obtained from the majority all patients (439 and 752/753 for uPA and PAI-1). Individually observed 10-year OS were also obtained and that compared to estimate. We compared individually observed and estimated OS according to the uPA and PAI-1 levels.

Results: The observed 10-year OS of the whole group of EBC patients was 61.5% while, estimated by Adjuvant! Online 65.5%. The difference between predicted and observed OS did not vary considerably in the subgroups of patients with low uPA or PAI-1 levels, while the differences became substantial in the subgroups of patients with either high uPA or PAI-1 levels.

	N	% Overall survival		
		Predicted	Observed	Predicted − observed
All patients	753	65.5	61.5	4.0
uPA low (⩽3 ng/mg)	195	62.8	62.6	0.2
uPA high	344	66.0	61.9	4.1
PAI-1 low (⩽14 ng/mg)	577	66.3	63.3	3.0
PAI-1 high	175	63.0	55.4	7.6

Conclusion: In high risk patients, defined by high uPA and/or PAI-1, the predicted 10-year OS calculated by Adjuvant! Online seems to be overestimated compared to observed patient outcome. Like in high risk patients defined by classical clinicopathological features, Adjuvant! Online could be unreliable tool for prognosis assessment in high risk patients defined by uPA/PAI-1 status. Using prognostic factor index calculation (PFIC) these differences could diminish.

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CEC and CTC in stage IV breast cancers treated with bevacizumab (Bv) and chemotherapy (CT)

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Introduction: The antiangiogenic agent Bv, in combination with CT, (i) improves progression free survival (PFS) of first line treatments, (ii) may modify tumor cell intravasation and CTC count, and (iii) may change CEC levels. We therefore investigated whether CTC and CEC counts could be early surrogate markers of time to progression (TTP) in MBC patients receiving a highly active anti-tumor treatment (HAATT) comprising taxanes combined with Bv.

Material and Methods: Eligible patients received Bv (10 mg/kg q2w or 15 mg/kg q3w) combined with a taxane-based CT or non-anthracycline CT, until disease progression, unacceptable toxicity or withdrawal. For patients participating in the sub-study, CTC and CEC were measured in 7.5 ml of blood at baseline and after cycle 2 or 3 of treatment. Analysis was performed using the CellSearch™ System, combining EpCAM immunomagnetic selection (IMS) followed by anti-cytokeratin (A45B/B3) staining for CTC and CD146 IMS and CD105 staining for CEC. VEGF-A constitutional polymorphisms were also analyzed. CTC and CEC counts at baseline and changes during treatment were correlated with TTP.

Results: Sixty-seven patients were included. There was no correlation between CTC, CEC levels and VEGF-A polymorphisms. At baseline, using the threshold of 5 CTC/7.5 ml which was previously defined with standard CT: (i) CTC positivity (54% of patients) was associated with elevated LDH ($p = 0.04$), elevated CA15.3 ($p < 0.001$) and high tumor burden (>3 metastatic sites) ($p = 0.03$); (ii) CTC was a significant prognostic marker for TTP at a threshold of 3 CTC/7.5 ml ($p < 0.05$) and not at 5 CTC/7.5 ml ($p = 0.09$). Baseline CEC levels were associated with age ⩾45y ($p = 0.01$), with elevated LDH ($p < 0.01$) and not with TTP at any threshold. In our series, changes of CTC count during treatment was not a surrogate for TTP, with any of the model tested (threshold-based or relative decrease in %). However, using a defined threshold, changes of CEC count during treatment was significantly associated with TTP ($p < 0.001$).

Conclusions: Our study is the first to monitor both CTC and CEC levels in the era of HAATT comprising an antiangiogenic agent combined with standard CT. We observed that previously reported CTC thresholds may be modified by antiangiogenic therapy, whereas changes in CEC levels are a promising early surrogate marker for TTP under HAATT.

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Expression and activation of protein kinases in Triple Negative Breast Cancer (TNBC)

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Background: Triple-negative breast cancers (TNBCs) are so named as they lack expression of estrogen receptor (ER), progesterone receptor (PR) and do not exhibit overexpression or amplification of the HER-2 gene. Unlike other subgroups of patients with breast cancer, targeted therapy is currently unavailable for patients with triple-negative disease.

Aim: Several protein kinases are causally involved in driving cancer cell growth, invasion and metastasis. Furthermore, protein kinases are amongst the most promising new therapeutic targets for cancer treatment. The aim of this investigation was therefore to examine the expression and/or activation state of 3 protein kinases in TNBC, i.e., mTor, Src and MAPK.

Materials and Methods: Tissue microarrays (TMAs) comprising cores from 89 TNBCs and 100 non-triple-negative breast cancers were constructed and then stained for mTor, phospho-mTor (activated mTor), Src and phospho-MAPK.

