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%)	

Results: TA+ tumors are 61% of 305 specimens. There was a statistical significant correlation only between TA and pN, G, and MIB1 value (see table).

Conclusions: In invasive breast cancer TA is significantly associated with nodal metastasis, cellular proliferation (Ki67/MIB1) and histological grade. The correlation between TA and clinical outcome are under evaluation.

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Expression profiling predicts poor outcome of disease in young breast cancer patients

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Purpose: Twenty percent of lymph-node negative breast cancer patients diagnosed at young age develops distant metastases at 5 years follow-up. We used expression profiling of the primary tumor for diagnostic classification and identification of an expression signature predictive for distant metastasis.

Methods: RNA of 97 breast tumors of LN0 patients (age < 55 yrs) with known clinical outcome was profiled on DNA microarrays that represent ~25,000 human genes fabricated with an ink-jet oligonucleotide synthesizer.

Results: Two-dimensional clustering displays two distinctive types of tumors based on differential expression of ~5100 genes. Discriminant and statistical analyses revealed sets of reporter genes for diagnostic subtypes, e.g. related to BRCA1 status (~300 genes). An expression signature for prediction of early distant metastasis was established (83% correct classification) and its predictive power was confirmed on an independent set of 19 tumors. In a multivariate model including known clinical parameters (logistic regression) the expression profile is a strong determinant of prognosis ($p < 0.001$).

Conclusion: Expression profiling is a powerful diagnostic tool in breast cancer and allows 'array-guided' tailored therapy.

Colo-rectal cancer

1001 ORAL

Continuous vs intermittant chemotherapy for advanced colorectal cancer: preliminary results of the MRC cr06b randomised trial

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A survey of UK clinicians suggested that there was no consistent policy regarding the duration of treatment for patients receiving chemotherapy for advanced colorectal cancer. Patients who were responding or had stable disease after receiving 12 weeks of de Gramont, Lokich or Raltitrexed therapy were therefore randomised to either 'continue' therapy until progression, or 'stop', re-starting on the same therapy on progression. The trial was closed in August 2000, when 354 patients had been entered from 42 UK centres in 4 years. Median age of patients was 64 years, 64% were male, 86% were WHO PS Grade 0/1, 65% had colon cancer, and 40% had responding disease, and these characteristics were well-balanced between the two policies. Of the 178 patients allocated to 'stop', 39% re-started treatment after a median of 134 days, mainly due to disease progression. Median time on re-started treatment was 83 days. The 'continue' group remained on treatment for a median of a further 91 days, stopping for progression (44%), toxicity (15%), or clinician or patient decision (35%). Similar proportions of patients on both groups received second-line therapy. Patients on 'continue' experienced significantly more serious adverse events and toxicity, and using patient-assessed EORTC QLQ-C30 and HADS reported significantly worse quality of life. There was no clear evidence of a difference in progression-free survival (HR 1.16 95% CIs 0.92-1.45, p=0.21) or overall survival (HR 0.87 95% CIs 0.68-1.12, p=0.28). From randomisation (after an initial 12 weeks of chemotherapy), median, and estimated 2-year survival were 11.8 and 11.2 months, and 18% and 14% for 'stop' and 'continue' respectively. The result of this trial, that there is no clear evidence of a benefit in continuing therapy indefinitely, and that there appears to be a gain in QL for the 'stop' policy, provides an evidence-base for stopping chemotherapy after 12 weeks

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5-FU-based adjuvant chemotherapy given after neoadjuvant chemoradiation and surgery for rectal cancer improves survival only among responders

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Purpose: To analyze the influence of adjuvant 5-FU-based chemotherapy (ADJ-CTX) on survival in rectal cancer patients treated with preoperative chemoradiation (CXRT) and surgery.

Materials and Methods: From 1990 to 1998, a total of 318 patients with Stage II-III rectal cancer were treated with CXRT followed by surgery with or without ADJ-CTX. CXRT (45 Gy/25fx with protracted venous infusion, 5-FU 300mg/m2) was delivered to 97% of patients (311/320). Patients were resected 4-6 weeks later. ADJ-CTX consisted of 5-FU and leucovorin for 4-6 cycles and was given in 181 patients (57%). Kaplan-Meier univariate and Cox Regression mutivariate methodology was used to evaluate survival.

Results: Median follow up was 59 months for living patients. Objective evidence of tumor response indicated by T-stage downstaging (TSD) occurred in 51% (161/318) of cases. ADJ-CTX was given in 48% (77/161) of TSD+ and 38% (60/157) of TSD- cases, respectively. On univariate analysis of all patients, age, gender, tumor length, clinical N-stage, and distance from the anal verge were not significant, whereas circumferential involvement (p=0.01), and high grade (p=0.02) were significant. Tumor fixation (p=0.07), T-stage (p=0.065), and ADJ-CTX (p=0.092) were borderline. None were independently significant on multivariate analysis. In every multivariate model that was attempted separately in the TSD+ and TSD- groups, ADJ-CTX was the only independently significant factor in the TSD+ group (p=0,044). Nothing was ever independently significant in the TSD- group, including ADJ-CTX. The 5-year actuarial survival for TSD+ patients who received ADJ-CTX was 94% verses 81% for those who did not.

Conclusions: In this large population of rectal cancer patients treated with neoadjuvant CXRT, post-operative adjuvant 5-FU-based chemotherapy did not independently improve survival in all patients. However, it independently improved survival in the subset of patients who responded to neoadjuvant therapy (TSD+), but not in those who did not respond (TSD-). The prospective investigation of novel adjuvant chemotherapy regimens is appropriate in patients who don't respond to neoadjuvant therapy.

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Definition of genetic instability as prognostic factor in colorectal cancer by microsatellite analysis and immunohistochemistry of an archival collection of patients. Comparison between sporadic and familial cases

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Background: Association between microsatellite instability (MSI) and favourable postoperative survival in patients with colorectal cancer (CRC) has been indicated. To confirm a prognostic role of MSI, we started screening a series of archival CRCs.

Patients and Methods: To date, DNA from paraffin-embedded paired samples of tumors and corresponding normal tissues from 369 CRC patients at various stages of disease was isolated. Patients [median age, 54 (range 22-90); male/female, 207/162] were surgically treated from 1987 to 2000, and disease stage recorded according to Dukes classification. PCR-based MSI analysis was performed using five microsatellite markers. Tumors were classified as MSI+ when > 2 markers were unstable. Involvement of the mismatch repair genes was evaluated by immunohistochemistry (IHC) using anti-MLH1 and anti-MSH2 antibodies on MSI+ tumor tissues.

Results and Conclusion: Among the 294 patients analyzed (March 2001), we found a similar distribution of the 95 (32%) MSI+ cases across the different disease stages (ranging from 31% of Dukes A to 34% of Dukes C cases), whereas a prevalence of MSI was observed for tumors of the right colon (20/34; 59%) in comparison to those of other sites [remaining colon (20/65; 31%) or rectum (55/195; 28%)]. Among the 174 patients whose family history was investigated, we found 11 (65%) MSI+ cases out of 17 familial CRC (> 3 affected members) versus 38/157 (24%)