PERGAMON

European Journal of Cancer 38 (2002) 880–886

European Journal of Cancer

www.ejconline.com

Prognosis and response to therapy in colorectal cancer

J. Walker, P. Quirke*

Pathology Department, Leeds University, Leeds LS2 9JT, UK

Received 18 December 2001; accepted 31 January 2002

Abstract

Colorectal cancer is Europe's second biggest cancer killer. Yet despite advances in knowledge and changes in chemotherapy practice, we have not seen great strides in improved survival. Histopathological staging is at present the most accurate prognostic factor for survival and recurrence. Improvements in staging have led to the recognition of the importance of the circumferential resection margin (CRM) and how the quality of surgery influences local recurrence rates. Further refinements in staging and increasing knowledge of tumour biology will have a large contribution to play in the future.

Keywords: Colorectal; Surgery; Tumour biology; Circumferential resection; Margin; Histopathological staging

1. Prognosis

For any individual patient, it is essential that their survival can be accurately predicted and the likely sites of recurrence identified. The methods of prediction should be simple, widely available, sensitive, specific and reproducible in any clinical setting in the world. Prognosis is affected by a large number of factors, the most important of which are the clinical stage of presentation, the choice of surgeon, their ability to perform a curative or palliative operation, histopathological stage and the appropriate type of treatment.

2. Clinical stage

It is clearly important whether a patient presents with early stage colorectal cancer that will allow the surgeon to remove all of the disease. It is also essential for the clinical stage of presentation to be accurately recorded so that surgical outcome can be compared between surgeons to identify where improvements are required. Good radiological staging must be available for the liver and pelvis. The former is widely available, but the latter is not and radiologists are only just recognising that high quality imaging of the boundaries of the mesorectum and anal canal underlies an informed decision-making process when managing rectal cancer [1]. Beets-Tan and colleagues [2] have reported a high accuracy (83%) in predicting involvement of the pathological CRM.

3. Surgical quality

There is now ample evidence of the importance of the surgeon in the outcome from colorectal cancer. McArdle and colleagues [3] showed variations between 20 and 63% for 10-year survival in a survey of 13 Consultant Scottish surgeons and the German colorectal cancer group [4–6] have reported wide surgical variation for both rectal and colonic cancer surgery. Much of this remains unexplained, but for rectal cancer the key feature appears to be adequate clearance of the local tumour by following the mesorectal plane of resection. Outcomes are markedly improved by mesorectal excision (TME) following the mesorectal fascia [7]. This has been clearly shown by the very low rates of local recurrence and improved 5-year survival when this operation is performed. It has now been proved beyond any doubt that most surgeons were not performing an adequate mesorectal clearance as shown by historical local recurrence rates of 20–30% in 'curative' operations and that with expert surgical tuition and pathological audit it is

* Corresponding author. Tel.: +44-113-233-3412; fax: +44-113-392-2834.

E-mail address: philq@pathology.leeds.ac.uk (P. Quirke).

0959-8049/02/$ - see front matter
PII: S0959-8049(02)00044-8

possible to decrease these rates to 6–8% [8–10] or even 0–3% [11,12] with the addition of short course Swedish radiotherapy. The frequency of circumferential margin involvement falls with the improvement in surgery and parallels the reduction in survival and thus the frequency of margin involvement can be used as an immediate predictor of the likely quality of surgery [13]. The quality of the surgical specimen is also a factor that will identify a higher rate of local recurrence. Incompletely removed surgical specimens with clear margins had a higher rate of local recurrence than nearly or totally complete specimens [14]. Thus, for rectal cancer, it is now possible to achieve a degree of quality control of surgery. The important factors for colonic cancer have not yet been determined, but do exist. Hermanek and colleagues [5,6] cites involvement of resection margin, timing of surgery, place of surgery, surgical volume and intraoperative local tumour spillage (confirmed by others [15–17]). The improvement in margin positivity and local recurrence rates with the adoption of TME has not been seen in abdomino-perineal resections, even Heald continues to have a high rate of local recurrence when performing this operation [18]. Recently, we have demonstrated a higher rate of CRM involvement and local recurrence with a poorer survival in these patients. We believe this is due to the anatomical restraints on surgery in the low rectum. The tapering end of the mesorectum with the surgical habit of taking the levator ani muscle close to the wall of the low rectum greatly reduces the possibilities for adequate surgical clearance. All patients who are being considered for an abdomino-perineal excision must be assessed for preoperative therapy. With the early results from the Dutch TME and radiotherapy study showing that involved margins are not salvaged by short course radiotherapy. We urgently need a study to identify whether such patients require either long course preoperative radiotherapy or radiochemotherapy for salvage. Investigation of wider surgical excision at the height of the levators is also required. The other possible cause of a higher rate of local recurrence is the possibility of spread to the iliac and obturator nodes. This clearly occurs, but in our opinion is currently less important than local incomplete removal of the tumour. The evidence for this is the higher rate of margin involvement in low rectal cancers and an analysis of the patterns of local recurrence by Suzuki and colleagues [19]. Hopefully the adoption of radiochemotherapy will allow treatment of both areas and improve outcome.

