Pathology – a molecular prognostic approach

N J Maughan and P Quirke

Academic Unit of Pathology, University of Leeds, and Department of Pathology, Leeds General Infirmary, Leeds, UK

Colorectal cancer affects 29,000 people and kills approximately 15,000 in England and Wales each year, most of these deaths resulting from the effects of local or distant recurrence. There is a need to give these patients accurate prognoses and individualised treatment regimens. At present, the best prognostic markers are clinicopathological. Post-genomic science and the new high throughput technologies offer unrivalled opportunities to understand the biology and molecular pathology of colorectal cancer. These technologies should be used in the context of large randomised controlled trials to identify new molecular prognostic and predictive markers and also new targets for therapy.
information. The most promising molecular targets at the moment are predictive factors such as thymidylate synthase levels and other enzymes in the 5-FU pathway which give information on patient response to 5-FU chemotherapy. New high throughput technologies should allow the identification of many more novel factors in the future and, simultaneously, further elucidate the processes of cancer.

Prognostic and predictive factors

It is important to understand the exact differences between prognostic and predictive factors (Fig. 1). A prognostic marker should give prospective information on patient outcome by which therapeutic decisions can be guided. Predictive factors on the other hand give information on likely tumour response to a single or a group of therapeutic agents. To be of

Fig. 1 Graphs showing difference between prognostic and predictive factors. (A) A typical Dukes’ stage C CRC survival curve; (B) a prognostic factor divides these patients into two groups with differing survivals. In this example, patients with the prognostic factor have worse survival; (C,D) show a predictive factor. For patients without the predictive factor (C), survival is similar whether or not they receive chemotherapy. In (D), however, possession of the predictive factor identifies a group of patients likely to benefit from chemotherapy. Reprinted with permission from Elsevier Science from *The Lancet*.2
genuine use, prognostic and predictive factors must ultimately be applicable in a clinical setting: they must be better than those currently available, sensitive, specific, reproducible, deliverable by readily available systems and cost effective.

Prognostic factors can be divided into two groups:

1. The ideal – giving novel information that does not overlap or replicate that derived from already existing clinicopathological parameters.

2. Those giving the same information as known clinicopathological factors. Most of these may represent potential targets for new therapies but add nothing to current prognostication. A few may be useful to provide early prognostic data from an initial small biopsy, etc. before a patient is fully investigated.

**Identification of molecular factors**

*Study design*

To identify prognostic and predictive factors effectively, the studies used must have enough statistical power, and be relevant and applicable to modern practice. These studies are best done prospectively in the setting of a large scale randomised controlled trial (RCT) and frequently are ‘piggy backed’ onto existing RCTs looking at treatment options.

The study population should also be relevant: Dukes’ stage A (pT1pN0) CRCs are usually cured by surgery alone; therefore, studies which minimise the number of these cases and instead concentrate on Dukes’ stages B and C CRCs are likely to yield more useful markers. Other clinicopathological parameters such as depth of penetration, extramural vascular invasion and axial resection margin involvement (UKCCCR QUASAR1 study) are now revealing poor prognosis sub-groupings in patients where clinicians were uncertain of the benefits of adjuvant chemotherapy.

Lastly, studies must be applicable to modern practice, especially in the case of predictive factors. Chemotherapy for Dukes’ stage C cancers only became standard in many oncology centres 5–7 years ago and it is important that we identify factors which apply to the most modern regimens given rather than outdated and presently unused ones. Unfortunately, these limits also tend to reduce the length of follow-up that is presently available on patients. As responses to chemotherapy become longer, studies of predictive factors in metastatic Dukes’ stage D (pTxpNxpM1) cancers will become increasingly important.
In the last year, there has been much focus on the evolving fields of genomics and proteomics and the techniques they embrace which allow high throughput of samples and the generation of massive amounts of data in relation to gene expression so offering the possibility of identifying novel prognostic and predictive factors (Fig. 2).

