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

E. De Kruijf[1], E. Bastiaannet[1], F. Ruberta[1], T. Sørlie[2], A.J.M. de Craen[3], P.J.K. Kuppen[1], V. Smit[4], C.J.H. van de Velde[1], G.J. Liefers[1]. [1]*Leiden University Medical Center, Surgery, Leiden, The Netherlands;* [2]*Norwegian Radium Institute, Genetics, Oslo, Norway;* [3]*Leiden University Medical Center, Gerontology & Geriatrics, Leiden, The Netherlands;* [4]*Leiden University Medical Center, Pathology, Leiden, The Netherlands*

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

ER, PR, HER2 was previously determined. IHC molecular subtypes were defined based on expression of these markers: Luminal A: ER+ and/or PR+, HER2− and Ki67−; Luminal B: ER+ and/or PR + and HER2+ and/or ki67+; ERBB2: ER−, PR− and HER2+; Basal-like: ER−, PR−, HER2− and EGFR+ and/or CK5/6+; Unclassified: ER−, PR−, HER2−, EGFR− and CK5/6−. IHC molecular subtypes were validated against gene expression defined molecular subtypes. Assessment of distribution and prognostic effect of molecular subtypes was stratified to age (<65 versus ⩾65 years).

Results: Validation of molecular subtypes determined by IHC against gene expression revealed a substantial agreement in classification (Cohen's kappa coefficient 0.77). A statistical trend to an association ($p = 0.056$) was found between molecular subtypes and age, where Luminal tumors were more often found in elderly patients, while ERBB2, basal-like and unclassified subtypes were more often found in young patients. Molecular subtypes showed a prognostic association with outcome in young patients concerning relapse free period (RFP) ($p = 0.03$) and relative survival (RS) ($p < 0.001$). No statistically significant prognostic effect was found for molecular subtypes in elderly patients (RFP $p = 0.7$; RS $p = 0.3$). Additional analyses showed that no molecular subtypes showed a statistically significant difference in outcome for elderly compare to young patients, apart from Luminal A tumor where elderly patients had a worse RS.

Conclusion: We have shown that molecular subtypes have a different distribution and prognostic effect in elderly compared to young breast cancer patients, emphasizing the fact that biomarkers may have different distributions and prognostic effects and therefore different implications in elderly compared to their younger counterparts. Our results support the premise that breast cancer clinical behavior is significantly affected by patient age, but we suggest that competing risks of death in elderly patients and ER-driven differences in biology are underlying these age-dependent variations in patient prognosis, rather than the general belief that elderly breast cancers are of a more indolent biological character.

301 Poster discussion

Survival in Early Breast Cancer Patients is Influenced by Circulating Tumor Cells

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Background: There is good evidence for Circulating tumor cells (CTCs) in the peripheral blood to be a predictor of shortened progression-free and overall survival in metastatic breast cancer patients. Now we evaluated whether the presence of CTCs in patients with early breast cancer before the initiation of systemic adjuvant chemotherapy increases the likelihood of subsequent relapse and death.

Methods: In 2,026 patients with early breast cancer, CTCs were analyzed using the CellSearch System (Veridex, USA) right after complete resection of the primary tumor and prior to the initiation of systemic adjuvant treatment. All patients were randomized in the SUCCESS A trial, which compared FEC-Docetaxel vs. FEC-Docetaxel-Gemcitabine and 5 vs. 2 years of treatment with zoledronic acid in primary breast cancer patients and node positive or high-risk node negative disease. Patients were followed for a median of 35 months (range 0 to 54 months). The prognostic significance of CTCs for disease-free and overall survival was assessed using the Cox regression models.

Results: CTCs were detected in 21.5% of patients (435 out of 2026; median 1.3, range 1–827). Axillary lymph node involvement was more prevalent in patients with CTCs ($p < 0.001$), but no association was found with tumor size, histopathological grading or hormone receptor status.

