ER-receptors, PgR receptor and several endocrine parameters in a prospective, randomised, double-blind, placebo-controlled, neo-adjuvant study in 15 pre- and 14 postmenopausal women with estrogen-receptor positive early breast cancer.

Results: Estetrol induced a significant increase of SHBG, a significant decrease of FSH in postmenopausal women and no increase of gonadotrophins in premenopausal women. Estetrol had no effect on Ki67 expression and on apoptosis-related Bax and Bcl-2, but the apoptosis index in tumor tissue increased significantly. Systemic IGF-1 levels decreased significantly. Surprisingly the intratumoral epithelial ER-alpha expression decreased significantly, whereas the ER-beta expression showed a trend to increase.

Conclusion: This data show that E4 has estrogenic endocrine effects. The data support the hypothesis that E4, may be suitable and safe for HRT in women with spontaneous or induced menopausal symptoms, since apoptosis increases, IGF-1 decreases and no unfavourable effects are observed on Ki67, Bax and Bcl-2. The decrease of ER-alpha and the increase of ER-beta suggest a mechanism of action, explaining why the natural fetal estrogen E4 has estrogen-antagonistic effects on breast cancer tissue.

298 Poster

Investigating the Effect of Extremely Low Frequency Electromagnetic Field On Recombinant Monoclonal Antibody Overall Expression in *E. coli*

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Background: In recent years, recombinant monoclonal antibodies and their derivatives have emerged as important targeted therapy agents and as the fastest growing group within pharmaceutical industry research. Despite benefits of these therapeutic agents, the cost of treatment is drastically high and many patients could not afford their prescriptions. The majority of therapeutic monoclonal antibodies are produced in mammalian cells such as Chinese hamster ovary (CHO). This is while the low yield in expression of active protein, high media costs, the complexity of mammalian production system, costly viral inactivation validation steps, and extremely long production time of mammalian cells increase imbursements. In this regard, we decided to use an alternative method in combination with classic antibody reproduction. Recently, Extremely Low Frequency Electromagnetic Fields (ELF-EMF), which has been known as a potential mutagen agent and in some cases a carcinogen agent, used as manipulating agent in cellular metabolism and signaling. Cooperation of an ELF-EMF generator with an unconventional bioreactor, results in yield improvement in expression of a recombinant protein cloned in *E. coli.* Therefore, we designed an observation in order to investigate the effect of ELF-EMF on overall expression of a recombinant monoclonal antibody in *E. coli.* expressing the protein under exposure of ELF-EMF.

Material and Method: A Helmholtz coil has been used in order to generate 50 Hz. electromagnetic field during 12 hr with the power of 10 to 100 mT. cDNA of monoclonal antibody cloned to the Origami and expression level measured by densitometry. Recombinant cells divided into two groups of test and control. Test group exposed to the field during the expression stage after induction and control was isolated from exposure. Also dried weight of cell plates measured in order to compare proliferation in same time.

Results: As it has been shown in previous studies, recombinant gp41 expression level in *E. coli* increased about 20 percent after exposing to the ELF. Therefore we propose that the expression level of recombinant monoclonal antibody would be increased in this system significantly.

Discussion: expression of recombinant monoclonal antibodies in bacterial host such as *E. coli* and also exposing the host cells during the expression under ELF-EMF eligibly reduces the extraordinary costs of mammalian cells. Also, because of proof reading enhancement effect of ELF-EMF, post translational properties of expressed protein, such as correct folding and bond formations, might become more reliable than mammalian expression system such as CHO cell. At last enhancing vitality of host cells and what mentioned before makes our new method as an economic procedure in order to produce anti cancer monoclonal antibodies with affordable cost for patients.

Thursday, 22 March 2012 12:30–13:30

POSTER SESSION

Predictive and Prognostic Factors

299 Poster discussion

Biomarker Discovery and Evaluation of Response to Anti-cancer Therapeutics in Breast Cancer Using a Novel Nanofluidic Immunoassay

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Our research efforts focus on the identification and detection of fundamental molecular differences between normal and tumor cells in breast, as well as differences among distinct breast cancer subtypes, especially in terms of signal transduction pathways that control cell cycle, apoptosis and cell growth. Cancer subtype specific molecular variations dramatically affect patient responses to already existing treatments. For example, the phosphorylation status of many proteins that are involved in signal transduction pathways perturbed in cancer cells is extremely important in determining whether these cells are susceptible to killing by available cancer therapeutics. Therefore, differentially phosphorylated protein isoforms can be a particularly useful prognostic biomarker of drug response in the clinic. However, accurate detection and quantitative analysis of cancer-related phosphoproteins in tumors is limited by current technologies.

Using a novel, fully automated nanocapillary electrophoresis technology (CB1000^{TM}) designed to separate protein molecules based on their isoelectric point (pI), we are currently developing highly sensitive assays for reliable assessment of the phosphorylation status of cancer-related phosphoproteins in tumors, before and during drug treatment.

We have developed and optimized assays measuring AKT1, AKT2, AKT3, ERK1 and ERK2, and their respective phosphoisoforms. Using these assays, we were able to measure levels of activated ERK1/2 and AKT1/2/3 in a breast cancer cell line panel developed in our lab, using protein extracted from as few as 50 cells. Based on RNA expression data, cell lines in this panel have previously been categorized in two distinct subtypes (Basal and Luminal) and their molecular phenotypes closely resemble the respective profiles of tumors obtained from breast cancer patients. This cell line panel is extensively used to measure cellular responses to breast cancer therapeutics, including drugs that target MEK, ERK, PI3K and AKT. Using CB1000 assays, we are currently measuring changes in the phosphorylation states of these targets during drug treatment, in order to completely characterize pharmacodynamic changes in these cells during treatment, and develop molecular profiles that predict response in breast cancer. We have also extended theses studies to include xenografts from *in vivo* experiments.

Since this technology enables accurate detection and quantification of protein isoforms and post-translational modifications from only very small amounts of tumor samples or serum, it promises to propel cancer biomarker discovery and enable the development of clinically useful prognostic and diagnostic assays that predict responses to drugs targeting cancer-specific molecular networks.

300 Poster discussion

Comparison of Frequencies and Prognostic Effect of Molecular Subtypes Between Young and Elderly Breast Cancer Patients

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Background: To compare the distribution and prognostic effect of the breast cancer molecular subtypes in young and elderly breast cancer patients.

Materials and Methods: Our study population (n = 822) consisted of all early breast cancer patients primarily treated with surgery in our center between 1985 and 1996. A total of 142/822 fresh frozen tissues were available with good quality RNA and analyzed by gene expression microarray. Gene expression molecular subtypes were determined by hierarchical clustering based on patterns of expression of 534 'intrinsic' classifications. Sections of a tissue micro array containing formalin-fixed paraffin-embedded tumor tissue of 714/822 patients were immunohistochemically (IHC) stained for Ki67, EGFR, CK5/6. Tumor expression of