different classifiers (70 genes "prognosis profile" (supervised on clinical outcome), Wound Signature (WS) and Hypoxia Signature (HS), both unsupervised) that separate patients into relatively good and poor prognosis groups. The supervised approach has an excellent sensitivity, but somewhat lower specificity for metastasis free survival. The unsupervised approaches have a higher specificity to identify patients with poor prognosis, but a relatively low sensitivity. In order to optimize both sensitivity and specificity, we combined the unsupervised profiles.
Methods: In a previously described series of 295 stage I and II breast carcinomas treated at the Netherlands Cancer Institute, we have obtained gene expression data of 25,000 genes using micro-array analysis. We have categorized the patients according to previously established groups. The first group consists of patient with a quiescent WS and a non hypoxic signature, patients in the second group have either an activated WS or hypoxic signature and the third group consists of patients with both an activated WS and a hypoxic signature.
Results: At a median follow up of 12 years for patients alive, the metastasis free probability (MFP) at 12 years is 79% for group 1 (n = 110), compared to 64% for group 2 (n = 103) and 45% for group 3 (n = 82) (log rank: p < 1*10−6. HR: 2.1 (95%CI: 1.6−2.7)); these figures were 87%, 68% and 37%, respectively, for overall survival (OS) (p < 1*10−12. HR: 2.6 (95%CI: 2−3.4). In subgroups with a favorable clinico-pathological characteristics (pT1N0, ER+ and pN0) the predictive power remains highly significant, as in patients with unfavorable clinico-pathological characteristics (pT2N+, ER− and pN+). The true negative predictive value for OS for group 1 is 87% and the true positive predictive value for group 3 is 60%. In multivariate analysis the combining the WS and HS signatures resulted in the best prediction of MFP and OS, which was independent of clinico-pathological variables (ER, TN-stage, Grade, Angioinvasion, Chemotherapy and age).
Discussion: In this consecutively treated series of breast cancer patients, the combination of the Wound Signature and Hypoxia classification stratifies patients that differ with respect to prognosis in three risk categories. Combining different gene expression signatures may result in improved classification of breast carcinomas.

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Utilisation of microarray technology to refine molecular classes and improve clinical management of breast cancer

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Background: The significant genetic heterogeneity among breast cancer patients is a primary obstacle to effective clinical diagnosis and management. Emerging technologies based on gene expression profiling (GEP) may provide clinically useful information to improve the management of breast cancer. GEP has been used to refine classification of previously undistinguishable tumour subgroups, and predict prognosis and response to anticancer agents. Here we report a multicentric GEP analysis to identify and validate predictors in order to improve tumour classification and predict patients most likely to respond to standard chemotherapy.
Material and methods: 323 patients with early breast cancer treated with adjuvant anthracycline-based chemotherapy were selected from Institut Paoli-Calmettes (IPC), Marseille and Centre Léon Bérard (CLB), Lyon. RNAs were analysed on 10 K nylon cDNA microarrays. Metagenes for tumour classification were identified based on adjusted t-test analysis and hierarchical clustering on an identification set. A Cox-based method was used to find predictors able to discriminate patients with favourable outcome (no metastasis) after chemotherapy, by combining validated metagenes with clinical factors on an identification set of 159 patients treated with anthracyclines (IPCa). The stability and robustness of these predictors were assessed on two different and independent validation sets (IPCb n = 54 & CLB n = 110). The best predictor was compared with the Nottingham Prognostic Index (NPI).
Results: A predictor was identified on patients treated with chemotherapy (anthracyclines). This predictor was based on a linear combination involving metagenes and clinical factors, i.e. A*(metagene 1)+B*(metagene 2)+C*(clinical factors). It classified patients in two groups with different outcome. The robustness of this predictor was then confirmed on the two validation sets of patients. Our predictor compared favourably with the NPI, improving the classification of the low-risk patients.
Conclusions: Our metagene-based predictor is highly efficient to discriminate patients with favourable outcome under adjuvant anthracycline-based chemotherapy. It uses a validated combination of genes known for their biological relevance, and is valid irrespective of the clinical centre. Additional clinical studies and technical developments are ongoing to translate this new tool into a decentralised test designed for routine clinical practice.