Results: Three distinct patterns of staining of phospho-mTor (activated mTor) were seen, cytoplasmic, peri-nuclear and nuclear. Cytoplasmic and perinuclear phospho-mTor levels were significantly higher in the non-TN group. ($p = 0.014$ and $p < 0.0001$, respectively) In contrast, nuclear phospho-mTor was predominantly seen in the TN group ($p = 0.0001$). A significantly higher proportion of TNBCs expressed cytoplasmic Src ($p = 0.012$) and membranous Src ($p < 0.0001$). With mTor and phospho-MAPK, there was no difference between the two groups.

Conclusions: These results suggest that the activation of mTor and Src play a role in the development and progression of TNBC. mTor and Src may therefore be new targets for the treatment of patients with TNBC.

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Randomised placebo controlled trial studying short term biological effects of the combination of letrozole and zoledronic acid on invasive breast cancer

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Background: To determine whether the addition of Zoledronic Acid to endocrine therapy increases apoptosis or decreases proliferation in early invasive breast cancer, a placebo controlled randomised trial comparing 14 days treatment with Letrozole or Letrozole and Zoledronic Acid pre-operatively was performed.

Materials and Methods: In total 109 postmenopausal women with early invasive hormone receptor positive breast cancer (grade I:29; grade II: 51; grade III:9) were randomised (1:1:1) to either placebo, Letrozole 2.5 mg/day or Letrozole with Zoledronic Acid 4 mg single dose intravenously 2−4 days before definitive surgical excision. Epithelial proliferation and apoptosis were measured on paired baseline and surgical biopsy specimens (after 14 days of treatment) using Ki67 and Activated Caspase 3 immunohistochemistry. Alterations in angiogenic markers (VCAM/VEGF and CD31) were also studied. The primary endpoint was fall in Ki67 between diagnosis and surgical excision. Sixteen percent were progesterone receptor negative.

	Placebo (n = 32)	Letrozole (n = 34)	Let + Zol (n = 35)
Baseline Ki67 level, median (range)	16.6 (1−39)	17.2 (2−40)	19.9 (3−68)
Absolute Ki67 change, median (range)	−0.8 (−12, 12)	8.6 (−14, 37)	12.9 (−12, 29)
Caspase 3 change, median (range)	0.1 (−3.8, 9.3)	0.4 (−2.7, −4.1)	0.2 (−10.9, −14.4)
Cell turnover index, absolute change	−0.3 (−142, −59)	18.9 (−201, 192)	17.7 (−14, 379)

Results: Overall 109 women were enrolled but paired biopsies were only available for 101 patients. Statistically significant reductions in Ki67 and Cell Turnover Index were seen with Letrozole and Let & Zol ($p \leqslant 0.001$) but

there was no significant difference between Letrozole and Letrozole plus Zoledronic Acid groups (p = 0.26). Apoptosis did not change between the three groups.

Conclusions: Letrozole reduces proliferation by 70% when used for 14 days prior to surgery. Zoledronic Acid administration prior to surgery is safe but when administered as a single dose at a median of 3 days before surgery does not alter apoptosis or proliferation compared to Letrozole alone.

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SOFIA: phase II study of neoadjuvant epirubicin, cyclophosphamide (EC) + sorafenib (S) followed by paclitaxel (Pw) + sorafenib (S) in women with previously untreated primary breast cancer (BC) (GBG 45)

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Background: Sorafenib is an oral RAF/RAS kinase inhibitor with antiangiogenetic potential. In metastatic BC it shows modest single agent activity but prolongs survival in combination with capecitabine. EC-P is a standard neoadjuvant chemotherapy regimen suitable to be combined with new drugs. Sorafenib might affect the pharmacokinetic (PK) of anthracyclines.

Materials and Methods: Sofia is an open-label, multicentre, single-arm, phase II study. Inclusion criteria were: uni- or bilateral, HER2−, cT3, cT4 or cT2 cN+, M0 primary BC. Pts received 4× E 90 mg/m^2 and C 600 mg/m^2 i.v. q3w followed by 12 weeks Pw 80 mg/m^2 q1w. S 800 mg was given concomitantly with the chemotherapy with a 3 day drug holiday around EC. PK samples were taken during the first 2 EC cycles. After an amendment in 9/2008 S dose started with 200 mg and escalated up to 800 mg at every EC cycle depending on pts' individual toxicity. Primary objective was the rate of pCR at time of surgery; main secondary objectives were the assessment of safety, DFS, OS and clinical response.