4. Histopathological staging

Staging of the received resection specimens by a trained histopathologist is essential for the proper management of colorectal cancer. The macroscopic description and dissection of the specimen are critical in obtaining adequate information for management. Time is needed for this examination. Adequate fixation takes at least 48 h, the pathologist needs to spend 20–40 min on the gross dissection with digital photography of the specimen and more than 10 blocks will need to be taken. Microscopic examination needs to concentrate on margin and lymph node involvement, depth of spread and peritoneal penetration [20]. Despite investigation of various immunohistochemical and molecular markers in large numbers of studies, none to date have as yet proved robust enough for routine clinical application. The above findings should be brought to the multidisciplinary team meeting of the surgeons, radiologists, oncologists and pathologists for discussion of patient management.

This is the ideal scenario. How good is routine histopathology? Audit has shown considerable variation in the standard of reporting between individual pathologists. Blenkinsopp and colleagues [21] reviewed the reports of some 2046 patients showing considerable variation in grading, staging and lymph node harvest. This was confirmed in a 1993 Welsh study looking at 1242 reports. 78% of colonic tumours and only 46.6% of rectal tumours had reports which met the minimum standards [22]. Both studies cited proforma reporting as a way towards improvement. Indeed, the introduction of such a style of reporting has been seen to be the only way to ensure all relevant information is included in a cancer staging report [23].

5. Which staging system to use?

There have been many debates over which staging system to use in routine practice. The major international choices are between Dukes and TNM, although the Australian staging system had many useful features. A staging system must not only allow accurate prediction of prognosis, but should identify the likely sites of failure, as well as the receipt of preoperative therapies such as radiation or chemotherapy. For example, TNM can identify cases at high risk of local recurrence via the R0/R1 stage [24], peritoneal recurrence via its T4 stage and liver metastases via M0/M1. The prefix y in front of TNM identifies a case which has had previous therapy. Dukes clearly fails to predict peritoneal recurrence or local recurrence of rectal cancer and there is no statement about preoperative treatment. Dukes is also of little value when trying to correlate imaging to the gold standard of pathological staging. TNM also now has the advantage of international acceptance and is increasingly reported in International trials. Dukes is an easy rapid way of describing cases and is widely understood, but should only be used as a second-line staging system and never on its own. The national UK

colorectal cancer proforma uses TNM as its primary staging system and this is now incorporated in all the UK national trials (www.rcpath.org/activities/publications/ccancer/html).

6. Accuracy of staging

There is little or no data on the reproducibility of pathological staging because of the impossibility of dissecting the same specimen twice. As stated above, we know pathologists fail to identify features and record them, but given excellent training how variable is staging between pathologists? We know that lymph node yields vary between pathologists and that this has been reported to impact on the treatment of patients [25], but the literature is very variable about the importance of finding large numbers of lymph nodes. Until this question is definitively answered, we should continue to make major efforts to recover all lymph nodes from resections and aim to meet the requirements of TNM of finding 12 lymph nodes on as many specimens as possible, however we all know that this is not possible on some cases, especially those who have undergone 6 weeks of radiotherapy. We also know little about the reproducibility of circumferential margin involvement and peritoneal involvement. These areas require urgent study if they are to be used as indicators of the need for treatment.

