and HER2 and 77% for PR (Table 1). All PR discordant cases (n = 6) originated from a single centre. Three samples (2 retrospective, 1 prospective) were excluded from concordance analysis as they were scored HER2IHC 2+ without additional FISH analysis. All three HER2 IHC 2+ samples were classified HER2 negative by TargetPrint. Prospective data collection is ongoing and more data will be presented at the meeting.

Table 1.

	ER	PR	HER2
7 centers retrospective	97% (n = 140)	86% (n = 140)	94% (n = 138)
4 centers prospective	100% (n = 26)	77% (n = 26)	100% (n = 25)

Conclusion: Microarray based readout of ER, PR and HER2 status using TargetPrint is highly comparable to local IHC and FISH analysis on retrospectively and prospectively analyzed samples in various hospitals. Using TargetPrint microarray readouts for hormone and HER2 receptor status in addition to standard IHC will improve the molecular characterization of breast cancer tissue.

5166 POSTER

The prognostic significance of age at diagnosis in patients with breast cancer younger than 35 years

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In breast cancer patients, age below 35 years (yr) is an independent risk factor of recurrence and death, even after correction for disease stage. However, as breast cancer (ca) occurs rarely in the mentioned age group (2–4% of all patients), prognostic factors for this population are not well understood.
It has been estimated that in general breast ca population the hazard of recurrence decreases with age by 4% per year of life. The aim of our study was to assess the association of age at diagnosis with disease outcome in women with breast cancer, aged 35 years or less.
Methods: The analysis was carried out retrospectively in 190 patients (pts) with breast cancer aged 35 years or less, referred to our Clinic between 1997–2006 (after exclusion of 10 patients with stage IV, 8 patients not treated surgically and 9 patients with incomplete clinical data). For all 190 patients the time to relapse (DFS, disease-free survival) was assessed. The median follow-up time was 47.7 months. Among this group there were 21.6% patients aged 34–35 years, 44.2% 30–33 yr, 25.2% 26–29 yr and 6.4% 25 yr and less.
Results: Relapse occurred in 36.6% of pts (20.5% distant metastases and 16.1% local recurrence only). 5-year recurrence-free survival was 57.2%, with estimated median survival time 10.9 yr. In univariate Cox analysis the most notable prognostic factors were nodal status (the most significant, both by clinical assessment and pathological analysis, $p < 0.005$), positive HER2 and negative ER/PR ($p < 0.05$). In the analyzed cohort of patients below age of 35 years, older patients showed poorer prognosis compared to very young women: patients aged 34–35 showed significantly worse 5-yr survival (41.9%), compared to younger patients (78.4%, $p = 0.004$). In Cox regression modelling, age at diagnosis increased the relapse risk by 7.6% per each year ($p = 0.07$), within the moderately narrow age frame assessed in our study. The effect of age group (34–35 vs younger) was significant also in multivariate analysis, in the context of nodal and hormone receptor status, with hazard ratio of 1.93 ($p = 0.016$).
Conclusions: In women below the age of 35 years, the increase of age seems to elevate the risk of disease relapse. This finding, contradictory to the generally observed poor risk in patients below 35 years and age-related decrease of risk in the whole population, warrants further investigation.

5167 POSTER

French cost effectiveness study of the MammaPrint 70-gene signature in early stage breast cancer patients

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Background: In early breast cancer, adjuvant chemotherapy increases the life expectancy of patients with high risk of developing metastases. However, for the other patients, chemotherapy-associated adverse effects outweigh the benefits.
Compared to clinicopathologic risk assessment, the MammaPrint 70-gene test has been shown to provide additional prognostic information for early stage breast cancer patients. However, the cost-effectiveness of this strategy is not well understood.
Materials and Methods: The budgetary impact of Mammaprint was studied using a Markov model. In France the initial target population for Mammaprint are stage I and II node negative breast cancer patients. Every year approximately 37,000 patients meet these criteria.
It has been demonstrated that Mammaprint can reduce the amount of unnecessary chemotherapy by 11% compared to Adjuvant!Online and by 27% compared to the St-Gallen criteria.
Results: In economic terms, we now show that the cost of MammaPrint was offset by the savings, due to a lower number of administered chemotherapies. The model estimates mean savings to be € 9,043 per 100 patients per year in the base case scenario. These results are sensitive to chemotherapy price, to relative usage of St-Gallen and Adjuvant!Online and to risk reduction associated with chemotherapy.
Conclusions: In summary, MammaPrint is a gene expression profiling test that has proved to be more accurate than current risk assessment tools. It helps oncologists to identify patients who may forgo unnecessary adjuvant chemotherapy in comparison to Adjuvant!Online or stGallen criteria. As patient's quality of life and rational use of resources are key factors in decision-making process, Mammaprint can be considered to be an efficient tool. As more costly systemic therapies are likely to become standard in the future, the economic advantages of Mammaprint might become even more apparent.

