Conclusions: In this study, obesity was associated with poorer outcome in node-positive BC patients. The role of CT dosing and/or patient co-morbidities in obese patients is currently being studied. Given the increasing prevalence of obesity worldwide, more research on improving the treatment of obese BC patients is needed.

30 Poster Discussion

Relevance of histological and molecular subtypes in the outcome of primary systemic therapy for operable breast cancer

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Objective: To analyse the relevance of histological subtypes, ductal versus lobular, and molecular subtypes using immunohistochemical profiles: luminal (estrogen-receptor positive and HER2-negative), basal (hormone receptor and HER2 negative) and HER2 positive, in the outcome of primary systemic therapy (PST).

Methods: Retrospective analysis of 254 patients treated with PST between 2000 and 2007 in the Netherlands Cancer Institute. Inoperable patients (T4, N3) were excluded. The majority of patients (70%) were initially treated with doxorubicin and cyclophosphamide and participated in two randomized studies in which antracycline and taxane based regimens were compared. Since 2005 HER2-positive patients received chemotherapy in combination with trastuzumab. The type of surgery feasible prior to neoadjuvant chemotherapy was compared to the actual surgery performed. Pathological complete remission (pCR) was defined as no evidence of invasive cancer in either breast and axilla.

Results: The increase in BCT was 32% (63/195) in patients with ductal carcinoma, and 17% (7/35) in patients with lobular carcinoma. Secondary mastectomy was required because of irradical resection in 2% and 33%, respectively. The pCR rate in ductal and lobular carcinoma was 12% and 2%, respectively. The overall pCR rate was 11%. The pCR rate in luminal, basal and Her2 positive patients treated with trastuzumab was 2%, 28% and 35%, respectively. Multivariate analysis indicated that molecular subtype was the only independent predictor of pCR. (P 0.004).

Conclusion: There is a clear difference in tumor response and surgical downstaging between histological and molecular subtypes. This result provides us another argument to select patients for PST on the basis of these subtypes in future trials.

		N	pCR	P value	Odds Ratio	95% CI
Age	<45 yrs	137	22	0.31	R	
	>45 yrs	114	7		0.42	0.077−2.27
Menopausal status	post	59	3	0.56	R	
	pre/peri	187	26		1.87	0.23−15.3
Histology	lobular	42	1	0.69	R	
	ductal	195	23		1.61	0.16−16.11
Molecular subtype	luminal	138	3	0.004*	R	R
	basal	57	16	0.01*	14.8	2.79−78.4
	Her2+	56	10	0.06	11.9	2.28−63.6
pN category prechemo	N1	169	13	0.10	R	R
	N0	43	13		3.07	0.95−9.94
T category	T1	12	2	0.27	R	R
	T2	151	19	0.76	0.33	0.04−2.76
	T3	88	8	0.11	0.16	0.02−1.62

31 Poster Discussion

Systematic validation of novel breast cancer progression-associated biomarkers via high-throughput antibody generation and application of tissue microarray technology

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Background: There now exist vast quantities of DNA microarray data defining differences in gene expression between different subtypes of breast cancer, including variations in invasiveness and metastatic capabilities. However, this type of genetic assay is of limited prognostic or predictive value in most clinical settings due to general requirements for fresh/frozen tissue. The aim of this project is to translate the genetic data available into a more clinically relevant form – that of immunohistochemistry – identifying from these gene datasets any independent biomarkers that may be potential biomarkers and/or drug targets.

Materials and Methods: Our approach involves the high-throughput validation of the affinity-purified, mono-specific antibodies created by the Swedish Human Proteome Resource (SHPR, www.proteinatlas.com) against candidate breast cancer progression-associated biomarkers selected from publicly available and in-house transcriptomic and proteomic datasets. Initial validation of these antibodies was performed by the SHPR using a variety of normal and cancer tissues. Of the 137 targets selected for production, 32 have begun specificity validation by Western blot analysis. Those that are successful are moved forward to immunohistochemical (IHC) validation using cell pellet arrays derived from different human breast tumour cell lines. Successful IHC validation leads to the use of tissue microarrays (TMAs) of clinical samples to assess the clinical relevance of the putative biomarkers, either individually or as a panel. For efficient validation of the candidate biomarkers a TMA is being used, constructed from a cohort of 512 consecutive breast cancer cases diagnosed between 1988 and 1992.

