MOLECULAR DISSECTION OF CANCER BY DIFFERENTIAL DRUG SENSITIVITY

In the clinic, we mainly use anticancer drugs based on outcomes of clinical trials that have been carried out in the general breast cancer population, whereas little is known about the molecular mechanisms underlying differential drug sensitivity. The same holds true for other cancer types, including non-small cell lung cancer (NSCLC), stomach cancer, ovarian cancer, and colorectal cancer. The focus of our research line is to unravel these molecular mechanisms in order to develop tests that may guide treatment decisions in the clinic and ultimately improve survival. For this purpose we use several genome-wide approaches and molecular techniques, in order to dissect the mechanisms that divide clinically well-defined cohorts of breast, colorectal, stomach, ovarian and NSCLC patients into resistant and sensitive to a particular drug. In addition, we have a close collaboration with the groups of Jos Jonkers and Piet Borst, who use conditional mouse models for breast cancer, and derived clonal cell lines, to study differential chemosensitivity in a controlled fashion.
A second research line focuses on the impact of prognostic molecular classifiers on adjuvant systemic treatment advice in breast cancer.

The 70-gene prognosis signature and outcome after tamoxifen (Collaboration with Division Experimental Therapy and Agendia BV)
Node-positive breast cancer patients can have an excellent outcome with tamoxifen only. It is unclear whether analyzing both the 70-gene-signature and hormone receptors provides superior prediction of outcome in tamoxifen-treated patients than standard measures. We evaluated three series: 121 patients (81% node positive; 98% > 50 years; > 80% grade I/II) received adjuvant tamoxifen, 151 patients did not receive tamoxifen (10% node positive; 44% > 50 years; 70% grade I/II) and 92 patients received tamoxifen for metastatic disease. The 70-gene signature was analysed using MammaPrint™. Oestrogen receptor (ER) and progesterone receptor (PR) immunohistochemistry was evaluated following St. Gallen Consensus (Highly Endocrine Responsive: ER and PR ≥ 50%, Incompletely Endocrine Responsive: ER and/or PR low or either one absent). In patients treated with adjuvant tamoxifen, both the 70-gene signature (adjusted for Endocrine Response Categories HR 2.17) as well as the Endocrine Response Categories (adjusted for 70-gene signature HR 6.35) were associated with breast-cancer-specific-survival. In metastatic disease, combined analysis of the 70-gene signature and ER/PR revealed also additional value. In patients who did not receive tamoxifen, only the 70-gene signature was associated with outcome. We concluded that the 70-gene signature and ER and PR provide independent information on outcome after tamoxifen for node positive ER-positive breast cancer.

Value of 70-gene prognosis signature in T1 breast cancer In collaboration with the Division of Diagnostic Oncology, Division of Surgical Oncology and Agendia BV, using a pooled database of ~1700 breast cancer patients with data on the 70-gene prognosis signature, we have demonstrated that the 70-gene prognosis signature retained its prognostic value in patients with T1 tumors.

Development of a predictive test for tamoxifen resistance in breast cancer
In collaboration with the group of Rob Michalides (Division Cell Biology II) and the group of Göran Landberg (Lund University, Lund, Sweden and Breakthrough Breast Cancer Research Unit, Paterson Institute for Cancer Research, Manchester, UK), we have shown that phosphorylation of ERa at serine 305 (ERaS305-P) causes tamoxifen resistance. Earlier, p21-activated kinase 1 (PAK1) nuclear expression has also been associated with tamoxifen resistance. We have collected primary breast cancer material of ~1000 postmenopausal patients who participated in a randomized trial of adjuvant tamoxifen versus no endocrine therapy started in the eighties, in order to confirm our earlier findings in a third, independent series. For ~ 600 patients we have also isolated tumor DNA to study the role of mutation, amplification and methylation of selected genes in tamoxifen resistance. Analyses are ongoing.
Based on our earlier findings we are now preparing a prospective randomized study

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Publications

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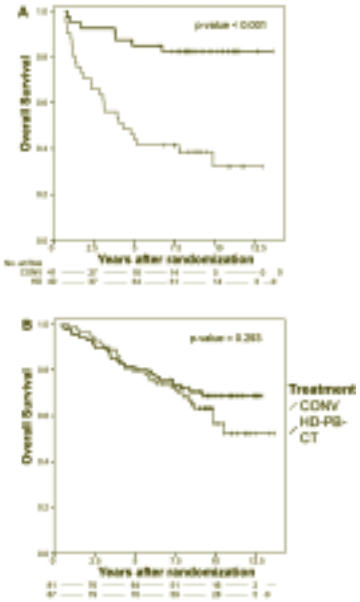