The map of the human genome is available and this together with techniques of large scale analysis such as single nucleotide polymorphisms (SNPs), high resolution comparative genomic hybridisation (CGH), cDNA arrays, serial analysis of gene expression (SAGE), quantitative mRNA analysis, *in silico* profiling, proteomics and quantitative tissue arrays can be used to give data on changes in gene and protein expression within a tumour and also to localise this within...
a particular cell group. However, these techniques are not the panacea to all ills and have drawbacks: a danger of data overload, incompatible databases, difficulties of comparison of data between different trials and centres and between targets studied by different techniques, the need for confirmation of data by a third party and the frequent absence of comparison of data to the gold standard of pathology.

These drawbacks must be addressed and this remit requires the collaboration of research groups and the application of powerful bioinformatic tools to extract meaningful conclusions from the large amount of data generated which can be applied to clinical practice and improve patients’ prognostication and treatment. Increases in the level of sharing of data will allow the accumulation of large volumes of data on small numbers of well-performed clinical trials and avoid time wastage in the repetition of work already done. Classically, many of these techniques require fresh tissue which has also limited their scope. Recently, steps have been made in using paraffin-embedded archival material and this will allow both the use of tissue with years of follow-up and also the use of collections of rarer tumour types.

The road to colorectal cancer

The great majority of CRC (95%) is sporadic with at least four morphological pathways: via polyploid adenomas, flat adenomas, serrated adenomas or the ulcerative colitis/Crohn’s dysplasia-carcinoma sequence. The remaining 5% of CRC is inherited, the two main causes being familial adenomatous polyposis coli (FAP; caused by an inherited mutation in one of the alleles of the tumour suppressor gene APC) and hereditary non-polyposis colorectal cancer (HNPCC) now known to be caused by mutations in any one of a number of mismatch repair genes. Also numbered within the hereditary group are Peutz Jeghers syndrome (LKB1 mutation) and juvenile polyposis (SMAD4 mutation).

Within the population, subtle and ill-defined polymorphisms also exist which increase an individual’s risk of sporadic CRC: this is seen in the 11307K APC gene polymorphism in Ashkenazi Jews.

Do these morphological pathways differ when analysed by thousands of markers and does this give us insight into prevention, prognosis and treatment?

Subtyping colorectal cancer

It is now apparent that subtypes of CRC exist. There are genomically unstable aneuploid cancers (60–70% of CRCs) with gross chromosomal
abnormalities and diploid cancers with minor chromosomal abnormalities of 44 to 48 chromosomes. The latter contain tumours with microsatellite instability (10–15% of CRCs) and without microsatellite instability\textsuperscript{15,16}. Those with microsatellite instability acquire it by inactivation of DNA mismatch repair mechanisms either by mutation within hMLH1, hMSH2, hMSH6, hPMS1 or hPMS2\textsuperscript{17}, or more frequently by hypermethylation of the hMLH1 gene promoter at the adenoma-carcinoma interface\textsuperscript{18}. Whether microsatellite stable diploid cancers arise by methylation of other key genes is uncertain. There is evidence for early methylation of some genes in these tumours (e.g. MGMT)\textsuperscript{19}. We also have evidence for loss of expression of MGMT in methylated hMLH1 cases suggesting that these microsatellite unstable tumours may have other methylated genes (CpG island methylator phenotype)\textsuperscript{20,21}.

This classification appears meaningful in that all components have characteristic demographic and pathological features and locations. To date, cDNA array data produced both by ourselves and others have not identified any other clinically relevant groupings, but no doubt these will emerge. Recently, Zhou et al\textsuperscript{22} have suggested a new classification comparing tumours with 18q/8p loss to either 18q or 8p alone or no loss at these loci. They suggest that tumours with both 18q/8p loss had a much worse prognosis than either alone or no abnormality at these sites. The scientific data within this study are good; however, the clinicopathological aspects are poor with samples used that were derived from several centres in different countries over a 12-year time period and no attempt to audit the quality of pathological evaluation or substage these tumours. Therefore, although this study showed 18q/8p lesions to predict prognosis more accurately than Dukes' stage A or B as determined by pathology, it seems unfair to draw this conclusion about a (well-performed) scientific test as opposed to a (potentially poorly performed) pathological assessment on a heterogeneous group of samples. Furthermore, as stated by the authors, the inherent increased chromosomal instability required to produce the loss of two different chromosomal regions (i.e. aneuploidy) is known to impact on prognosis and this, rather than the specific lesions noted, may have caused the higher recurrence rates seen\textsuperscript{23,24}.