7. Key features of staging

Pathological staging informs the decision-making process on postoperative therapy with respect to adjuvant therapy and postoperative radio- or radio-chemotherapy. It is accepted that N1/N2 or Dukes' stage C patients benefit from chemotherapy (absolute survival benefit of 5% [26]). Thus the identification of lymph node involvement is essential and a failure to do so is a disservice to the patient. Most of the early trials relied on standard pathology and thus it is likely that only the cases with a relatively large burden of disease were identified. It was not unusual for some of these studies to have a lymph node yield of five nodes where these important factors were audited. The benefit to patients with a lesser degree of metastatic burden is unclear as is the benefit to T3/T4 N0 cases (Dukes' B). In many centres, these are being treated, especially where adverse pathological factors exist but this haphazard approach to adjuvant therapy hinders progress and needs to be informed by prospective randomised trials such as QUASAR1. We need to know which pathological factors are indeed the best predictors of a poor outcome, which factors predict for each type of recurrence and whether these different modes of spread are amenable to adjuvant chemotherapy. A large number of potential pathological staging factors are cited. Which of these are truly adverse and which are treatable? Is lymphatic permeation less important than vascular invasion; does extensive extramural spread matter if there is no vascular invasion; what is the relative response of cases with peritoneal involvement to intravenous adjuvant therapy? These questions are currently under study in QUASAR1 and initial results should be available early next year.

Patients with microscopic involvement of the circumferential margin of resection in rectal cancer have a high rate of local recurrence [27,28]. Margin involvement has been used as the indicator for postoperative radiotherapy in the Dutch colorectal study group and for postoperative radio-chemotherapy in the MRC CR07 trial. Early results from the Dutch study indicate that postoperative radiotherapy alone does not lessen the recurrence rates of margin-positive rectal cancer. We await the results from CR07 to identify whether these patients are salvageable by radio-chemotherapy. If we are moving routinely to such therapy, we must ensure that circumferential margin involvement (CRM involvement) is as accurate and reproducible as possible. Surgeons can be assessed on the basis of the frequency of circumferential margin involvement. In an audit of our local surgeons, the frequency of CRM positivity predicted the survival of patients [13]. Reduction in the frequency of margin involvement led to an improvement in 5-year survival for individual surgeons. The current frequency of CRM positivity in curative cases in MRC CR-07 is 16% and in the Dutch trial data 16–19%. A second way of assessing the quality of surgery developed in Leeds, has been recently presented in the results from the Dutch TME/RT-TME study where a poor quality of resection as judged by a pathologist assessing the completeness of removal of the mesorectum also identified a group at a higher risk of local recurrence. A poor resection is one where there is little bulk to the mesorectum with defects down onto the muscularis propria and/or a very irregular CRM [29]. Currently, this group forms approximately 15% of all cases in the Dutch study and in CR-07. Improvements in surgery in these cases would lead to a further reduction in local recurrence of rectal cancer.

Identification of peritoneal involvement is clearly important with respect to prognosis [20], but we do not know how they respond to intravenous chemotherapy. Scheithalier [30] has shown that intraperitoneal chemotherapy is beneficial to these patients and further studies of directed chemotherapy are required. It does seem likely that we require anatomically-directed therapies guided by pathological or molecular staging and knowledge of the response rates of the different sites of failure. Individualised therapy is required.

8. Post-therapy staging

With the advent of preoperative chemoradiotherapy, we will increasingly encounter the phenomena of downstaging of the primary tumour occurring before staging of the resected specimen is undertaken. This does not appear to be a major problem after short course radiotherapy if the patient is operated on within 7 days, but with increasing delay the tumour may show increasing effects of radiotherapy. A tumour response is seen as a diminution in the number of tumour cells and increased fibrosis. There may even be a complete loss of tumour in 23% of cases after preoperative long course chemo-radiotherapy [31]. At present, there is no objective way of scoring this response, only a subjective description. It may be useful to apply a grading system, such as that proposed by Mandard for the oesophagus [32], so that the patient's response can be more accurately graded or a simple quantitative assessment of the density of tumour cells may be advantageous. We also do not know the importance of the mucoid lakes that may be left behind post-treatment. Do the borders of these accurately reflect the pretherapy stage, what is the importance of CRM positivity by these possibly tumour-sterile lakes, do they indicate a good response and thus a low risk of recurrence? It must be borne in mind that the stage given by the pathologist will be a reflection of treatment response rather than the stage of the disease and disentangling the two with respect to prognosis is difficult. How we plan further treatment on the basis of rectal cancers treated by preoperative chemo-radiotherapy is as yet unsure.