Results: Between 11/2007 and 5/2009, 24 patients (pts) were recruited in 6 centres. 12 pts received EC-P starting with 800 mg S (cohort 1) and 12 pts EC-P with a dose escalation of S during EC (cohort 2). The whole population median age was 43 (range 28−67). 87.5% had a T2 tumour, 54.2% were N+. 54.2% with a G3, ductal invasive (78.3%), hormone receptor pos (58.3%) tumour. In cohort 1 the median daily dose was 400 mg and the cumulative dose 611,400 mg. The following grade 1−4 toxicities were reported: hand-foot syndrome (HFS) 11, other skin reactions 15, diarrhea 2, mucositis 6, hypertension 1; 12 pts started therapy and 4 pts discontinued early due to AEs; 2× due to progression, interrupted treatment (9). In cohort 2 the median daily S dose was 700 mg and the cumulative dose 892,400 mg. Toxicities grade 1−4: HFS 6, other skin reactions 7, diarrhea 2, mucositis 7. 12 pts started therapy. 1 pt discontinued early due to allergic reaction. No patients stopped due to skin toxicities. The maximum tolerable dose was 400 mg (N = 2), 600 mg (N = 4), 800 mg (N = 6).

Conclusion: This is the first study evaluating S in a neoadjuvant setting in pts with primary BC. The individual dose escalation of S offers is an excellent tool to reduce treatment discontinuation and HFS and will increase the cumulative dose S given by 46%. Two additional cohorts of EC-P and P-EC with S with a reduced dose escalation phase are ongoing. PK results will be presented at the meeting.

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Luminal-like oestrogen receptor-positive breast cancer: identification of prognostic biological subclasses

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Background: Gene expression studies have been able to classify breast cancer (BC) into a number of different classes. The luminal BC group is complicated by biological heterogeneity indicating the presence of subclasses. The biological characterisation of ER-positive luminal-like subtypes could have important implications in patient management.

Methods: Our study aimed at subclassifying ER-positive luminal-like cancers according to their gene and protein expression characteristics. Using gene microarray experiments in 128 frozen invasive BC, 47,293 gene transcripts were analysed using different bio-statistical models. In addition, we used immunohistochemistry and high throughput tissue microarray technology to study the protein expression of 16 biomarkers with strong relevance to ER including FOXA1, TFF1, CD71, CARM1, PELP1, RERG, TK1, TFF3, XBP1, BCL2, Cyclin B1, FOXO3a, P27, C-MYC, BEX1 and AGTR1 in a well characterised consecutive series of invasive BC (n = 1902). The data were analysed using artificial neural network and different clustering methods including Hybrid hierarchical, K-means and Partitioning Around Medoids. Kaplan Meier plots with Log-rank test (LR) were used to model clinical outcome.

Results: We identified a transcript signature for ER positive BC including RERG, GATA3, IGF1R, CA12, and others by a supervised classification analysis using 10-fold external cross-validation of the gene microarray data. Immunohistochemical validation study was done for RERG and confirmed its association with ER positive BC. GATA3 expression was validated using QPCR. Through a consensus between different clustering techniques applied over protein expression data, three biological clusters in ER positive breast cancer, with significant difference in patient outcome (LR = 8.084, $p = 0.018$), have been identified. Decision tree analysis of the protein expression identified a minimal protein signature of the identified clusters including TFF3 and P27. Importantly, the poor prognosis cluster was significantly characterised by a high MIB1 proliferation index ($p = 0.013$).

Conclusion: In conclusion, our results emphasise the biological and behavioural heterogeneity of ER-positive luminal BC. More importantly, we have identified a signature for ER-positive luminal-like BC and the existence of luminal subclasses that differ with respect to patient outcome.

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Is the breast-conserving treatment with radiotherapy appropriate in BRCA1/2 mutation carriers? Long term results and review of the literature

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Background: Because tumours in *BRCA1/2* mutation carriers might be more sensitive to radiation, we investigated after long term follow-up whether mutation status influenced the rate of ipsilateral and contralateral breast cancers after breast-conserving treatment (BCT).

Material and Methods: *BRCA1* and *BRCA2* genes were screened for germline mutations in 131 patients with a family history of breast and/or ovarian cancer who had undergone BCT and radiotherapy. Patients were matched to 261 controls with sporadic breast cancer according to age at diagnosis and year of treatment. Controls were followed up for at least as long as the interval between diagnosis and genetic screening in familial cases. Rates of ipsilateral and contralateral cancer between groups were compared by the log-rank test.

Results: *BRCA1/2* mutations occurred in 20.6% of tested patients. Tumours in mutation carriers were more likely to be grade III ($p < 10^{-4}$) and estrogen-receptor negative ($p = 0.005$) than in non-carriers and controls. Overall median follow-up was 161 months. There was no significant difference in ipsilateral tumours between mutation carriers, non-carriers and controls ($p = 0.13$). On multivariate analysis, age was the most significant predictor for ipsilateral recurrence ($p < 10^{-3}$). The rate of contralateral cancer was significantly higher in familial cases: 40.7% (mutation carriers), 20% (non-carriers), and 11% (controls) ($p < 10^{-4}$).

Conclusion: After 13.4 years of follow-up, the rate of ipsilateral tumours was no higher in mutation carriers than in non-carriers or controls. Because tumours in *BRCA1/2* mutation carriers might be more sensitive to radiation, BCT is a possible treatment option.