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Standardisation of HER2 testing: results of an international proficiency testing ring study

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Background: HER2-positive breast cancer indicates aggressive tumour growth, poor prognosis and treatment response to trastuzumab. Early and accurate determination of HER2 status is essential for optimal management of breast cancer. Because current HER2 tests (immunohistochemistry [IHC], fluorescence in-situ hybridisation [FISH], and chromogenic in-situ hybridisation [CISH]) are reader-dependent, validation by laboratory proficiency testing is important to improve standardisation. The study compared IHC and FISH testing between five international pathology reference centres.
Methods: A total of 20 IHC and 20 FISH breast cancer specimens were evaluated separately in five testing rounds (8-week intervals). In each round, a designated laboratory selected two sets of four different invasive tumour specimens (set A for IHC [HercepTest™]; set B for FISH [PathVysion]). The lab sent samples from each set to the other four testing centres in a blinded fashion, while retaining samples for its own evaluation. IHC scores were stated as negative (0, 1+), ambiguous (equivocal, 2+) or positive (3+). FISH scores were based on the ratio of HER2 signals to chromosome 17 centromere signals: negative (<2.0) or positive (⩾2.0). At the end of each round, an independent co-ordinator analyzed and discussed the results among the centres.
Results: All centres reported the same findings for nine out of 20 IHC specimens (45%). Although reports differed in the remaining 11 specimens, there were no cases wherein a laboratory reported a specimen as HER2 positive and another reported it as negative. At least one laboratory reported an ambiguous HER2 status in each of the 11 specimens. Sixteen out of 20 (80%) FISH specimens had similar scores from all the centres. The four cases in which the centres did not agree had mean amplification levels of 1.95, 1.48, 1.72 and 1.82. In the second of these cases, the difference in the report was due to one centre reporting a value of 2.0, while the others reported <2.0.
Conclusions: Equivocal IHC and borderline FISH cases are difficult to interpret, even for highly experienced and validated laboratories. To help determine the treatment, FISH retesting of IHC 2+ samples and retesting of FISH borderline cases with FISH, IHC, or CISH is recommended. As a follow-up of this study, equivocal IHC samples will be retested by FISH. Each testing laboratory should regularly validate their HER2 testing to ensure proper reporting of test results.

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Comparison of her2/neu expression on the primary tumor and on isolated tumor cells in the bone marrow of breast cancer patients

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Background: There is growing evidence that the presence of isolated tumor cells in the bone marrow (ITC) of breast cancer patients, both at primary diagnosis and during follow-up, indicates an increased risk for subsequent recurrence (Braun, NEJM 2000; Janni, Cancer 2005). Therefore, ITC might be a potential target for tailored treatment options in these patients. Aim of this study was to establish a new method to analyse cytokeratin-positive (CK+) cells for her2/neu gene amplification.
Methods: ITC were detected using a standardized immunoassay with monoclonal antibody A45-B/B3, directed against cytokeratin 8, 18, 19 (CK) and stained according to the APAAP-technique. 2×10^6 cells per patient were screened by bright field microscopy. Subsequently, cytospins with CK-positive cells were further characterized by fluorescence in situ hybridisation (FISH) using a her2/neu DNA probe (Zymed, Germany) or a multi-colour probe (Vysis, Il., USA) for hybridisation of centromer 17 (polyploidy) and the her2/neu growth factor gene. A ratio of her2/neu and centromer chr. 17 signals of at least two was regarded as amplification.
Results: 232 bone marrow aspirates of 156 patients with breast cancer were analyzed at the time of primary diagnosis and during follow-up. ITC were detected in 68 samples (29%) in this patient group. The median number of detected cells was 2 (range 1−58). In 45 randomly assigned aspirates with ITC, the her2/neu status on these cells was evaluated and