Results: PDZK1, an estrogen-responsive gene, was previously found to be associated with good prognosis (interval to distant metastasis) at the transcript level in breast tumours. Our TMA IHC results showed PDZK1 protein to be associated with improved breast cancer-specific survival ($p = 0.0247$), ER positivity ($p = 0.041$) and low grade ($p = 0.002$). Another promising putative biomarker undergoing validation according to this schema is PDZ-binding kinase (PBK).

Conclusion: We have developed a comprehensive biomarker pathway that extends from discovery through to validation on TMA and is yielding clinically relevant biomarkers.

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Down regulation of angiogenesis antagonist EFEMP1 is associated with unfavourable prognosis in sporadic breast cancer patients

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Background: EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1) was recently described to be an angiogenesis antagonist and to act as a suppressor of formation and progression of human malignancies.

Materials and Methods: Immunohistochemistry on tissue microarrays of 203 clinically well characterized primary breast carcinomas was used to assess the potential clinical relevance of reduced EFEMP1 protein expression regarding patient outcome. Cox regression for multivariate survival modelling as well as univariate analyses were performed. Next to immune reactivity score for EFEMP1 expression, tumor grade, hormone receptor status, lymph node status, Her-2 status, tumor size, and type of adjuvant systemic therapy were included into analysis.

Results: Multivariate regression analyses in the 186 node-positive cases revealed that next to tumour size and grade EFEMP1 expression remained in the survival model as relevant factor influencing disease-free- and overall survival at borderline significance (DFS: $p = 0.14$; OS: $p = 0.077$). Further analysis of patient subgroups with homogeneous adjuvant systemic therapy revealed a significant correlation of low EFEMP1 expression with poor DFS and OS survival ($p = 0.037$ and $p = 0.032$) only in those node-positive patients who had received adjuvant anthracycline-containing

chemotherapy (n = 31), whereas no significant impact was seen in node-positive patients treated either by CMF chemotherapy (n = 49) (DFS: p = 0.605; OS: p = 0.934) or by adjuvant endocrine therapy (tamoxifen) alone (n = 106) (DFS: p = 0.735; OS: p = 0.275). While median DFS and OS in anthracycline-treated patients was >10 years if tumours showed high EFEMP1 expression, it was only 3.1 years (DFS) respectively 4.5 years (OS) in cases with low EFEMP1 expression.

Conclusions: The results point to a predictive value of EFEMP1 expression regarding anthracycline response in node positive patients, which needs to be further validated in larger collectives of homogeneously treated breast cancer patients. In view of clinically emerging angiogenesis inhibitors, identification and characterization of components of the angiogenic pathway as specific prognostic as well as predictive markers is of great relevance for the success of this treatment option. EFEMP1, with its anti-angiogenic properties, may serve here as an important molecular marker for defining an adequate tumour-biology oriented therapeutic strategy.

33 Poster Discussion

AlphaB-crystallin predicts poor breast cancer survival in basal-like tumors

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Background: Basal-like breast cancers are high grade tumors with poor prognosis (Perou et al. Nature 406:747−52, 2000; Nielsen et al. Clin Cancer Res 10: 5367−74, 2004). In gene expression profiling studies, α-basic (αB)-crystallin, a small heat shock protein with anti-apoptotic and oncogenic activity, is commonly expressed in basal-like tumors. We previously reported that tumors over-expressing this protein had a poor prognosis, and that approximately half of basal-like breast cancers express αB-crystallin (Moyano et al. J Clin Invest 116:261−70, 2006). In this study, we aim to validate the prognostic value of αB-crystallin in a regional population-based series of 4000 breast cancers, powered for subset analysis.

Materials: Tissue microarrays were constructed using 4046 invasive primary breast cancers referred to the British Columbia Cancer Agency from 1986 to 1992 with clinical outcome. Breast cancer subtypes were defined using the immunopanel of ER, PR, HER2, epidermal growth factor receptor (EGFR) and cytokeratin 5/6. Immunohistohemistry of αB-crystallin was scored as diffuse positive (⩾30% of cancer cells positive), focal positive (<30% of cancer cells positive), or negative. Univariate survival probabilities were estimated using Kaplan–Meier method. Multiple Cox regression analyses and likelihood ratio tests (LRT) were used to determine the independent prognostic significance of αB-crystallin.

