The most common reasons for not receiving Trastuzumab were node negative small tumours considered to be at low risk of recurrence and the patients' age. Recent studies have demonstrated that being HER2+ is a significant risk factor for relapse in patients previously perceived to be at low risk i.e. small node negative grade 1 or 2 tumours and no HER2+ patient should now be considered low risk [1]. Further trials are required to evaluate whether elderly HER2+ patients who are only eligible for Trastuzumab after adjuvant chemotherapy may derive benefit from Trastuzumab alone or in combination with endocrine therapy.

Table 1: Reasons for not receiving Trastuzumab (n = 148)

Reasons	Number of patients (%)
Tumour size <10 mm, node negative	53 (35.8)
Small node negative tumours (size 11−20 mm)	16 (10.8)
Age	27 (18.2)
Comorbidities	20 (13.5)
Patients refused therapy	19 (12.8)
Miscellaneous reasons	13 (8.8)

References

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95 Poster

Transfection of the gene e and later application of cytotoxic drugs in the treatment of breast multicellular tumour solid cancer

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Background: The low efficiency of conventional therapies in achieving long-term survival of breast cancer patients calls for development of novel options. The potential use of combined gene therapy is under intensive study. One approach uses the expression of genes encoding cytotoxic proteins that affect cellular viability. The *E* gene from >X174 encodes for a membrane protein with a toxic domain which leads to a decrease in the rate of tumour cell growth. To improve the antitumoral effect of the doxorrubicin in breast cancer cell, we investigated a combined suicide gene therapy using this drug and *E* gene *in vitro*, using MCF-7 breast cancer multicellular tumour spheroids (MTS).

Materials and Methods: We cloned the gene E >X174 genome and tested the possibility of using it as an anticancer reagent in multicellular tumour spheroid of breast cancer (MTS). We investigated a suicide gene therapy using gene E in vitro using MCF-7 breast cancer cells forming MTS. In order to determine the effect of the combined therapy (gene therapy and citotoxics) transfected MCF-7 MTS were treated with gradient concentrations of the drug diluted in the culture medium: paclitaxel, docetaxel and doxorubicin. We studied the action mechanism of the combined therapy: study of apoptosis and cellular cycle, and the modulation of the volumes of the MTS of tumour cells.

Results: Our results showed that the use of doxorrubicin in MCF-7 breast cancer MTS transfected with E gene enhanced the chemotherapeutic effect of this drug. This inhibition was greater than that obtained using the gene therapy or chemotherapy alone.

Conclusions: The transfection of gene E in MCF-7 MTS is able to increase the chemotherapeutic effect of drugs and specially is able to enhance the anticancer effect of the doxorubicin in comparison to the growth inhibition obtained using the gene therapy or chemotherapy alone. These results indicate that this combined therapy may be of potential therapeutic value in breast cancer.

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Hsp90 inhibition with 17AAG can sensitize human breast cancer cells to taxol

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Background: Taxol (paclitaxel) is a potent cytotoxic and cytostatic drug which is used against breast cancer. Unfortunately, some drug-treated tumor cells survive being selected for adaptive mutations or involving alternative pathways that confer drug resistance. Because multiple heat shock protein 90 (Hsp90)-dependent pathways ensure tumor cell progression and survival, Hsp90 inhibitors, such as 17-N-allilamino-17-demethoxygeldanamycin (17AAG), may be synergistic with other anticancer drugs. Here, we examined effects of combining of 17AAG and taxol on human breast cancer cells.

Materials and Methods: Paclitaxel conjugated to a fluorescent label, rhodamine-123, and 17AAG were used as single agents or in combination (each at nanomolar concentrations) in the experiments with cultured breast cancer cells (MCF-7 line). The drug-induced cytotoxicity was assessed in TUNEL, annexin V-staining, and clonogenic or MTT-assays. The Akt and Raf-1 levels were analyzed by immunoblotting. The pumping-drug-out function of P-glycoprotein was evaluated on duration of retaining of paclitaxel-rhodamine-123 inside the drug-treated tumor cells which are gradually liberated from the label.