There were 114 events of recurrence and 66 patients died of their disease. The presence of CTCs before systemic treatment was an independent predictor of poor disease-free survival (DFS) ($p < 0.0001$), distant disease-free survival (DDFS) ($p < 0.001$) and overall survival (OAS) ($p = 0.0002$). Patients with at least 5 CTCs had the worst prognosis with a four-fold increased risk of recurrence and a three-fold increased risk of death (hazard ratio (HR) 4.0 for DFS and 3.1 for OAS).

Conclusions: This is the first study to prospectively evaluate in a large patient cohort with early breast cancer the relevance of CTCs observed in the peripheral blood prior to the initiation of systemic treatment to the prognosis of early disease recurrence. CTC detection may be a clinically useful tool for monitoring treatment and should be tested as an indicator for secondary adjuvant treatment interventions in clinical trials.

302 Poster discussion

Using the 21-gene Breast Cancer Assay in Adjuvant Decision-making in ER-positive (ER+) Early Breast Cancer (EBC) is Cost-effective: Results of a Large Prospective German Multicenter Study

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Background: The Onco*type* DX® Recurrence Score (RS) is accepted as a predictive marker of adjuvant chemotherapy benefit in patients (pts) with ER+ EBC. We performed a clinical study to evaluate the impact on treatment decisions when using the RS including a pharmacoeconomic assessment of cost-effectiveness of using the assay in Germany.

Materials and Methods: Pts with ER+, HER2-negative N0 and N+ (1−3 positive lymph nodes) EBC and no contraindication for chemotherapy were included. Treatment recommendations before and after knowledge of the RS and actual treatment data were recorded. A Markov model was developed to estimate the long term costs and life expectancy associated with chemotherapy decisions in ER+, N0 and N+ EBC including 3 health states (recurrence, no recurrence and dead). Transition from one state to another was based on published recurrence risk data. The model compared costs and life expectancy associated with treatment decisions either based on criteria currently used in German clinical practice or on the RS. The study was conducted in the perspective of German sick funds' and over a 30 year time frame. Costs and outcomes were discounted at 3% per year. One-way sensitivity analyses were conducted on key variables.

Results: Of the 366 evaluable pts 244 were N0 and 122 N+, 54.1% had low, 38.0% intermediate and 7.9% high RS values. Initial recommendation changed in 33.1% of all cases.

Prior to the RS 50.5% of low, 62.6% of intermediate and 75.9% of high RS pts were recommended chemotherapy. Net changes in chemotherapy use from the study were −18.9% for all pts, −36.9%, 1.4%, and +20.7% for pts with low, intermediate and high RSs. Using Onco*type* DX to guide chemotherapy decisions was associated with an increase in survival (4.83 life years) due to the high number of pts reclassified by the RS as likely to benefit from chemotherapy and an incremental cost of €757 per patient. Thus, using the test in Germany is expected to be cost-neutral to the sick funds (i.e. incremental cost-effectiveness ratio of €206/life year). Considering the societal perspective, the incremental cost-effectiveness ratio associated with the use of the test is €6/life year. One-way sensitivity analyses confirmed the robustness of the main results.

Conclusions: Onco*type* DX guided chemotherapy decision-making for ER+ EBC resulted in a significant reduction of adjuvant chemotherapy usage and was cost-neutral versus current clinical practice.

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Could the Axilla Be Managed Less Aggressively in Selected Node-positive Breast Cancer Patients?

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Background: With advances in staging, and increasingly effective systemic treatments, management of the axilla is becoming less aggressive. Until recently, when sentinel lymph node biopsy became the standard axillary staging technique, our institutional policy has entailed four-node sampling, with node-positive (<4) patients receiving axillary radiotherapy (ART) without axillary clearance. This has been retrospectively evaluated for outcomes of regional recurrence, in a consecutive cohort with over 10 years of follow-up. Our hypothesis is that this management protocol is efficient and associated with low risk of regional recurrence (RR), and that there is a difference based on the number of positive nodes.

Methods: The study population was selected from 2607 consecutive patients with operable cT1-T2 breast cancer at our institution between the years 1990 and 2000. Surgery and radiotherapy to breast or chest wall, and systemic therapy, were given according to standard local guidelines.