9. Response to therapy

It is clear there are different molecular subtypes of colorectal cancer. These can be classified on the basis of their cytogenetic and molecular defects or their pharmacogenetic patterns. It is currently unclear as to which is the most important. It is likely that a mixture of both will ultimately be used to determine treatment.

Current molecular classifications concentrate on identifying chromosomally unstable cancers that are easily revealed by flow cytometry where non-diploid populations are shown. These cancers have triploid to tetraploid populations and are grossly abnormal on comparative genomic hybridisation. They usually have an abnormal p53 pathway and loss of multiple tumour suppressor genes. The chromosomally stable cancers are comprised of the microsatellite unstable tumours accounting for 15% of all colorectal cancers and other tumours that do not show microsatellite instability, but may show tumour suppressor gene methylation (*p16*, *MGMT*, *THBS1* [33]). Unpublished work from our laboratory on 400 randomised patients prospectively investigated in the AXIS study shows a poorer prognosis for DNA aneuploid tumours when compared with DNA diploid tumours, but no difference in the response to therapy when using intraportal 5-FU chemotherapy. Unlike the retrospective analysis by Elasaleh and colleagues [34], we saw only a trend to a better prognosis in microsatellite unstable cancers, but no difference in response to therapy between those cases with microsatellite instability and those without. This was confirmed with hMLH-1 immunocytochemistry [35] with identical survival curves to those with microsatellite instability for prognosis and response to therapy. Thus, in our hands, this classification does not seem to be of great value in predicting the response to therapy. Intriguingly, we saw a good response to intraportal 5-FU in patients with evidence of normal 18q and 17p when assessed by the use of microsatellites to three markers in this area. Those cases with allelic imbalance in this area did not show any response to therapy. This finding needs confirmation in further series with more modern chemotherapy.

An alternative approach is a pharmacogenetic one studying the levels of important proteins that control metabolic pathways, either creating active agents or inhibiting the breakdown of important chemotherapeutic agents such as 5-FU/folinic acid, oxaliplatin or irinotecan. This approach has been highly successful in breast cancer when used to determine patients who require antibody-directed therapies such as Herceptin against amplified HER-2/c-erbB2. There are reports in the literature using antibodies to thymidylate synthase, thymidine phosphorylase, dihydropyrimidine dehydrogenase and deoxyuridine thymidine phosphorylase to predict response, but these are all small retrospective studies conducted outside of randomised trials and are thus unreliable as to whether they should be used in routine practice [36,37]. What are required are large-scale studies in the context of randomised clinical trials to finally evaluate the role of such markers. These should be easier to perform with the advent of tissue microarrays [38,39].

10. Molecular staging

Molecular methods have been used in an attempt to screen patients for colorectal cancer looking for the presence of mutations in the stool or molecular abnormalities in DNA released from tumours into plasma and to identify patients with micrometastatic disease in lymph nodes. Many of these studies are proof of the principle of the test, but do not inform the debate about their robustness in routine use. We have known for many years that tumour cells can be found in the peripheral blood and that further testing of lymph nodes by techniques such as immunocytochemistry can

identify involved nodes. What has not happened is the testing of these new techniques against the current gold standard to prove their benefit. Common molecular targets are Ki-*ras*, *TP53* mutations and the presence of microsatellite instability. The drawback of such tests is the limited presence of the molecular abnormality under study, e.g. 40% for Ki-*ras*, 70% for *TP53* and 15% for microsatellite instability. One test does not identify all cases. Promising studies include the following. Thebo and colleagues [40] looked at Ki-*ras* mutations in lymph nodes to upstage Dukes' B patients. None of 4 patients with mutation-free nodes developed recurrence, whereas 37.5% of those with positive lymph nodes did. Ki-*ras* mutations identified in plasma DNA have also been shown to be strongly associated with the presence of a colorectal neoplasm, bearing such mutations [41,42]. p53 antibodies have been demonstrated to be present in 14 of 54 colorectal cancer patients (26%) by an enzyme-linked immunoabsorbant assay, with none being present in 24 patients with non-malignant digestive disease [43]. The identification of *TP53* [44] and Ki-*ras* mutations [45,46] from DNA shed from tumour into the stool has also proved possible. A small number of such samples, 22, have also been analysed using a panel of assays (including Ki-*ras*, *TP53*, *APC* and a micro-satellite marker). This demonstrated a sensitivity of 91% and specificity of 93% in identifying malignancy [47].

