CHEK2*1100delC mutation and for 48 BRCA1 and 2 founder or recurrent mutations.

Results: So far we determined 15 CHEK2 1100delC mutations among 233 (6.4%) clbc patients, 13 among 169 (7.7%) patients who did and 2 among 64 (3%) who did not receive RT. We identified 30 BRCA1/2 mutations among 188 (16%) clbc patients, 23 among 128 (18%) patients who did and 7 among 60 (11.7%) who did not receive RT. ATM truncating germline mutations were determined among 4 out of 188 clbc patients (2.1%), all were detected among those who received RT. The ATM missense mutations spectrum is under analysis.

Conclusion: In the subset of 188 clbc patients with complete mutation data, 26% carries a germline mutation in one of the tested genes. Thirty percent of the women who received RT carried a germline mutation versus 15% among those who did not receive RT, OR=2 (95% CI 1.045–3.888, p=0.03). Our results suggest that ionizing radiation treatment might be a risk factor for breast cancer development in these mutation carriers. The excess risk for heterozygotes to develop radiation induced contralateral breast cancer provides a scientific basis for mutation analysis and subsequently an intensified follow-up protocol for mutation carriers.

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Changes in gene expression profiling due to primary chemotherapy in patients with locally advanced breast cancer

J. Hannemann[1], R. Oosterkamp[2], C.A.J. Bosch[1], A.T. Witteveen[3], A.A.M. Hart[4], A. Velds[5], E.J.T. Rutgers[7], S. Rodenhuis[2], M.J. van de Vijver[3]. [1]*Netherlands Cancer Institute, Experimental Therapy, Amsterdam, The Netherlands;* [2]*Netherlands Cancer Institute, Medical Oncology, Amsterdam, The Netherlands;* [3]*Netherlands Cancer Institute, Pathology, Amsterdam, The Netherlands;* [4]*Netherlands Cancer Institute, Radiotherapy, Amsterdam, The Netherlands;* [5]*Netherlands Cancer Institute, Central Microarray Facility, Amsterdam, The Netherlands;* [7]*Netherlands Cancer Institute, Surgical Oncology, Amsterdam, The Netherlands*

Background: Patients with locally advanced breast (LABC) cancer often undergo neoadjuvant chemotherapy treatment. At present, commonly used regimens of neoadjuvant chemotherapy include the combination of adriamycine and cyclophosphamide (AC). Good responses have also been observed with adriamycine and docetaxel (AD).

It is currently not possible to predict sensitivity or resistance of tumors to specific drugs. Some patients may therefore undergo the toxicity of these drugs, but do not benefit from the therapy. This study was designed to identify gene expression patterns that can predict which tumors will respond to a combination of AC and which tumors to a combination of AD.

Material and Methods: We started a prospective phase III trial for patients diagnosed with locally advanced breast cancer, randomizing between six courses of adriamycine (60 mg/m^2) and cyclophosphamide (600 mg/m^2) or adriamycine (50 mg/m^2) and docetaxel (75 mg/m^2) respectively. Chemotherapy was administered every three weeks.

Total RNA was isolated from a frozen 14 G core needle biopsy obtained from the tumor before treatment. All patients underwent surgery after completing chemotherapy. If there was residual tumor after completion of chemotherapy, RNA was isolated from frozen tissue sections. Amplified mRNA was hybridized on human 18k cDNA microarrays obtained from the NKI microarray facility. Supervised and unsupervised classification have been used to analyze differences between gene expression before and after treatment and to correlate gene expression profiles to patient's response to the chemotherapy administered.

Results and Discussion: Thus far 62 patients with LABC have been randomized in the study. Good quality RNA from tissue with more than 50% tumor cells was obtained from 46 biopsies and 18 tumors. In total, data from 49 patients (three tumors without biopsy) could be included into the analysis. From these patients 25 were treated in the AC arm, 24 in the AD arm of the study.

Preliminary analysis indicates that there are significant differences in gene expression in the tumor before and after chemotherapy treatment. There does not appear to be a major difference in gene expression between tumors from patients with a complete pathological remission compared to all other patients. There are subtle differences in gene expression between these two groups, but the identification of a reliable "response signature" probably requires a higher number of patients for analysis.

