Methods: Thirteen primary breast cancers from a large randomized treatment trial for metastatic disease were studied. Laser capture microdissection was used to obtain 90%-pure tumour samples from frozen and paraffin sections. Genomic DNA was labelled using nick translation from frozen tissues and degenerate oligonucleotide-primed PCR (dop-pcr) from paraffin tissues. Tumour DNA was cohybridized with normal peripheral blood lymphocyte DNA onto arrays containing 60 candidate oncogenes. Ratios were normalized and two-dimensional hierarchical clustering was used to reorder patient and amplicon data into new classifications. Clustering was also performed with aCGH data from 40 breast cancer cell lines to correlate key copy number changes.

Results: We compared results from nick translated and dop-pcr labelled DNA from the same cell line (n=18) or breast cancer (n=2). Clustering of aCGH data showed complete concordance indicating that dop-pcr can faithfully represent gene copy number. The analysis also correctly clustered cell lines derived from the same patient (n=2) and closely linked probes (n=2). This indicates that, as for expression microarray analysis, clustering of aCGH data may reveal new classifications. Examination of the 13 primary breast cancers showed striking clustering of MYC and ERBB2 along with NRAS and WNT1 amplification (n=4). Separate clusters included genes from the 20q13 amplicon. Clustering of both primary cancers and cell lines showed clustering of the same ERBB2/MYC cancers together with cell lines SKBR3, SKBR7, OCUB-F, OCUB-M, SUM190, SUM225 and MDA-MB361. Validation of these results are now being carried out using FISH probes for ERBB2 and MYC on cell lines and tissue microarrays containing 250 high risk cancer patients.

Conclusion: ERBB2/MYC coamplification has been independently identified by Southern analysis and shown to be associated with a significant reduction in patient survival [1]. Our study indicates that the combination of aCGH and clustering analysis can identify important prognostic classifications.

References

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HER-2 amplification evaluated by fluorescence in-situ hybridization (FISH) as a predictive marker in node-positive (N+) breast cancer (BC) patients (pts) randomly treated with CMF or an anthracycline-based therapy

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Purpose: FISH is a reliable technique for HER-2 testing. We have investigated the predictive value of HER-2 evaluated by FISH in a population of 777 N+ BC pts aged ≤70 yrs randomly treated either with CMF or with an anthracycline (A) - based therapy. Treatment arms of the clinical trial were as follows: a) classic CMF × 6; b) HEC × 8 (epirubicin [E] 100 mg/m^2 + cyclophosphamide [C] 830 mg/m^2, d 1 q 3 wks); c) EC × 8 (E 60 mg/m^2 + C 500 mg/m^2, d 1 q 3 wks). The median study follow-up is of 6 yrs.

Methods: Archival primary rumor samples were collected for 625 of the 777 eligible pts. Of the 625 available samples, 354 were fixed in formalin and appropriate for FISH evaluation by the Path Vysion kit from Vysis. FISH was unfeasible in the remaining 271 samples mainly because they were fixed in bouin. HER-2 amplification (ratio > 2) was found in 21% of the 354 evaluable cases. Our primary results are reported below:

Study comparison	Hazard ratio (95% CI) for event-free survival		
	HER-2+ (73 pts)	HER-2− (281 pts)	All pts (777 pts)
CMF vs HEC	1.42 (0.54–3.76)	0.84 (0.49–1.44)	1.08 (0.81–1.44)
CMF vs EC	1.65 (0.66–4.13)	0.66 (0.39–1.10)	0.84 (0.65–1.10)

Conclusion: Although the number of evaluable pts is limited and no statistical significance is reached, these results suggest that when HER-2 is evaluated with a highly reliable technique like FISH, HER-2 positive pts derive the highest benefit from an A-based regimen, while HER-2 negative pts have a better outcome if treated with CMF. Because of the limited statistical power of individual studies, largely due to the low prevalence of HER-2 amplifications in BC pts, only a meta-analysis with centralised HER-2 testing could properly define the predictive value of HER-2 in the adjuvant therapy of BC.

