algorithm using Her-2-FITC fluorescence of leukocytes to determine the Her-2-expression threshold in each sample.

Results: Her-2 expression of CTC varied greatly within and between patients compared to Her-2 expression of leukocytes. In M1 patients, a threshold of 75% of Her-2 positive CTC in patients with $\geqslant$5 CTC showed a relatively low discrepancy rate between the primary tumor and CTC Her-2 status. Applying this threshold, 9% of M1 patients with Her-2 negative primary tumors had Her-2 positive CTC status and 29% of M1 patients with Her-2 positive primary tumors had Her-2 negative CTC status. No Her-2 discrepancy was observed between CTC and primary tumor in M0 patients.

Conclusions: Our findings demonstrate the feasibility of real-time quantitative and reproducible assessment of treatment targets on CTC, opening a path towards personalized treatment. Her-2 expression is heterogeneous among CTC within each patient. Overall, M1 patients with Her-2 positive primary tumors exhibited Her-2 negative CTC frequently, whereas discrepancies in Her-2 status were limited in other clinical settings.

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Effects of MTor and Insulin Receptor Inhibition in Tamoxifen Resistant Breast Cancer Cells

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Background: PI3KCA-mutations occur frequently in hormone-receptor positive breast carcinomas and may be targeted using mTOR inhibition. In the present study, we aimed to analyse the effects of mTOR inhibition as well as possible interactions with insulin receptor signalling in Tamoxifen-resistant breast cancer cells.

Material and Methods: MCF7 breast cancer cells, harbouring an activating PIK3Ca mutation (Exon 9 1633G>A), and tamoxifen-resistant MCF7 cells (T-MCF7) were treated with the allosteric mTOR complex 1 (mTORC1) inhibitor Everolimus and the active-site mTORC1/mTORC2 kinase inhibitor PP242. In this setting, the effects of insulin receptor signalling on cell growth, motility and viability were investigated by stimulation with insulin or IGF1 and in the presence of siRNA inhibition of the insulin receptor (IR) and insulin like growth factor 1 receptor (IGF-1R).

Results: T-MCF7 showed elevated level of IR/IGFR expression as well as an activated (phosphorylated) ERK1/2 in contrast to the untreated MCF7. The addition of insulin resulted in an increased signal transduction via AKT and ERK1/2. Simultaneous inhibition of mTORC1/2 through PP242 abolished AKT-phosphorylation and led to a complete cell cycle arrest in G0/G1 as well as a substantial decrease of cell viability in MCF7 and T-MCF7. However, mTORC1-inhibition alone using Everolimus resulted only in a partial G0/G1-arrest which could be reversed by addition of insulin. siRNA inhibition of IR demonstrated an effective reduction of MAPK-signalling in both MCF7 and T-MCF7 while siRNAs against IR or IGF1R resulted in an additional decrease of cell viability.

Conclusions: Inhibition of mTOR-signalling reduced cell viability and proliferation in PIK3CA-mutated breast cancer cells independent of an acquired Tamoxifen resistance. However, our data indicate that IR and IGF1R-conferred cell growth may reduce the effects of isolated mTOR inhibition in tamoxifen-resistant breast cancer cells and that additional targeting of the insulin receptor pathway may prove useful in this setting.

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Study of the Effect of Concurrent Use of Letrozole with Radiotherapy to the Cell Death Mechanisms in the Breast Cancer Cell Line

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Purpose: Studies have shown that hormone receptor-positive tumors have molecular, biological and clinical differences. Using hormonal treatment prolongs survival for the majority of hormone receptor positive breast cancer patients. Postoperative radiotherapy (RT) decreases the risk of locoregional recurrence. Studies show that concurrent use of tamoxifen sensitizes cells to RT and increases RT induced pulmonary fibrosis. Whether letrozole sensitizes breast cancer cells to RT has not been determined with sufficient number of studies. There is a single experimental study of breast cancer cells that revealed increased radiation sensitivity with letrozole. The purpose of this study is to investigate the effect of the concurrent use of letrozole with RT, on cell death in the breast cancer cell lines MCF7, and MCF7aro.