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Gompertzian effect of tumor size on mortality in early breast cancer

V. Vinh-Hung, G. Storme. *Oncologisch Centrum, AZ-VUB, Radiotherapy, Jette, Belgium*

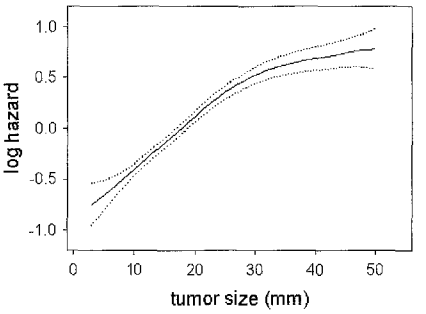
Purpose: To determine the type of relationship between tumor size and mortality in T1-T2 breast carcinoma.

Methods: Data on 83,686 women from the Surveillance, Epidemiology and End Results (SEER), diagnosed 1988–1997, no metastases, in whom axillary node dissection was performed: 58,070 node-negative (N0), 25,616 node-positive (N+). Endpoint is death from any cause. Tumor size in millimeters is modeled as a continuous variable by proportional hazards (PH), using a Generalized additive models procedure.

Results: Functionally, the same Gompertzian expression **exp(−exp(−(*size* − 15)/10))** provided a good fit to the effect of tumor size on mortality, irrespective of nodal status, and irrespective of the number of nodes involved (npos).

Quantitatively, for tumor size from 5 mm to 50 mm, the crude death rate increase was (figures rounded) from 10% to 25% in N0, 15% to 35% with npos=1, 29% to 40% with npos=2, 15% to 30% with npos=3, 30% to 50% with npos>3.

Conclusion: The effect of tumor size is functionally and quantitatively independent of nodal involvement. This is in contradiction with the Halstedian concept of a sequential involvement of nodes.



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Tissue inhibitor of metalloproteinases-1 (TIMP-1) and prognosis in primary breast cancer: an EORTC–RBG collaborative validation study including 2984 patients

A. Schrohl[1], M.N. Holten-Andersen[1], H.A. Peters[2], M.P. Look[2], M.E. Meijer-van Gelder[2], J.G.M. Klijn[2], N. Brünner[1], J.A. Foekens[2]. [1]*The Royal Veterinary and Agricultural University, Dept. of Pharmacology and Pathobiology, Frederiksberg C, Denmark;* [2]*Erasmus MC, Josephine Nefkens Institute, Dept. of Medical Oncology, Rotterdam, The Netherlands*

Introduction: Previous studies have indicated that TIMP-1 levels in primary breast tumours may be related to patient prognosis. To validate this, in the present EORTC–Receptor and Biomarker Group (RBG) collaborative study we investigated the association between total tumour tissue levels of TIMP-1 and prognosis in 2984 patients with primary breast cancer. We also analysed whether TIMP-1 may be useful as a prognostic marker in combination with urokinase plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1).

Experimental Design: In cytosolic extracts of 2984 primary breast tumours, total levels of TIMP-1 were determined using an established, validated TIMP-1 ELISA and the results were analysed statistically. TIMP-1 was analysed as a continuous, log-transformed variable and as a dichotomised one. Dichotomisation of data was done using a statistically identified cut-point. Median follow-up time was 98 months. 51% of the patients were lymph node-negative and 48% lymph node-positive; median age of the patients was 57 years. Levels of uPA and PAI-1 have previously been determined in the extracts.

Results: The cut-point identified and used in the analyses was 11.71 ng/mg of total protein. In univariate survival analysis, high levels of tumour tissue TIMP-1 were associated with a poor prognosis [recurrence-free survival (RFS), overall survival (OS), P<0.001], both when including TIMP-1 as a continuous and as a dichotomised variable. Also in subgroups of lymph node-negative and lymph node-positive patients, high TIMP-1 levels were associated with a significantly shorter RFS (dichotomised variable, both groups P<0.05). In a multivariate model including established prognostic parameters (lymph node status, age and menopausal status, tumour size, grade of malignancy, hormone receptor status), high tumour tissue levels of TIMP-1 (dichotomised variable) were associated with a significantly shorter RFS (P<0.001) and OS (P=0.003). When adding uPA and PAI-1 to the multivariate model, TIMP-1 still added significantly to the model for RFS (P=0.002).

Conclusion: This study supports previous findings, namely that high levels of tumour tissue TIMP-1 are associated with poor prognosis in patients with primary breast cancer. It also shows that TIMP-1 may be useful as a prognostic marker in combination with components of the urokinase-plasminogen activation system (uPA and PAI-1), which have been established as strong prognostic markers in breast cancer.