11. The future

We are now no longer limited to the study of a few markers when attempting to predict the biological behaviour and response to therapy of colorectal cancers. It is now possible to study the patterns of expression of thousands of genes using cDNA arrays, of thousands of single nucleotide polymorphisms using DNA chips or thousand of proteins using proteomic technologies. New data is emerging from large-scale studies of gene expression. In collaborative studies with Genentech on Affymetrix chips obtained data showed that roughly 5% of 6000 genes were overexpressed at a much higher level and 5% underexpressed when comparing colorectal adenocarcinomas with their corresponding normal mucosa (data not shown). Publications by Notterman [48] and Kitahara [49] used different cut-offs. Notterman [48] using the Affymetrix 6500 chips showed 19 (0.48%) of transcripts had at least 4–10.5-fold higher mRNA expression and 47 (1.3%) 4–38-fold lower expression. They identified a large number of individual genes some of which could be hypothesised to be involved in colorectal cancer. Reverse transcriptase-polymerase chain reaction (RT-PCR) confirmation of some of these was obtained. Hierarchical clustering appeared to be able to separate the tumours, but this was after significant data removal with only 1096 genes and EST's included and stripping out of muscle- and connective tissue-associated genes. Hierarchical clustering usually requires a large amount of data manipulation and the robustness of these assays remains to be seen when tissue and tumour heterogeneity is not rigidly controlled. Kitahara and colleagues [49] used a printed cDNA array of 9216 genes and explored the cDNA expression patterns of laser capture microdissected tissue. They reported upregulation of 44 genes and downregulation of 191 genes for more than half of their 8 cancers analysed. RT-PCR data were consistent in 64 of 74 experiments revealing a concordance of 86.5%. They went on to test the other 12 collected tumours and confirmed the data. Several of the genes identified by both sets of workers were also abnormal in our data.

These techniques have unparalleled power, but also immense complexity. They will surely identify new molecular abnormalities and important genes, which determine response to therapy, but whether large cDNA or DNA chips will ever be introduced into routine practice is another question. They would have to demonstrate superiority over the information provided by routine histopathology and simple immunocytochemical tests. Early reports on small numbers of cases are appearing and target genes are being identified for further testing. It is important that such targets are vigorously tested. Current indications are that they will not be. Genes such as *TP53* were described several decades ago, but we still do not have the answers required as to the value of these genes. Major improvements in translational research are required to reap the benefits from such new technologies. The widespread adoption of tissue microarrays and the sharing of such material will hopefully lead to a more systematic approach to the investigation of target genes cDNA arrays.

References

1. Brown G, Radcliffe A. Will MRI provide maps of lines of excision for rectal cancer. *Lancet* 2001, **357**, 495–496.
2. Beets-Tan RGH, Beets GL, Vliegen RFA, *et al*. Accuracy of magnetic resonance imaging in prediction of tumour-free resection margin in rectal cancer surgery. *Lancet* 2001, **357**, 497–503.
3. McArdle CS, Hole D. Impact of variability among surgeons on post-operative morbidity and mortality and ultimate survival. *Br Med J* 1991, **302**, 1501–1505.
4. Hohenberger W. The effect of specialization or organization of rectal cancer surgery. In Soreide O, Norstein J, eds. *Rectal Cancer Surgery. Optimisation-Standardisation-Documentation*. Berlin, Springer-Verlag, 1997, 353–363.
5. Hermanek PJR, Wiebelth H, Riedl S, Staimmer D, Hermanek P. Long-term results of surgical therapy of colon cancer; results of the colorectal cancer study group. *Chirurgury* 1994, **65**, 287–297.
6. Hermanek PJR, Mansmann U, Staimmer DS, Riedl S, Hermanek P. The German experiences: the surgeon as a prognostic factor in colon and rectal cancer surgery. *Surg Oncol Clin North Am* 2000, **9**, 33–49.

