

## **New possibilities for combined therapy of breast cancer**

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In spite of the wide spectrum of available tumour treatment modalities, cancer therapy is still a major clinical challenge and none of the therapies can be considered fully effective when applied alone. In our studies, trastuzumab and photodynamic therapy, both used clinically for tumour therapy, were combined with novel small molecule inhibitors to test the efficacy of their combination in the treatment of breast cancers.

Trastuzumab (TR) is a humanized antibody against ErbB2 used in the therapy of ErbB2 positive breast cancer. To investigate the ability of HSP90 inhibitor 17-AAG to downregulate ErbB2 and the possibility of 17-AAG and TR potentiating each other's effect, the recently established trastuzumab resistant breast cancer cell line, JIMT-1 was compared to the known trastuzumab sensitive SKBR-3 line. Baseline and stimulus-evoked dimerization and activation levels of ErbB2, and the effects of trastuzumab and 17-AAG alone and in combination on cell proliferation and apoptosis, as well as on ErbB2 expression and phosphorylation have been measured. Baseline activation and amenability to activation and downregulation by trastuzumab was much lower in the resistant line. However, 17-AAG enhanced ErbB2 homodimerization after 5-10 minutes of treatment in both cell lines, and decreased their proliferation with an  $IC_{50}$  of 70 nM for SKBR-3 and 10 nM for JIMT-1. Thus, 17-AAG may be a useful drug in trastuzumab resistant ErbB2 overexpressing tumors. The antiproliferative effect of 17-AAG was positively correlated with phosphorylation and downregulation of ErbB2 and was dominated by apoptosis, although, especially at higher doses, necrosis was also present. Interestingly,  $IC_{50}$  values for ErbB2 downregulation and phosphorylation, in the 30-40 nM range, were not significantly different for the two cell lines. This observation and the negative correlation between resting ErbB2 levels and the antiproliferative effect of 17-AAG may indicate that activation of ErbB2 to some extent could counteract the overall cytostatic effect, especially at higher levels of ErbB2 expression. The usual therapeutic dose of trastuzumab did not change the  $IC_{50}$  of 17-AAG on the proliferation of either cell line, but nevertheless decreased overall ErbB2 phosphorylation and at low doses of 17-AAG further decreased cell growth in the sensitive SKBR-3, thus trastuzumab may be a good combination partner to counteract undesired activating effects of 17-AAG.

Photodynamic therapy (PDT) and inhibition of cathepsin B proteases by cystatin (CPI) are potential new tumour treatment modalities. We have investigated the efficacy of PDT and CPI alone and in combination on a solid mammary carcinoma transplanted in Wistar rats. Intraperitoneally injected single doses of chlorine e6 or HpD as photosensitizers were excited at 630 nm (90 J/cm<sup>2</sup>). CPI (500 µg/animal) was injected around the tumour daily during the 8-day treatment. Inoculation of tumour was either on day 1 of the protocol, or 8 days before the protocol. On day 8, tumour size was measured, tumour necrosis and vascularization determined based on HE stained sections and serum VEGF levels measured using an ELISA kit. No differences (2-way ANOVA) were found for treatments started at various lags. At doses where CPI or PDT alone had no or negligible effect, their combination caused a marked ( $p < 0.001$ ) decrease of serum VEGF, paralleled by a significant decrease of tumour size and number of capillary vessels, and a significant increase of necrosis up to 80% of the tumour tissue. Thus, the combination of PDT and CPI could be a useful approach in tumour therapy as the two agents appear to be synergistic and probably decrease VEGF production by the tumour tissue.