patients each divided into two classes. Notably, most of the genes expressed more highly in the CI-1040-resistant cell lines formed one cluster, while most of the genes expressed more highly in the sensitive cell lines formed another cluster ($p<<0.001$; Fisher Exact Test), a strong indicator that these genes are similarly co-expressed in both cell lines and tumor samples. Preliminary results indicate that roughly a third of patients show a pattern of gene-expression that is similar to the CI1040-sensitive cell lines.

Conclusion: The expression of genes identified to associate with CI1040 sensitivity *in vitro* show a similar pattern of co-expression in I-SPY patient samples. These data suggest that this CI1040-sensitivity predictor should be tested for its ability to predict the therapeutic response among patients receiving CI1040 (or other drugs in this class). The I-SPY 2 trial will include phase II targeted drugs for which predictor gene sets will be derived from our *in vitro* system and prospectively tested in patients receiving that treatment.

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Influence of EIF4E and HER2 Protein Expression on the Response to Neoadjuvant Chemotherapy and Trastuzumab in Breast Cancer.

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Background:
Resistance to trastuzumab (TTZ) is still poorly understood. Many proteins implicated in signaling pathways of breast cancer cells have so far been studied as potential markers of TTZ resistance in preclinical models.

Method:
63 consecutive patients were treated for HER2 + breast cancer with a neoadjuvant chemotherapy regimen containing TTZ between December 2003 and July 2008. This regimen was mostly 4 cycles of FEC 100 (5FU, Epirubicin and Cyclophosphamide) followed by 4 cycles of TTZ – Docetaxel 100mg/m².

Immunohistochemistry (IHC) was used to analyse, in the initial biopsy, the expression of HER family receptors (EGFR, HER3, HER4 and HER2), PTEN, cMyc and p27. We also analyzed the expression of two proteins (eIF4E and 4EBP1) involved in translational control of mRNAs. 4EBP1 is a small protein that represses the initiation of protein translation through its association with eIF4E, the mRNA cap-binding subunit of the eIF4F complex. mTOR-dependent phosphorylation of 4E-BP1 decreases the affinity of the protein for eIF4E, which facilitates the formation of eIF4F complex. Furthermore, the expression of eIF4E is elevated in many cancers.

We analysed the pathologic response rate (pRR) after chemotherapy + TTZ, and we established correlations between the expression of these markers and pRR.

Results:
Among the 63 patients, 54 had a valuable pre-chemotherapy biopsy.

For those 54 patients, median age was 48 years (range [21;80]), median tumor size was 50 mm clinically (range [20;120]), and 26 mm by ultrasound (range [10;80]). Respectively 63%, 57% and 52% were estrogen receptor (ER) negative, progesterone receptor (PR) negative and ER/PR negative. Histology type was ductal infiltrant carcinoma for 91% of patients. Respectively 28% and 72% were grade SBR (EE) II and III.

A pathologic complete response (pCR) (Sataloff TA) was observed on 63% of patients, 28% had a partial response but superior to 50% (Sataloff TB) and 9% had a poor or absent pathologic response (Sataloff TC and TD).

This pCR was not statistically linked to any initial clinical characteristics (age, grade, hormonal status and tumoral size).

pCR was strongly correlated with weak eIf4E and with percentage of HER2 strong-stained cells (respectively p=0,0114 et 0,0056). 93% of tumors with an eIF4E IRS <7 (n=13/14) had a pCR, and only 5% (1/19) with an incomplete pRR had an eIF4E IRS <7. Moreover, 78% (25/32) of tumors with 100% of tumoral cells harboring a strong intensity of HER2 had a pCR versus 41% (9/22) of tumors with less than 100% of cells expressing HER2 with a strong staining.

Conclusion:
Our study shows a strong correlation between eIF4E expression or HER2 expression and pRR in patients with HER2 + breast treated with a neoadjuvant TTZ-containing regimen. IHC of eIF4E might be a powerful test to predict sensitivity to TTZ.

