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