5168 POSTER

Circulating tumor cells (CTCs) in peripheral blood of breast cancer (BC) patients two years after primary diagnosis – Results from the German SUCCESS trial

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Background: While CTCs have shown promising results as marker of treatment efficacy and early recurrence in MBC, there is a lack of data in the adjuvant setting. The SUCCESS trial evaluates the role of persisting CTCs at primary diagnosis and after chemotherapy as well as two years after diagnosis in primary BC patients treated with zoledronate.
Methods: We analyzed 23 ml of peripheral blood in N+ and high risk N− primary BC pts receiving 3×FEC(500/100/500)-3×Doc100 q3w vs. 3×FEC(500/100/500)-3×DocGemcitabine(75/1000 d1+8) chemotherapy followed by 2 yrs (4 mg q3m×24 m) vs. 5 yrs (4 mg q3m×24 m followed by q6mx36 m) of zoledronate. CTC results after two years are shown. CTCs were assessed with the CellSearchSystem (Veridex, Warren, USA). After immunomagnetic enrichment with an anti-Epcam-antibody, cells were labelled with anti-cytokeratin (8, 18, 19) and anti-CD45 antibodies.
Results: The data of 579 pts at the mean of 29 months (range 20–43) after diagnosis are available. 4.3% of pts (n = 25) presented with >1CTC in peripheral blood. In pts with the detection of CTCs, the mean number of cells was 1 (range 1–29). While we found 1 CTC in 5.9% and 2 CTCs in 1.6% of pts, 1.5% had 3–5 CTCs, 1.2% >5 CTCs. We found no correlation between the presence of >1CTC with tumor size ($p = 0.41$), nodal status

(p = 0.41), grading (p = 0.45), hormonal status (p = 0.92) or Her2-Status of the tumor (p = 0.59).
In this patient group, 9.7% and 6.9% of pts had presented with >1CTC at primary diagnosis and after chemotherapy, respectively. We found no correlation of CTCs after chemotherapy with the results at primary diagnosis (p = 0.08) or at two years (p = 0.23). However, the presence of CTCs at diagnosis was associated with CTCs after two years (p = 0.03).
In 184 postmenopausal HR+ pts endocrine treatment data was analyzed. CTCs at two years were detected in 6.8% of pts on tamoxifen (n = 9), while 1.9% of pts were positive on anastrozole treatment (n = 1; p = 0.19).
Conclusions: In the SUCCESS trial we observed persisting circulating tumor cells in a relevant number of recurrence-free breast cancer patients after cytostatic, endocrine and zoledronate treatment. Longer follow-up will deliver insight if these cells can identify patients with increased risk for recurrence who might benefit from additional treatment approaches.

5169 POSTER

Comparison and combination of gene-set classifiers for prediction of localized breast cancer survival

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Background: Genome-wide expression profiling has been used to identify gene sets and corresponding gene signatures associated with clinical end-points of breast cancer patients. Here, we assessed and compared the risk predictive ability of 13 published gene sets in localized breast cancer patients.
Materials and Methods: The training set was data from a recent microarray study of 123 early-stage breast cancer patients. A clinically similar cohort of 80 localized breast cancer patients was used as test set. As clinical end points both systemic recurrence status and breast cancer specific death were considered. A Cox model was used to model the relationship between survival and gene expression of selected genes. Penalized likelihood regression (using an L2 penalty) was used to estimate regression parameters. Cross-validation was used to determine the penalty parameter. A Cox model for specific gene set was developed from the training set; this model was further applied to test set to calculate a Prognostic Index (PI) for a patient. The derived PIs from individual gene set were then used for comparison of the predictive performance across gene sets, as well as for combination of multiple gene-set predictors. The performance of specific gene set for risk prediction was also compared with the Adjuvant! Online model.
Results: A Gene-Set expression prognostic model was developed for individual gene set from the training set and a PI as risk indicator for each patient in test set was predicted using the gene-set specific model. The patients were then classified into different prognostic groups based on the derived PIs. The survival probabilities among the patient-groups were found significantly different and various clinical indications were explored in these groups. Each gene set in our study was assessed for risk prediction performance using PIs both in the univariate setting and compared with clinical information added by Adjuvant! Online. The results showed that the overall predictive information added by multiple gene sets was significant and the model incorporated gene-set predictors outperformed Adjuvant! Online model.
Conclusions: The proposed method can be used to quantify the potential contribution of a predefined gene set for breast cancer risk prediction and multiple gene sets as well as clinical parameters can be integrated in a risk prediction model to improve the prediction performance.