Materials and Methods: In our study, in vitro cell culture methods were used. Aromatase expressing MCF7aro breast cancer cell line was chosen as a model and aromatase non-expressing MCF-7cells were used as control. Letrozole was used with varying doses of 100, 500, 1000 nM, and cells were exposed to letrozole for 24–72–144 hours. Irradiation was performed using a Co-60 source with doses 2−4 Gy. Cell death determination experiments were held 24 hours after RT. Cell death was evaluated by measuring caspase-3 activation in cell-lysates and by cell surface annexin V/propidium iodine (PI) staining detected by flow-cytometry. Beclin expression levels known to elevate in autophagy was determined by western blot, The experiments were done in triplicates.

Results: We evaluated caspase-3 and annexin-V/PI results after 24−72–144 h of incubation with letrozole. There was no significant difference for early apoptosis, late apoptosis and necrosis between the letrozole treated and untreated MCF7 cells. In the aromatase expressing MCF7aro cells, we observed that there was a general reduction in cell death in cells treated with letrozole; with a trend towards apoptosis as a cell death modality rather than necrosis.

Also we observed increased autophagy in the MCF7 cells incubated with letrozole only for 24 h and have received 4 Gy irradiation. There were no differences in Beclin levels when these cells received 2−4 Gy irradiation, and were incubated for 72–144 h. On the other hand MCF-7Aro cells which received 2−4 Gy irradiation and were incubated with letrozole showed increased autophagy in all experimental groups.

Conclusion: In conclusion we observed a general reduction of cell death, in hormone-sensitive, receptor-positive and aromatase enzyme expressing cells, after concurrent use of letrozole and radiotherapy; with apoptosis being the primary cell death modality. This observation also correlates with our findings that autophagy which is primarily a survival mechanism may have also increased in these cells. More extensive studies are needed to be able to evaluate the effects of the concurrent use of letrozole and radiotherapy on tumor cell death.

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Expression of Cancer-testis Antigens in Breast Cancer

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Background: Cancer testis (CT)-antigens predominantly expressed in human germ cell lines, but not in somatic tissues, become activated in different cancer types. Several CT-antigens have been shown as possible prognostic marker and therapeutic target for cancer immunotherapy, although the biological functions in cancer are largely unknown. In this study, we investigated the expression of CT-antigens in breast cancer phenotypes to develop strategy of CT-antigen targeting immunotherapy.

Materials and Methods: Expressions of CT-antigens (i.e. NY-ESO-1, MAGE-A, and MAGE-C1) were characterized by immunohistochemistry (IHC) in 100 patients with primary invasive breast carcinoma. Aldehyde dehydrogenase (ALDH)-1 expression, which have been reported as predictive marker of cancer stem cells in terms of resistance to chemotherapy, were also examined. The IHC findings were statistically analyzed with clinical profiles and prognosis of the patients.

Results: NY-ESO-1, MAGE-A, and MAGE-C1 antigens were expressed in 6%, 15%, and 12% of tumor specimens, respectively. NY-ESO-1 and MAGE-A were preferentially expressed in triple negative ($p < 0.01$) or ER negative breast cancers ($p < 0.05$). ALDH-1 expression was observed in 22% of tumor specimens, and was most prevalent in the triple negative breast cancers ($p < 0.001$). Moreover, 41% of ALDH-1 positive specimens were accompanied with expression of any of CT-antigens, some of which showed concomitant expression of CT-antigens and ALDH-1. There was no significant association between the CT-antigen expressions and clinical prognosis (e.g. OS and RFS) possibly due to small sample size in this study.

Conclusion: CT-antigens were expressed in a large proportion of triple negative- and ALDH-1 positive breast cancer specimens. Because of the limited therapeutic modalities for these phenotypes, significance of CT-antigen expressions should be further studied for beneficial immunotherapy in breast cancer patients.