Results: Among breast tumors interpretable for αB-crystallin, 11% (361/3285) of cases are positive. Consistent with the previous report, 55.4% (175/316) of basal-like tumors, defined as ER/PR/HER2 negative and (EGFR or cytokeratin 5/6) positive, express αB crystallin. αB-crystallin positive tumors are associated with 11% absolute decreased breast cancer survival [10-yr BCSS (95% CI) 75% (73−77) versus 64% (58−68)]. In the Cox regression model including lymphovascular invasion, tumor size, grade, nodal involvement, age at diagnosis and breast cancer subtypes, αB-crystallin remains as an independent poor prognostic marker with a hazard ratio of 1.310 (LRT p = 0.02113). Within the subset of basal tumors, αB-crystallin positive tumors are also independently associated with poorer breast cancer survival (Hazard Ratio 1.63, LRT p = 0.02).

Conclusion: αB-crystallin independently predicts poor survival in a large population based cohort and among basal-like tumors, suggesting its role as a novel biomarker that identifies a particularly aggressive subset of basal-like tumors.

34 Poster Discussion

Monoclonal antibodies specific for Phospho-4E-BP1 (Thr 70) and phospho-AKT (Ser 473) indicate prognosis in breast cancer

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Background: The activation of cell surface growth factor receptors initiates a cascade of signalling through overlapping signalling pathways via the phosphorylation of signalling proteins. Previous studies have suggested that the phosphorylation of 4E Binding Protein 1 (4E-BP1), Mitogen Activated Protein Kinase (MAPK) and v-akt murine thymoma viral oncogene (AKT) proteins has value as a prognostic indicator in breast cancer. However these studies have been limited by small patient groups and, in some cases, complicated by the interaction of systemic treatment on outcome.

Materials and Methods: Paraffin embedded invasive tumour samples from 430 patients who had received no adjuvant chemo or hormonal therapy were used to construct a Tissue MicroArray (TMA). Median follow up was 21 years. Tissue sections from TMAs were stained using monoclonal antibodies to ER, PgR, Ki67, phospho-AKT, Phospho-4E-BP1 and polyclonal HER2 and phospho-MAPK antibodies using standard immunohistochemistry methods. Nuclear markers (ER, PgR, Ki67 and Phospho-4E-BP1 (Thr 70) were scored with validated algorithms on an Ariol imaging system (Applied Imaging). All other markers (HER2, phospho-AKT (Ser 473) and phospho MAPK (Thr202/Tyr204)) were scored manually.

Results: ER and PgR histoscores were significantly positively correlated (p < 0.001). A significant inverse correlation observed between both ER and PgR histoscores and Ki67 (both p < 0.001). Analysis indicated a significant correlation between high phospho-4EBP1 staining and reduced recurrence free survival (p < 0.05). Conversely, high phospho-AKT staining was correlated with longer overall survival (p < 0.05).

Conclusions: We have found that the phosphorylation of 4E-BP1 and AKT proteins are prognostic for disease free and overall survival respectively in breast cancer patients in the absence of systemic therapy suggesting these are true prognostic markers in breast cancer. Further analysis of their significance in the context of other known markers of breast cancer prognosis will be performed and presented.

Wednesday, 16 April 2008 12:30–14:30

POSTER SESSION

Detection, diagnosis and imaging

35 Poster

Axillary study before surgery in patients with breast cancer

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Background: The axillary study with ultrasound and cytological puncture with fine needle aspiration of suspicious nodes are new diagnostic methos.

Material and Methods: We study 159 patients with axillary ultrasound and cytological puncture with fine needle aspiration (FNA) of suspicious nodes. Suspicious nodes were those with at least one of the following signs: long to short axis ratio less than 1.5, absence of hilius and cotical disrruption. If the results were compatible with metastases (positive) then we performed axillary lymphadenectomy, if it was found to be benign (negative) then we conducted sentinel node study.

Results: In the group of ultrasound positive plus ultrasound positive FNA positive was 54 patients (33.96%) when we conducted axillary lymphadenectomy, 13 patients (24%) were found to have one positive node, 7 patients (13%) two positive nodes, 9 patients (16%) three positive nodes, 25 patients (45%) more than three positive nodes.

The other group (ultrasound negative plus ultrasound negative plus FNA negative) was 105 patients (66.03%), the sentinel node study was: in 76 patients (72.38%) pNOi−, in 9 patients (8.57%) pNOi+, in 7 patients (6.66%) pN1mic, in 12 patients (11.42%) pN1a, in 1 patient (0.95%) pN2a.

Conclusion: The axillary study with ultrasound and FNA before surgery allows excluding a group of patients to make the sentinel node study.

36 Poster

Magnetic Resonance Imaging to predict pathological response in neoadjuvant chemotherapy for breast cancer

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Background: The rationale of neoadjuvant chemotherapy in patients with locally advanced breast cancer is to achieve down-staging of the tumour to enable breast conservative surgery. The objective of this study is to