**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.

1000 ORAL

### 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

1002 ORAL

### 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.

1003 ORAL

### 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%)

in the remaining sporadic CRCs. No differences in disease-free survival (DFS) and overall survival (OS) were observed between MSI- and MSI+ patients with localization not including the rectum (median DFS: 18 and 19 months, respectively; median OS, 21 months in both groups), whereas a favourable prognostic value was found for rectal cancer patients with MSI+ (median DFS and OS: 30 and 34 months vs 22 and 25 months for MSI- cases, respectively). Preliminary data from IHC revealed a high concordance between down-regulation of MLH1 expression and presence of MSI+ phenotype. Significant CRC cases to be screened for mutations in mismatch repair genes have been thus identified.

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1004 ORAL

## Clinical determinants of tumor response in patients with 5-FU based treatment for metastatic colorectal cancer. Results of a multivariate analysis of 3825 patients

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**Purpose:** To identify clinical prognostic factors predictive for response rate (RR) in previously untreated patients with metastatic colorectal cancer.

**Methods:** Source data of 3825 patients from 19 randomized trials have been analyzed using recursive partitioning and amalgamation (RECPAM) and cluster by response (CBR) methodology on a learning (LER) and validation (VAL) sample. Variables were grouped into laboratory (WBC, PLT, Hb, AP, LDH, Bilirubin, ALAT, ASAT, protein, albumin, CEA) or tumor burden (colon or rectal primary, grading, No of metastatic sites [NSites], presence of liver, lung, lymphnode or peritoneal metastases) or clinical parameters(ECOG, weight loss, tumor related symptoms). A minimum of 100 patients had to remain in any prognostic subgroup throughout the analysis. In the first step the analysis was performed in each group and the "winners" of each group then entered the final model.

**Results:** The final prediction model using CBR was as follows: good risk (n=956)(LER RR 29.9%; VAL RR 24.3%): ECOG(0/1) AND NSites(1 only) AND Hb (>11gpt/l); poor risk (n=734)(LER RR 15.8%; VAL RR 14.8%): all other patients. Using the RECPAM methodology good risk patients (LER RR27.8%; VAL 23.4%) had platelets ≤400x109/L AND Hb >11 gpt/l AND Nsites=1 AND no peritoneal mets AND ECOG=0,1; all other patients were poor risk patients (LER RR17.4%; VAL 14.5%). If treatment failure (prevention of early PD during chemotherapy) is the endpoint, patients with ECOG 0 or 1 AND Nsites=1 had a 71.2% chance of no early PD while all other patients had a 52.1% chance of no early PD.

**Conclusions:** Patients can be divided into at least 2 risk groups depending on initial performance status, WBC, number of metastatic sites and hemoglobin levels, parameters that were also identified as independent predictors for survival (ASCO 2000). Consistency between the two statistical methods is high. However, the prognostic power of these clinical parameters comes out to be considerably weaker for the prediction of response or prevention of early PD as compared to the prediction of survival. Other parameters like level of intratumoral thymidilate synthase may be more useful to predict response.

1005 ORAL

## Capecitabine in combination with oxaliplatin as first line therapy for patients (pts) with advanced or metastatic colorectal cancer (ACRC): preliminary results of an international multicenter phase II study

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**Introduction:** Xeloda (capecitabine) is a novel oral fluoropyrimidine, which is converted to 5-fluorouracil (5FU) at the tumour site by exploiting the higher activity of thymidine phosphorylase in malignant tissue. It has demonstrated superior activity and an improved safety profile compared with iv bolus 5-FU/leucovorin (LV) Mayo Clinic in two large phase III trials. Oxaliplatin combined with 5FU/LV is also a recognised first line therapy option for ACRC, with superior response rates and time to progression compared with 5FU/LV alone in phase III trials.

**Methods:** Following a phase I study, the current phase II trial evaluated the efficacy and safety of oral capecitabine (1000 mg/m2 twice daily d1-14, q3 weeks) and intravenous oxaliplatin (130 mg/m2 d1, q3 weeks) as first line therapy for pts with ACRC.

**Patient characteristics:** At present enrolment is completed with 96 pts. For this preliminary report pts enrolled during the first 4 months of the study were included: 37 are assessable for safety and 34 for efficacy. The median number of treatment cycles per pt was 6 (1-10). Twenty-six men and 11 women, with a median age of 65 years (48-79 years) and a median Karnofsky Performance Status of 100 (80-100) were included. 28 (76%) pts had liver metastases.

**Safety:** Grade 3/4 adverse reactions reported in more than one pt were vomiting (14%), nausea (11%), diarrhea (8%), neutropenia (8%) and thrombocytopenia (5%). Of note, no pts had grade 3 hand-foot syndrome (24% grade 2) or grade 3/4 peripheral neuropathy (8% grade 2). There were two cases of laryngo-pharyngeal dysesthesia (grades 2 and 3). Six (16%) pts withdrew from study due to thrombocytopenia, asthenia, hemiparesis, diarrhea, lethargy, flushing. The most common grade 3/4 laboratory abnormality was increased bilirubin, found in 19% of pts, which was not associated with grade 3/4 elevated liver enzymes. Elevated bilirubin is a known phenomenon with oral fluoropyrimidines. One death was attributed to study treatment: respiratory failure in a pt with pre-existing pulmonary fibrosis.

**Efficacy:** The objective response rate was 50%, including 2 complete and 15 partial responses (3 PR not yet confirmed). A further 10 pts (33%) had stable disease. Of 11 pts who had received prior adjuvant fluoropyrimidine treatment, 6 pts achieved a PR (55%).

**Conclusion:** These data indicate that the capecitabine and oxaliplatin combination is highly active and has an acceptable safety profile as first line therapy in ACRC.

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## Marked differences in tumour-associated protein expression and genetic stability between proximal and distal colon tumours

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**Purpose:** There is increasing evidence that proximal and distal colon tumours comprise distinct diseases, which may impact on the clinical outcomes of colon cancer patients. The aim of this study was to characterise phenotypic and genomic differences between proximal and distal colon tumours.

**Methods:** IHC was carried out on 189 colon tumours (n=90 proximal and n=99 distal tumours), to evaluate the expression of the following proteins: beta-catenin, cyclin D1, pRb, p53, p21, p27, p16, PCNA and EGFR. In addition, CGH was used to evaluate genetic aberrations in a subset of 25 samples (n=9 proximal and n=16 distal tumours). Analysis of protein expression with respect to tumour site was carried out using the chi-squared test, while patient survival was tested using Kaplan-Meier survival plots and analysed using the log rank test.

**Results:** Several of the proteins evaluated by IHC demonstrated distinct differences in expression between proximal and distal colon tumours. Nuclear accumulation of beta-catenin was markedly less frequent in proximal tumours compared to distal lesions (P=0.002), as was aberrant expression of p53 (P=0.028). Conversely, p21 protein was more commonly expressed in proximal tumours (P=0.018). While EGFR expression did not vary significantly between the tumour sites, expression was associated with patient survival in proximal tumours (P=0.018), but not in distal lesions (P=0.807). Further disparity was observed between proximal and distal tumours using CGH, where the total number of genetic aberrations in proximal tumours (n=8.7) was notably higher than in distal lesions (n=4.8).

**Conclusion:** Marked differences in the expression of tumour-associated proteins, and also in the level of genomic aberration, have been demonstrated between proximal and distal colon tumours. These dissimilarities may have an effect on the clinical outcomes of colon cancer, including patient survival. Elucidating such differences might therefore assist in selecting the treatment options most likely to be effective.