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Differences in MicroRNA Expression Pattern Predetermine Receptor Phenotypes of Breast Cancer Cells

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Background: Tumor growth is tightly associated with regular shifts in microRNA (miRNA) expression pattern. More than 50% of miRNA genes are located in fragile chromosomal regions that are susceptible to

amplification, translocation and other damages during the carcinogenesis. Usually, expression of miRNAs miR-16, miR-122, miR-125a/b, miR-31, miR-143, miR-145 and miR-205 is down-regulated in breast cancer cells whereas expression of miRNAs miR-20b, miR-21, miR-155, miR-19, miR-148, miR-210 and miR-221 is up-regulated. This investigation aims to identify in what way these shifts in miRNA expression pattern contribute to the differences in receptor phenotype of breast cancer cells.

Material and Methods: miRNA targets within gene transcripts were predicted *in silico* using TargetScan software.

Results: miRNAs miR-122, miR-145, miR-148, miR-181, miR-204, miR-18a/b, miR-19, miR-22, miR-221/222 can target transcript of gene ESR1 encoding estrogen receptor alpha (ER). Transcript of gene *PGR* encoding progesterone receptor (PR) carries miR-23a/b, miR-26a/b, miR-135 and miR-181 binding sites. miRNAs miR-125a/b, miR-193, miR-331/331−3p can suppress gene encoding ErbB2 receptor. miR-19, miR-143, miR-145, miR-148, miR-205 silence gene encoding ErbB3 receptor. Transcript of gene encoding ErbB4 receptor carries miR-19, miR-23a/b, miR-125a/b, miR-135, miR-145, miR-193a/b, miR-205, miR-221/222 binding sites. Disregulation of above miRNAs is essential for tumor growth, because downregulated miRNAs silence expression genes of proliferative components E2F1, E2F2, E2F3, RASA1, RASA2, CDK6 as well as gene of main antiapoptotic factor Bcl-2. At the same time, up-regulated miRNAs silence genes encoding cell cycle inhibitors p27 and p57, TGF-β receptor, tumor suppressor pTEN, proapoptotic factors Bak1, FAS as well as genes encoding key elements of cell differentiation network.

Conclusions: Transcripts of genes encoding ER, PR as well as ErbB2, ErbB3 and ErbB4 receptors carry the binding sites for both downregulated and upregulated miRNAs. Therefore, expression or silencing of these genes is a result of balance between shifts in miRNA expression, which are individual for each case of tumor transformation. Different sets of shifts in miRNA expression pattern can cause reactivation of cell oncogenes and antiapoptotic genes as well as repression of proapoptotic, antimetastatic genes and genes of cell cycle inhibitors. Consequently, receptor phenotypes of breast cancer cells can mirror distinct modes of abnormal epigenetic activation of the signal pathways that leads to the tumor growth.

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Overexpression of PGRMC1 ñ a Potential Mechanism for Increased Breast Cancer Risk During Combined Treatment with Estrogen and Norethisterone

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Background: Epidemiological studies suggest that the addition of progestin to estrogen therapy negatively influences breast cancer development. In a recent study from Finland increased breast cancer risk has been observed especially under a combined estrogen (E2)/norethisterone (NET) treatment. Up to now no increased proliferation rate has been detected *in vitro* under this combination. The present study investigates the effects of an E2/NET combination on the proliferation of MCF-7 breast cancer cells overexpressing the progesterone receptor membrane component 1 (PGRMC1). In addition this combination was tested in a mouse transplantation model.

Materials and Methods: MCF-7 cells were stably transfected with a PGRMC1 expression plasmid (MCF-7/PGRMC1−3HA). E2 in concentrations of 10^{-12} and 10^{-10} M was combined with 10^{-7} M NET in a sequential regimen. Proliferation was determined by MTT assay. In transplantation experiments nude mice were injected with 17-beta-E2 pellets (0.72 mg/60 day release) on both flanks 24 h before inoculation of tumor cells. Tumor cells were injected subcutaneously into each flank. After approx. 8 days animals were injected with NET pellets (10 mg/60 day release) or with control pellets. Tumor volumes were recorded twice per week.

Results: Sequential combination of E2 and NET increases the proliferation rate of PGRMC1 overexpressing MCF-7 cells by a factor of 2−3. When the concentration of E2 is reduced the proliferation rate decreases by approx. 50%. Tumors growing from MCF7/PGRMC1 cells in the recipient nude mice are more sensitive to E2. Developed tumors are approx. 4-fold larger compared to transplanted MCF-7 cells, transfected with an empty vector control. NET almost doubles the tumor volume of the MCF-7/PGRMC1 cells compared to animals treated with control pellets.