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Chemosensitivity Testing of Circulating Epithelial Cells (CETC) in Breast Cancer Patients and Correlation to Clinical Outcome.

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In spite of ample prognostic markers available in breast cancer, still a considerable proportion of patients with good prognostic markers suffers relapse whereas patients with poor prognostic markers may remain disease free. It would, therefore, be desirable to control, at the individual patient level, whether the applied therapy is effective. Our previous work indicates, that in cancer patients most of the epithelial cells circulating in peripheral blood (CETC) are part of the tumor and that the response of these cells reflects the response of the tumor to the applied therapies.

Therefore, these cells were used to assay chemosensitivity in short time cultures analyzing the percent of cell killing during short time incubation and monitoring the decrease or increase in numbers of these cells during treatment with the respective agents providing a unique tool for therapy surveillance. Patients were prospectively analysed for the number of CETC before each new combination of chemotherapy. 1ml of blood was drawn into EDTA vials, red blood cells lysed and the white blood cell pellet stained with FITC-labelled anti-Epcam. Green fluorescent cells were detected by image analysis and dead cells excluded due to red PI fluorescence. Activity of individual compounds was determined using three different concentrations of each compound at 3h, 6h and 12hs and displayed as % cell killing. The in vitro results were then compared to in vivo reduction of CETCs and to the reduction of a marker lesion.

215 patients have been investigated so far. Cell killing was dose and time dependent. The highest killing rates were observed with Epirubicin and Taxanes, agents which are known for their high activity in breast cancer. Less than 20% killing activity was termed marginal activity. In vitro sensitivitity was highly significantly predictive of in vivo CETC reduction. In some cases an increasing resistance could be shown to develop during repeated cycles of the same combination therapy. CETC reduction was correlated with a prolonged progression free survival.

Thus this approach can in the future be used to test in advance the sensitivity of the circulating tumor cells to chemotherapy and at the same time monito the current response of the cells to therapy in vivo in order to optimize an individualize therapy.

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Biomarkers ER, PR, HER-2 En Topoisomerase II alpha i Correlation with Response to Neoadjuvant Chemotherapy fo Primary Breast Cancer.

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Background: Neoadjuvant chemotherapy (CT) is widely accepted for patien with primary breast cancer (BC) not eligible for breast conservative surger (BCS). Docetaxel, when used in combination with or when sequentiall added to an anthracycline-based regimen, is active in the adjuvant setting. Th objectives of this trial were to assess the activity of sequential anthracyclin docetaxel chemotherapy in the neo-adjuvant setting and to evaluate thes markers as predictors of response.

Patients and methods: Patients with unilateral BC > 2 cm (with or witho positive sentinel lymph node (LN)), adequate liver, kidney and bone marro function and no evidence of distant metastasis were eligible for enrolmer Patients with stage T4d or inflammatory BC were excluded. Treatme prior to surgery consisted of 4 cycles of Adriamycin (60 mg/m²) pl cyclophosphamide (600 mg/m²) (AC) administered intravenously every weeks (q3wk), followed by 4 cycles of docetaxel (D) (100mg/m²) q3w Patients were clinically evaluated after 4 cycles of AC and again after 4 cycl of D. Pathological evaluation was performed after definitive surgery. Hormon receptor, HER2 and topoisomerase II alpha (topoII) status and tumor gra were evaluated for their ability to predict response to treatment.

Results: Fifty-three patients with a mean age of 49 years (range 31-69), we included in this analysis. Mean largest tumor diameter before treatment w 49.1 mm (range 25-90 mm). There were 12 grade 2 and 30 grade 3 tumou representing 46 ductal carcinomas, 5 lobular carcinomas and 2 mixed tumo Eleven tumors (21%) were not evaluable (NE). LNs were positive in 62% patients. Forty patients were evaluable for HER-2 and topoII. Thirteen patie (33%) were HER2-positive by immunohistochemistry (IHC) and 11 of the