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