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Stage migration in breast cancer after biopsy of internal mammary lymph nodes

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Purpose: Although dissection of the internal mammary chain nodes (IMNs) in breast cancer does not improve prognosis, their involvement is associated with poorer prognosis. With the development of lymphoscintigraphy to visualize sentinel nodes in breast cancer it has become evident that the IMNs often receive lymph from the breast area containing the tumor. We performed a pilot study to assess the feasibility of biopsying IMNs, to determine how often they were metastatic, and to assess the impact of their status on disease stage and consequent adjuvant therapy decisions.

Methods: We biopsed IMNs in 137 consecutive patients with either radioactive uptake to the IMN region as revealed by lymphoscintigraphy following injection of radiotracer close to the breast, or tumor location in the medial portion of the breast. After tumor removal, the longitudinal fibres of the pectoralis major were divided exposing the intercostal muscle, a portion of which adjacent to the sternum was removed to access to the subcostal space. Fatty tissue there was carefully freed from the blood vessels taking care not to damage these or the underlying pluera. All material removed from the subcostal space was sent for histological analysis.

Results: In 122/137 patients IMNs were found on histological examination. Of these, 110 (90.2%) had negative IMNs and 12 patients (9.8%; who received RT to the internal mammary chain) had a metastatic IMN. In four of these 12 cases the axilla or axillary sentinel node was negative and in eight the axilla was positive. Four patients had an involved IMN but a negative axillary sentinel node. The pleural cavity was breached in 3 cases (2.2%) with spontaneous resolution and no sequelae.

Conclusions: We found that IMNs can be easily removed through the incision used for breast conservation, even when the tumor is in the lateral part of the breast. The sampling method is simple and quick to perform, and was often done while waiting for the result of the intraoperative analysis of the axillary sentinel node. The risks of the procedure also proved to be insignificant and did not increase the postoperative hospitalization period. The twelve cases with a positive IMN migrated from N0 (4 cases) or N1 (8 cases) to N3 in all cases prompting modification of the treatment plan. If the sampling had not been performed they would have been understaged. It remains to be seen whether this additional information can lead to better survival.

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Telomerase activity (TA) in breast cancer and its correlation with other biological and pathological parameters

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Introduction: Telomerase is a ribonucleoprotein enzyme that appears to play an important role in carcinogenesis. TA has been detected in a wide range of human malignancies and its association with prognostic factors has been investigated. We have studied TA in breast cancers and analyzed its correlation with tumor size (pT), tumor grade (G), nodal status (pN), expression of ER, PgR, P53. C-erb B-2 and ploidy.

Methods: TA was studied in 305 frozen human invasive breast cancer specimens by use of telomeric repeat amplification protocol (TRAP). The TRAP assay standardization was performed using the 'Biorad protein assay'. The ER, PgR status and P53 and c-erbB-2 expression were evaluated by IHC (clone 6F11, 1A6, CB11, DO-7, Mib-1, Neomarkers), while ploidy by citofluorimetry. TRAP was applied on 6, 0.6, 0.06 mg/ml concentration of protein extract for each sample. We considered TA positive (TA+) the tumors with TA detectable at 0.6 and/or 0.06 mg/ml and TA negative (TA-) the others. The association between TA and other parameters was analyzed using c2 test and a P value of 0.05 was considered significant.

Parameters	Cases	TA-	TA+	P value
N −	132	61 (46%)	71 (64%)	0.002
+	116	32 (28%)	84 (72%)	
G 1	34	20 (60%)	14 (40%)	0.0009
2	133	51 (38%)	82 (62%)	
3	102	32 (31%)	70 (69%)	
MIB1 < 25%	97	49 (50%)	48 (50%)	0.003
> 25%	105	32 (30%)	73 (70%)	