7. Heald RJ, Husband EM, Ryall RDH. The mesorectum in rectal cancer surgery—the clue to pelvic recurrence. *Br J Surg* 1982, **69**, 613–616.
8. Martling AL, Holm T, Rutqvist LE, Moran BJ, Heald RJ, Cedemark B. Effect of a surgical training programme on outcome of rectal cancer in the county of Stockholm. Stockholm colorectal cancer study group, Basingstoke Bowel Cancer research project. *Lancet* 2000, **356**, 93–96.
9. Dahlberg M, Pahlman L, Bergstrom R, Glimelius B. Improved survival in aptients with rectal cancer: a population based register study. *Br J Surg* 1998, **85**, 515–520.
10. Havenga K, Enker WE, Norstein J, Moriya Y, van Houwelingen HC, van de Velde CJ. Improved survival and local control after total mesorectal excision or D3 lymphadenectomy in the treatment of primary rectal cancer: an international analysis of 1411 patients. *Eur J Surg Oncol* 1999, **25**, 368–374.
11. Dahlberg M, Glimelius B, Pahlman L. Changing strategy for rectal cancer is associated with improved outcome. *Br J Surg* 1999, **86**, 379–384.
12. Graf W, Dahlberg M, Osman MM, Holmberg L, Pahlman L, Glimelius B. Short-term radiotherapy in down staging of rectal cancer: a study of 1316 patients. *Radiother Oncol* 1997, **43**, 133–137.
13. Birbeck KF, Macklin CP, Dixon MF, *et al*. Circumferential margin status in rectal cancer.Variation depending on surgeon, and relationship to local recurence. *Dis Colon Rectum* 1999, **42**, A8.
14. Nagtegaal I D, van der Worp E, Quirke P, *et al*. The importance of the correct total mesorectal excision technique—prognostic value of the macroscopic analysis of the resection analysis of the resection specimen by pathologists. *J Clin Oncol*, in press.
15. Kockerling F, Reymond MA, Altendorf-Hofmann A, Dworak O, Hohenberger W. Influence of surgery on metachronous distant metastases and survival in rectal cancer. *J Clin Oncol* 1998, **16**, 324–329.
16. Holm T, Johansson H, Cedermark B, Ekelund G, Rutqvist L-E. Influence of hospital and surgeon related factors and outcome after treatment of rectal cancer with or without pre-operative radiotherapy. *Br J Surg* 1997, **87**, 657–663.
17. Porter GA, Soskolne CL, Yakimets WW, Newman SC. Surgeon related factors and outcome in rectal cancer. *Ann Surg* 1998, **227**, 157–167.
18. Heald RJ, Smedh RK, Kald A, Sexton R, Moran BJ. Abdominoperineal excision of the rectum-an endangered operation. *Dis Colon Rectum* 1997, **40**, 747–751.
19. Suzuki K, Muto T, Sawada T. Prevention of local recurrence by extended lymphadenctomy for rectal cancer. *Surg Today* 1995, **25**, 795–801.
20. Shepherd NA, Baxter KJ, Love SB. Influence of local peritoneal involvement on pelvic recurrence and prognosis in rectal cancer. *J Clin Path* 1995, **48**, 849–855.
21. Blenkinsopp WK, Stewart-Brown S, Blesonsky L, Kearney G, Fielding LP. Histopathology reporting in large bowel cancer. *J Clin Pathol* 1981, **34**, 509–513.
22. Bull AD, Biffin ANH, Mella J, Radcliffe AG, Stamatakis JD, Steele RJ, Williams GT. Colorectal cancer reporting: a regional audit. *J Clin Pathol* 1997, **50**, 138–142.
23. Cross SS, Feeley KM, Angel CA. The effect of four interventions on the informational content on histopathology reports of resected colorectal carcinomas. *J Clin Pathol* 1998, **51**, 481–482.
24. Wittekind C, Hermanek P. Residual tumour classification. *Adv Anat Pathol* 1995, **2**, 277–279.
25. Wong JH, Severino R, Honnebier MB, *et al*. Number of nodes examined and staging accuracy in colorectal carcinoma. *J Clin Oncol* 1999, **17**, 2896–2900.
26. Midgley R, Kerr D. Colorectal cancer-review. *Lancet* 1999, **353**, 391–399.
27. Quirke P, Durdey P, Dixon MF, Williams NS. Local recurrence of rectal adenocarcinoma is due to inadequate surgical excision. Histopathological study of lateral tumour spread and surgical excision. *Lancet* 1986, 996–999.
28. Adam IJ, Mohamdee MO, Martin IG, *et al*. Role of circumferential margin involvement in the local recurrence of rectal cancer. *Lancet* 1994, **344**, 707–711.