Results: Longer intracellular retaining of paclitaxel-rhodamine-123 nicely correlated with the increased percentage of apoptosis in MCF-7 cells treated with both the drugs. The same tendency was also found in clonogenicity and MTT-assay. Such enhanced cytotoxicity seems to be partly associated with the 17AAG-induced inhibition of the Hsp90-dependent pumping-drug-out function of membrane P-glycoprotein. As generally accepted biomarkers of the Hsp90 inhibition, we revealed the specific depletion of Akt and Raf-1 in the 17AAG-treated cells. In addition to the P-glycoprotein dysfunction, the down-regulation of Akt and/or Raf-1 can also contribute to the intensification of apoptosis conferred by the drug combination.

Conclusion: The synergism in cytotoxicity under combining of paclitaxel and 17AAG appears to be due to (i) overcoming the P-glycoprotein-mediated multidrug resistance and (ii) promoting the apoptotic scenario in the drug-treated tumor cells, while both these causes are a result of the functional inhibition of Hsp90. Co-administration of 17AAG and taxanes may therefore be effective in combinatorial schemes of chemotherapy of breast cancer.

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Novel gold speckled silica nanoparticle (GSS) as madiators of tumour imaging and photothermal ablation (University of Florida and Kurume University, Cancer Nanotechnology Study Group)

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Background: Our group has recently developed Gold Speckled Silica nanoparticles (GSS) as multimodal contrast agents for fluorescence, magnetic resonance and photoacoustic tomographic (PAT) imaging. The near infra red (NIR) optical absorption property of these particles makes them potentially useful for therapeutic applications such as for thermal ablation of tumors

Hypothesis: GSS nanoparticles are biocompatible and can be used for *in vivo* photothermal ablation.

Methods: Fluorescent (Fluorescein isothiocyanate doped)-GSS nanoparticles were synthesized using the water-in-oil microemulsion method. The average particle size was determined to be ~100 nm by Dynamic light scattering and Transmission Electron Microscopy. For *in vitro* experiments, human breast cancer cells (BT474) were incubated with pegylated and Fluorescent-GSS nanoparticles (PF-GSS). The tumor cell uptake was assessed using flow cytometry and fluorescence microscopy experiments. After GSS exposure, cell viability was assessed by propidium iodide exclusion. For *in vivo* experiments, BT474 cells were implanted subcutaneously in nu/nu mice and tumors were allowed to grow to 0.5 cm. PF-GSS (30 μL, 10g/mL) or saline (30 μL as control) were injected intratumorally in animal models. In vivo imaging was performed using PAT and ablation was achieved by exposure to near infrared red (NIR) laser (500mW, 10min). Tumor ablation was determined by histologic analysis with hematoxylin/eosin to assess the distribution and extent of tumor ablation, 24 hours following photothermal ablation.

Results: (i) In *in vitro* experiments dose related uptake of PF-GSS by BT474 cells (20μl, 25.4±7.4%; 40μl, 46.4±6.6%, p = 0.001) was observed. PF-GSS were non-toxic to cells in culture as evidenced by propidium iodide assays. (ii) In vitro, GSS (10 mg/mL) increased the temperature by nearly 15ºC, when exposed to NIR laser (785nm, 350mA) x 300 seconds vs. only 1ºC for plain water. (iii) Following intratumoral injection of PF-GSS, particles could be clearly imaged with PAT. (iv) Histological analysis showed significant photothermal tumor ablation in treated tumors after illumination with NIR light that was not seen in control treated tumors.

Conclusions: GSS are novel, biocompatible nanoparticles, which are able to generate heat in response to NIR laser stimulation. In addition, these nanoparticles can be imaged by multiple imaging tools such as fluorescence and PAT. Our experiments demonstrate that GSS can be used as mediators of image guided non-invasive cancer therapy by photothermal ablation.