Conclusions: For the first time we demonstrate in cell experiments and in an animal model that by combining E2 and NET the proliferation of breast cancer cells overexpressing PGRMC1 is increased. In a next step breast tissue or epithelial cells from nipple aspirates from women should be investigated for increased expression of PGRMC1 and women's risk to develop breast cancer after treatment with E2 and NET should be evaluated.

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Cytochrome P450 2D6 Gene Copy Number as an Indicator for Tamoxifen Resistance in Breast Cancer

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Background: Tamoxifen is one of the most effective adjuvant breast cancer therapies available worldwide.

The rate of metabolism of this medicine is mainly determined by the amount of cytochrome p450 2D6 **(CYP2D6)** enzyme expressed in the liver, which is highly variable due to its extensive genetic polymorphisms and copy number variation. So *copy number variation* maybe one of the most important mechanisms of resistance to Tamoxifen. Since there is limited information about CYP2D6 in resistant patients, we aimed to determine copy number of this gene in Tamoxifen resistant Iranian breast cancer patients.

Materials and Methods: *Samples*: The samples of two sensitive and resistant groups were obtained from Iranian Center for Breast cancer Bio-Bank (ICBC-BB). DNA extraction was done using phenol chloroform method and the extracted DNA concentration was quantified using spectrophotometery.

Copy number analysis: Establishment of standard curves for copy number determination was done by cloning of CYP2D6 fragment as the gene of interest and albumin gene as a copy number control in TA cloning vector. PCR Primers were designed using primer express V.3.0 software. Real-time PCR was performed using the ABI 7500 system apparatus. Amplification reactions (20 ul) were carried out in triplicate with 40 ng of template DNA, SYBR Green Master Mix buffer (PrimerDesign Ltd, UK) and 300 nM of each primer. Each sample was run triplicate with 4 fold serial dilutions in the same plate. Samples with standard deviation greater than 0.5 from the mean threshold cycle of the triplicates were excluded from the analysis. Copy number calculation was done using applied biosystems SDS software ver2.0.

Result: Primer efficiency for both CYP2D6 and Albumin was about 100%. The copy number range was 0.4 to 3 and no significant difference was seen between resistance and sensitive groups in this phase of the study.

Conclusion: Although no significant difference was detected between two groups in this phase of the study, it does not mean that copy number variation play no role in resistant group in the samples. Further analysis including genotyping and multivariate analysis considering other factors for tamoxifen resistance and also increasing sample size must be done in order to decide about CYP2D6 status in tamoxifen response in Iranian samples.

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Changes in Estrogen Receptor (ER), Progesterone Receptor (PgR) and HER2/neu Status During Primary Systemic Chemotherapy in Breast Cancer

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Background: The influence of primary systemic chemotherapy (PST) in breast cancer on expression of biological marker is still unclear. In this study, we investigated whether there were any differences in estrogen receptor (ER), progesterone receptor (PgR) and HER2/neu status between core needle biopsies before PST and surgical specimens after PST.

Patients and Methods: One hundred patients with invasive ductal carcinoma who have received PST (PST group) at our hospital were included in this study. The immunohistochemical (IHC) analysis was performed on both core needle biopsies before PST and surgical resection specimens after PST. We also evaluated the expression of ER, PgR and HER2/neu using core needle biopsies and surgical specimens in sixty nine patients without PST (control group).

Results: ER discordance was 4.0% in the PST group, and 2.9% in the control group. PgR discordance was 10% in the PST group, and 7.2% in the control group. HER2/neu discordance was 9% in the PST group, and 2.9% in the control group. HER2/neu expression changed to positive in three cases, and changed to negative in six cases after PST. There were no significant differences in the change of biological marker pattern between the PST and the control group. Discordance of HER2/neu expression tended to be high in the PST group, compared to the control group.

Conclusion: Discordance of biological markers was observed before and after PST. To select optimum adjuvant therapy, HER2/neu status as