5170 POSTER

Breast cancer - Clinical early disease Neoadjuvant versus adjuvant chemotherapy for women with operable breast cancer: a matched-pair analysis and prognostic factors on overall survival

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Background: Neoadjuvant chemotherapy (NAC) for patients with localised breast carcinoma became a standard approach although several phase II trials failed to demonstrated higher overall survival rates, when compared with the adjuvant chemotherapy (AC) while improving rates of breast-conserving surgery. Registry-base Study of potential prognostic factors on overall survival (OS) whether chemotherapy was delivered in the NAC or the AC setting are valuable for optimizing the therapeutic strategy.
Methods & Materials: From 1982 until 2005, 184 women with localised breast carcinoma were retrospectively selected: 92 patients were treated with NAC followed by surgery and then radiotherapy (RT) and 92 matched-pair patients were treated with surgery followed by AC and then RT. Criteria for matching were: clinical AJCC stage at baseline, age ($\leqslant$50 *vs* >50 years), diagnosis period (1982–1999 *vs* 2000–2005) and oestrogen receptor status (OR). The Kaplan Meier methods were used to estimate OS and DFS. Cox multivariate model was applied to assess independent prognostic factors of NAC for OS and DFS.
Results: Patients' characteristics were similar between the two groups, especially for SBR. All women received RT. No difference was found between irradiated lymph node (LN) stations. Median follow-up were 7 years and 6 years for the NAC and AC groups, respectively. Rates of breast-conserving surgery (BCS) were 57.6% and 48.9% for AC and NAC groups, respectively (p = 0.24). DFS rates at 1, 5 and 10 years were 91%, 73% and 53% for the NAC group and 96%, 70% and 52% for the AC group, respectively (p = 0.85). OS rates at 1, 5 and 10 years were 96%, 82% and 64% for the NAC group and 97%, 72% and 62% for the AC group, respectively (p = 0.2). With univariate analysis prognostic factors for OS were OR, SBR and post operative staging AJCC. With multivariate analysis, OR and SBR remained significant. Timing of chemotherapy did not impact on OS. If NAC, the pathological complete response rate was 13% with OS rates at 1, 5 and 10 years: 100%, 83%, 83%, respectively.
Conclusion: This first registry-based case-control study failed to demonstrate improvements in breast-conserving surgery, DFS and OS rates with NAC compared with AC for women with localised breast carcinoma. Although, patients with a pathological complete response had excellent survival rates at 10 years, new phase III trials testing NAC strategies should be done to definitely answer on its impact on quality of life and/or in a medico-economic perspective.

5171 POSTER

Neo-adjuvant anthracycline based chemotherapy in locally advanced breast cancer: assessment of topoisomerase IIA with response

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Background: The anthracycline group of chemotherapy drugs are reported to target DNA Topoisomerase IIA (TopoIIA), indicating that breast cancers which over express TopoIIA are more likely to respond to anthracyline based chemotherapy. We sought to test this hypothesis by studying a number of biomarkers, including TopoIIA, in biopsies taken from patients with locally advanced breast cancer treated with neo-adjuvant anthracycline based chemotherapy.
Method: Between July 2001 and November 2006, thirty seven consecutive breast cancer patients with locally advanced inoperable (T2-T3) or locally advanced inoperable (T4) were treated with primary neo-adjuvant chemotherapy.
Analysis was carried out to assess response using the following methods: clinical measurement, mammogram, ultrasound, magnetic resonance imaging and histopathology. Chemotherapy treatment was according to protocol: 2000–2002 Six cycles Fluorouracil, Epirubicin, Cyclophosphamide (FEC).
2002–2006: Four cycles (FEC); followed by four cycles taxotere. Pre and post operatively, specimens were analysed for size, type, nodal status, oestrogen receptor, progesterone receptor, HER2 status. Patients were asked, with informed consent, for retrospective analysis biopsy specimen for TopoIIA using fluorescent in situ Hybridisation (FISH).
Results: Of 37 patients, 28 consented for retrospective Topo IIA specimen testing. All those that were Topo IIA amplified were also HER2 amplified. Histological complete response (CR) rates were 9/37 (24%). Of 7/28 patients that were TopoIIA positive, 2 had histological CR (29%). Of 14/28 patients that were Topo IIA negative, 4 had Histological CR (29%).
Conclusion: In this small neoadjuvant study, Topo IIA tumour testing did not predict for likelihood of complete histological response to anthracycline based chemotherapy.