29. Colorectal Cancer Working Party. CR07 Pathology guided treatment in rectal cancer. a randomized trial comparing pre-operative radiotherapy and selective post-operative chemoradiotherapy in rectal cancer. *Clinical Protocol*. Medical Research Council.
30. Scheithalier W, Kornek GV, Marczell A, *et al*. Combined intravenous and intraperitoneal chemotherapy with fluorouracil and leucovorin Vs fluorouracil and levamisole for adjuvant therapy of resected colon carcinoma. *Br J Cancer* 1998, **77**, 1349–1354.
31. Cooper R, Finan P, Sagar P, *et al*. Pre-operative chemoradiotherapy for locally advanced carcinoma of the rectum. Submitted for publication.
32. Mandard AM, Dalibard F, Mandard J-C, *et al*. Pathological assessment of tumour regression after preoperative chemoradiotherapy of esophageal carcinoma. *Cancer* 1994, **73**, 2680–2686.
33. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci USA* 1999, **96**, 8681–8686.
34. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet* 2000, **355**, 1745–1750.
35. Cawkwell L, Gray S, Murgatroyd H, *et al*. Choice of management strategy for colorectal cancer based on a diagnostic immunohistochemical test for defective mismatch repair. *Gut* 1999, **45**, 409–415.
36. Lander RD, Lynch FJ, Groshen S, *et al*. DUTP nucleotidohydrolase isoform expression in normal and neoplastic tissue: association with survival and response to 5-fluorouracil in colorectal cancer. *Cancer Res* 2000, **60**, 3493–3503.
37. Edler D, Hallstrom M, Johnston PG, *et al*. Thymidylate synthase expression: an independent prognostic factor for local recurrence, a distant metastasis, disease-free and overall survival in rectal cancer. *Clin Cancer Res* 2000, **6**, 1378–1384.
38. Moch H, Kononen T, Kallioniemi OP, Sauter G. Tissue microarrays: what will they bring to molecular and anatomic pathology. *Adv Anat Pathol* 2001, **8**, 14–20.
39. Budendorf L, Nocito A, and Moch H, Sauter G. Tissue microarray technology: minaturized pathology archives for high-throughput insitu studies. *J Pathol*. In press.
40. Thebo JS, Senagore AJ, Reinhold DS, Stapleton SR. Molecular staging of colorectal cancer: K-ras mutation analysis of lymph node upstages Dukes' B patients. *Dis Colon Rectum* 2000, **43**, 155–162.
41. Kopreski MS, Benko FA, Borys DJ, Khan A, McGarrity JJ, Gocke CD. Somatic mutation screening: identification of individuals harbouring K-ras mutations with the use of plasma DNA. *J Natl Cancer Inst* 2000, **92**, 918–923.
42. De Kok JB, van Solinge WW, Ruers TJM, *et al*. Detection of tumour DNA in serum of colorectal patients. *Scand J Clin Lab Invest* 1997, **57**, 601–604.
43. Hammel P, Boissier B, Chaumette M-T, *et al*. Detection and monitoring of serum p53 antibodies in patients with colorectal cancer. *Gut* 1997, **40**, 356–361.
44. Euchi S, Kohara N, Komuta K, Kanematsu T. Mutations of the p53 gene in the stool of patients with resectable colorectal cancer. *Cancer* 1996, **77**, 1707–1710.
45. Villa E, Dugani A, Rebecchi AM, *et al*. Identification of subjects at risk for colorectal carcinoma through a test based on K-ras determination in stool. *Gastroenterology* 1996, **110**, 1346–1353.

46. Puig P, Urgell E, Capella G, *et al*. Improved detection of K-ras codon 12 mutations in fecal exfoliated cells. *Lab Invest* 1999, **79**, 617–618.
47. Ahlquist DA, Skoletsky JE, Boynton KA, *et al*. Colorectal cancer screening by detection of altered human DNA in stool: feasibilty of a multitarget assay panel. *Gastroenterology* 2000, **119**, 1219–1227.
48. Notterman DA, Alon U, Sierk AJ, Levine AJ. Transcriptional gene expression profiles of colorectal adenoma, adenocarcinomas and normal tissue examined by oligonucleotide arrays. *Cancer Res* 2001, **61**, 3124–3130.
49. Kitahara O, Furukawa Y, Tanaka T, *et al*. Alterations of gene expression during colorectal carcinogenesis revealed by cDNA microarrays after laser-capture microdissection of tumour tissues and normal epithelia. *Cancer Res* 2001, **61**, 3544–3549.