positive was defined as ER/PR positivity. Breast cancer were calssified into different molecular subtypes as follows: Luminal A (HR+/HER2−), Luminal B (HR+/HER2+), Triple-negative (HR−/HER2−) and HER2 positive (HR−/HER2+) subtype. 11 candidate molecular biomarkers including Tau, β-Tubulin III, PTEN, MAP4, Thioredoxin, MDR1, Ki67, p53, Bcl-2, BAX and ERCC1 were detected by IHC in pre-NCT core needle biopsy specimens and analyzed the relationship between these markers and pCR. Logistic Regression Models including routine clinical, pathological markers and candidate molecular markers in various combinations were built to compare different predictive accuracy of models for predicting pCR.

Results: 91 patients had available core needle biopsy specimens for evaluation, and 18 patients achieved pCR with pCR rate 19.8%. Univariate analysis showed that ER, PR, molecular classification (clinicopathological markers) and Tauβ-Tubulin III?p53?Bcl-2?ERCC1 (candidate molecular markers) were associated with pCR; Multivariable analyze revealed that β-Tubulin III, Bcl-2 and ERCC1 were independent pCR predictive markers, β-Tubulin III-negative, Bcl-2-negative or ERCC1-negative was associated with higher pCR rate, with OR 6.03 (95% CI, 1.44–25.24, $P = 0.014$), 7.54 (95% CI, 1.52–37.40, $P = 0.013$) and 4.09 (95% CI, 1.17–14.30, $P = 0.028$), respectively. To compare different Logistic Regression Models built with different combination of these variables, we found the model including routine clinical, pathological and β-Tubulin III, Bcl-2, ERCC1 these three candidate molecular markers had highest predictive power, area under ROC (Receiver Operating Characteristic, ROC) curve was 0.900 (95% CI, 0.831–0.968).

Conclusion: β-Tubulin III, Bcl-2 and ERCC1 were independent pCR predictive factors among breast cancer patients treated with weekly PCb regimen as NCT. Patients with β-Tubulin III-negative, Bcl-2-negative or ERCC1-negative tumors had a higher pCR rate. Model integrating routine clinical, pathological and β-Tubulin III, Bcl-2, ERCC1 candidate molecular markers had highest predictive power of predicting the pCR rate of this weekly PCb regimen.

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Association of the germline MDM2 SNP309 and TP53 R72P variants with breast cancer survival in specific tumour subgroups

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Germline and somatic mutations in genes such as those of the "DNA damage response pathways" are associated with development of specific tumor subtypes and influence breast cancer outcome. One of those pathways includes the tumor suppressor gene *TP53* and its regulator *MDM2*. Interaction between *TP53* germline mutation status and *MDM2* SNP309 carriership is known to accelerate tumor development. Moreover, somatic inactivation of the *TP53* gene in breast tumors is related to a poor prognosis. Here we inverstigated wether common germline polymorphisms in genes of the 'TP53 response pathway' might affect breast cancer outcome. In particular we evaluated their contribution to the prognosis of tumors that harbor specific additional somatic changes. We determined the effect of the germline *TP53* R72P and *MDM2* SNP309 polymorphisms on breast cancer survival in distinct tumor subgroups in a consecutive cohort of breast cancer patients (age at diagnosis <53 years, n = 295). Tumors were classified in subgroups according to *TP53* somatic mutation status (n = 209) (wild type (including silent mutations) versus missense and non-missense mutations) or the 70-gene prognostic (good versus poor prognosis) profile (n = 295). Analyses were performed using Cox regression models adjusting for clinico-pathological characteristics and treatment. A decreased breast cancer-specific survival was found for carriers of the germline *MDM2* SNP309 GG genotype compared to those carrying the common TT genotype, only within those patients having *TP53* mutated tumors (HR 3.35 (95% CI: 1.02–11.0) compared to *TP53* wild type tumors: HR 1.01 (0.31–3.29)). Additionally the same effect was seen within those patients with a poor prognosis profile tumors (HR 2.39 (1.21–4.73) compared to the good prognosis profile tumors: HR 0.55 (0.10–3.22)).