Figure 4: Association of BRCA-like^{CGH} status with overall survival after HD-CTC-chemotherapy and conventional chemotherapy. Kaplan-Meier survival curves for overall survival were generated separately for patients with BRCA-like^{CGH} (A) and with Non-BRCA-like^{CGH} (B) tumours, who had been randomly assigned to HD-CTC-chemotherapy or conventional chemotherapy.

to test whether biomarker-guided adjuvant endocrine therapy improves recurrence-free survival in premenopausal, hormone-receptor positive breast cancer patients when compared to standard care.

Molecular mechanisms underlying sensitivity for high dose alkylating agents The inability of breast cancer cells deficient in homologous recombination (HR), such as *BRCA1/2*-mutated cells, to repair DNA double strand breaks (DSBs) appears to offer a target for DSB-inducing therapies, such as platinum agents, intensified alkylating therapy, and poly(ADP)ribose polymerase (PARP) inhibitors. Our group previously employed array Comparative Genomic Hybridization (aCGH) to assess the genomic profiles of *BRCA1*- and *BRCA2*-mutated breast cancers. We hypothesized that this so-called BRCAness is present in sporadic breast cancers and might predict sensitivity to DSB-inducing agents in hormone-receptor positive and negative patients.
We tested the performance of these BRCA1-like and BRCA2-like classifiers in the context of a randomized clinical trial that compared 5 x fluorouracil-epirubicin-cyclophosphamide (FEC) with 4 x FEC followed by 1 x high-dose (HD) carboplatin-thiotepa-cyclophosphamide (CTC) with autologous stem cell rescue for high risk, primary operable breast cancer [Rodenhuis et al., New Engl J Med, 2003]. Patients with breast cancers with a BRCA1-like and/or BRCA2-like CGH pattern were labeled BRCA-like^{CGH}. We had chosen to only evaluate HER2-negative patients as in the original study HER2-positive patients had appeared not to derive any benefit from HD-CTC. We evaluated whether the effect on recurrence-free and overall survival (RFS, OS) of HD-CTC compared to conventional chemotherapy differed by BRCA-like^{CGH} status. Approximately one third of patients were scored as BRCA-like^{CGH} and these had a greater benefit of HD-CTC compared to conventional chemotherapy regarding OS (adjusted HR 0.19). In patients with Non-BRCA-like^{CGH} tumors this benefit was not significant (adjusted HR 0.90). The difference observed between treatment arms was significantly different (p-interaction: 0.004) (figure 4). Using aCGH we were able to select hormone-receptor positive and triple negative breast cancer patients who had selective benefit of intensified DSB-inducing chemotherapy. (Collaboration with Petra Nederlof, Division of Diagnostic Oncology, and Sjoerd Rodenhuis, Division of Medical Oncology).
In collaboration with the Jonkers and Borst groups we are now evaluating potential biomarkers that may cause resistance to DSB-inducing agents in patients with BRCA-like^{CGH} tumors.

Translation of the BRCA-like^{CGH} classifier to other cancer types HR deficiency is not limited to breast cancer. Although less obvious than breast and ovarian cancer risk, other cancers also occur at increased rates in hereditary breast and ovarian cancer families with germline BRCA1 and -2 mutations. These other cancers include gastric (BRCA2-associated, RR:2.59), pancreatic (BRCA2-associated, RR:3.51), and colon cancer (BRCA1-associated, RR:2.03) (Thompson and Easton; The Breast Cancer Linkage Consortium). In collaboration with the NKI Familial Cancer Clinic possible BRCA-associated tumors will be collected to investigate a causal effect as well as generate tumor type specific array CGH profiles. These profiles will subsequently be tested in defined cohorts of patients who had been treated with either DSB-inducing therapy or other therapy and outcome correlated with BRCA-like^{CGH} status.

Identification of druggable targets in lobular breast cancer and triple negative breast cancer As participant of the FP7 RATHER consortium, together with the Bernards group (Division of Molecular Carcinogenesis), we have initiated a project to assess the presence of druggable targets in 150 lobular and 150 triple negative breast cancers. We are using high-throughput next generation sequencing, together with exon capture technology, to sequence genomic regions of interest in breast cancer in all 300 tumor samples. The final goal is to deliver diagnostic tests along with the right targeted therapy – chemotherapy combination to improve treatment options and outcome for these difficult to treat breast cancer subtypes.