Do these genetic pathways differ when analysed by thousands of markers and does this give us insight into prevention, prognosis and treatment?

The pathway a tumour has evolved through as well as many other factors from inherent ones such as age, gender and ethnic background\textsuperscript{25} to clinicopathological to molecular factors can all affect the prognosis of an individual patient and therapeutic decisions such as surgery type and chemoradiotherapy regimen. As discussed, the molecular derangements in colorectal cancer are wide-spread, affecting many cell functions and these derangements offer targets both for prognostic and predictive information and new treatments.
All of these factors must be considered when working to give the most accurate prognostic information possible for an individual patient.

Clinicopathological prognostic factors

The gold standard for prognostication is clinicopathological stage\textsuperscript{3,4}. This gives information on depth of invasion of the tumour through the bowel wall, peritoneal involvement and also on the presence of lymph node or distant spread. This is the benchmark against which newly identified factors must be measured although, as mentioned above, these novel factors should also give new information rather than overlap with that given by clinical stage.

Clinical factors have been found to be independent prognostic indicators although to a lesser extent than tumour stage. These include: (i) inherent factors such as age, gender and ethnic background\textsuperscript{25}; (ii) presentation type (\textit{e.g.} bowel obstruction\textsuperscript{4}); (iii) intra-operative factors (\textit{e.g.} the surgeon and surgical technique used – total mesorectal excision \textit{versus} conventional operations in rectal cancer)\textsuperscript{26}; and (iv) whether blood transfusion was required.

Assessment of certain pathological features may also give independent prognostic information (\textit{e.g.} tumour site, grade, peritoneal invasion and lymphovascular invasion/extramural vascular invasion)\textsuperscript{4}.

Recent research has found that quality of clinical care, surgery and pathological assessment may all impact on patient survival. McArdle \textit{et al}\textsuperscript{27} show that, after adjustment for case mix and extent of deprivation, the hospital the patient is treated in can still significantly effect the outcome. The importance of quality of surgery has been recognised for several years and this can be monitored by the pathologist: the quality of total mesorectal excision surgery in colorectal cancer may be assessed by looking at rates of circumferential margin positivity\textsuperscript{28} and macroscopic appearances of the resection\textsuperscript{29}. Obviously, since pathologists are essential – both in providing feedback on quality of surgery and also in assessing other independent prognostic factors such as nodal involvement – they must also perform their job well\textsuperscript{30}.

It is important to give the patient the most favourable and accurate prognostic picture possible and this means that the performance of the clinical team, surgeon and pathologist must be carefully audited and poor performers identified and retrained. Quality assurance of both surgery and pathological assessment should include the number of nodes retrieved and the frequency of extramural vascular invasion, peritoneal involvement and circumferential resection margin involvement. It is essential that we use only patients receiving optimal clinical, surgical and pathological care in trials to test hypotheses on new prognostic and
predictive factors or we run the danger of only assessing the impact of substandard care as opposed to actual tumour biology.

**Molecular prognostic factors**

At present, it is hoped that the most accurate prognostic information will be achieved by combining both clinicopathological and molecular data. In the future, there may come a time when a small biopsy of a tumour can be run on cDNA microarrays or analysed by proteomic methods to give an individualised gene expression fingerprint leading to an accurate prognosis and personalised treatment regimen.