A similar trend was seen for carriers of the germline *TP53* R72P GC genotype versus those carrying the common GG genotype (*TP53* mutated tumors compared to *TP53* wildtype tumors: HR 2.01 (0.87–4.64) and HR 1.16 (0.53–2.54); poor compared to good prognosis profile tumors: HR 1.57 (0.98–2.51) and HR 0.33 (0.08–1.37)). These results support our hypothesis and are in line with the available biological evidence. Common polymorphisms in specific pathways in combination with somatic changes (in the same pathway) in the tumor may become of importance in predicting prognosis of breast cancer patients.

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Locoregional recurrence after breast conserving therapy remains an independent prognostic factor even after an event free interval of ten years in early stage breast cancer

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Background: Locoregional recurrence after breast-conserving therapy is a well-known independent risk factor associated with unfavourable long-term outcome. Controversy exists however of the prognostic impact of a locoregional recurrence after a very long event free interval.

Materials and Methods: Data were pooled from 4 EORTC Breast Group trials, which accrued patients with early stage breast cancer. Only locoregional recurrences as first event were taken into account. Univariate and multivariate Cox regression analyses were conducted using locoregional recurrence as a time-dependent variable in a landmark analysis to study the independent prognostic impact of locoregional recurrence on long-term outcome after different event-free time intervals. Long-term outcome was defined as distant disease-free survival and overall survival. Three different landmark analyses were undertaken. One analysis including all breast conserved patients, one analysis that included only patients with an event-free interval of at least 5 years, and one analysis including patients who were event-free for at least 10 years after primary diagnosis.

Results: In total, 7749 early stage breast cancer patients who underwent breast-conserving therapy were included. At time of the analysis, the median follow up was 10.9 years. In the multivariate analysis, including all patients, locoregional recurrence, tumour size, nodal status, young age, oestrogen receptor status and chemotherapy remained independent prognostic factors with a significant impact on long-term outcome. Locoregional recurrence was the strongest prognostic factor for overall survival (HR 5.07 95% CI 4.37–5.88, P < 0.01) and distant disease-free survival (HR 5.24, 95% CI 4.50–6.10, P < 0.01). In the multivariate analysis including patients that had an event-free interval of at least 5 years, locoregional recurrence remained the strongest independent prognostic factor for overall survival (HR 3.69, 95% CI 2.64–5.19, P < 0.01) and distant disease-free survival (HR 3.87, 95% CI 2.84–5.27, P < 0.01). In patient that were event free f more than over ten years after primary treatment, locoregional recurrence remained the only independent prognostic factor with a significant impact on both distant-disease free survival (HR 4.08, 95% CI 1.25–13.27, P 0.02) and overall survival (HR 8.38, 95% CI 2.54–27.63, P < 0.01).

Conclusions: Locoregional recurrence after breast-conserving therapy is a very strong time dependent independent factor for long term outcome even after a very long event-free interval in early stage breast cancer patients. These findings suggest that even after a long event free interval, locoregional recurrence seems to be associated with distant disease rather than a cause of subsequent distant disease.

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Comparison of Adjuvant! Online prediction with 10-year follow-up results according to the uPA and PAI-1 levels in Slovenian early breast cancer patients

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Background: Adjuvant! Online is very useful tool for prognosis assessment of early breast cancer (EBC) patients. It is very consistent for the low risk patient according to the classical clinicopathological criteria (eg. small tumors, node negative, grade 1−2), as it was presented in our previous work. uPA and PAI-1 are level 1 prognostic markers in EBC and when high they characterize poor prognosis.

Methods: 753 EBC patients diagnosed and treated in the Institute of Oncology from 1996–1999 and with 10-year follow-up were included into the study. The basic clinical-pathological characteristic were assessed for each patient and entered into the Adjuvant! Online (Version 8.0) to calculate estimated 10-year OS. uPA and PAI-1 levels were measured routinely using ELISAs (American Diagnostica Inc.; CT) in tumor tissue extracts

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