Currently, there are few recognised specific molecular prognostic factors in CRC (e.g. carcino-embryonic antigen [CEA])\(^4\), none of which are ideal. CEA is used as a serum marker of CRC recurrence in patients; however, staining of tumour sections with CEA antibodies has not been found to be particularly helpful in prognostication. Aneuploidy, a more general reflection of gross chromosomal disarray, has been shown to be a prognostic factor\(^23,24\). To progress from this state of affairs to our vision of the future, we must concentrate on looking at gene mutations or functional derangements in every step of the neoplastic and metastatic pathway. The functions which must become deranged for a cell to evolve from normal to cancerous include proliferation and the cell cycle, apoptosis, DNA repair, angiogenesis, cell adhesion and recognition, lytic enzyme production and cell motility\(^10\). Cells may also develop mechanisms to resist certain therapeutic agents. A single cell will not necessarily have to acquire all of these properties. The various subtypes of CRC show certain characteristic gene derangements which allow a cell to acquire some of these properties. Also, certain pathways may be targeted at more than one site by different subtypes of CRC. Below is a list of potential molecular prognostic factors, known to be deranged in CRC and grouped by CRC subtype, functional pathways and specific cellular functions. Work has already been done to link some of these genes to prognosis.

**Classification by CRC subtype**

**CRCs showing chromosomal instability**

Classically, CRC has been believed to develop from normal mucosa through the premalignant adenoma by the step-wise accumulation of mutations in several key tumour suppressor genes\(^11\). Loss of heterozygosity of the second allele of some of these mutated genes allows their complete inactivation, but necessitates the cell to develop a
‘chromosomal instability’ phenotype. Genes classically involved in this pathway in sequential order are those coding for APC, k-ras, SMAD4 and p53. APC and SMAD4 tumour suppressors are inactivated while k-ras becomes mutated at either codon 12 or 13 to alter gene function and p53 becomes mutated, gaining a longer half-life and increasing the tumour cell’s resistance to apoptosis. Recently, the sequential pathway of APC and ras has been questioned. Three of these genes have been investigated in relation to prognosis. The RASCAL I and II large, retrospective, meta-analysis, multicentre studies focus on the importance of k-ras mutation. They found that different gene mutations had varying impacts on survival even when these mutations occurred at the same site. In particular, a glycine-to-valine mutation at codon 12 of the k-ras gene led to a significantly more aggressive tumour and poorer survival and this effect was particularly pronounced in Dukes’ stage C patients. SMAD4 is one of several closely linked genes (including SMAD2 and DCC) on 18q, a region frequently showing loss of heterozygosity in CRC. Studies of this area have shown that its loss may be an adverse prognostic sign, particularly in Dukes’ stage B patients, the most likely candidate for this effect appearing to be SMAD4. Zhou et al, as mentioned previously, have also suggested that loss of 18q in conjunction with 8p is an independent adverse prognosticator. As mentioned, p53 mutations are often acquired by neoplastic cells to allow them to resist apoptosis induced by DNA damage. Mutations in this gene which lengthen its half-life have been shown to be related to increased tumour aggressiveness and poorer survival in some studies. When both k-ras and p53 mutations are found within a tumour these may have an additive effect on reducing survival.

**CRCs showing microsatellite instability**

Nearly all CRCs from patients with HNPCC and 15% of sporadic CRCs show microsatellite instability – replication errors in repetitive small DNA sequences. While these microsatellite lesions are used to detect this subtype of CRC, they merely reflect similar mutational events occurring within the coding sequences of genes carrying these repetitive sequences. These lesions are caused by a defect in mismatch repair which may either be inherited or acquired.

*Inherited:* Patients with HNPCC inherit a mutation in one of several mismatch repair genes – hMLH1, hMSH2, hMSH6, hPMS1 or hPMS2. Of these, mutations in hMLH1 or hMSH2 make up 95% of cases. The tumours in these patients show mutations in genes carrying short repetitive sequences and particularly polyA stretches: CTNNB1 (β-catenin), Bax, Tcf4, CDX2, E2F4, TGF-βRII and even other mismatch repair genes such as hMSH3 and hMSH6. Although some of the detected mutations may be ‘bystander’ mutations (just like the random
mutations in non-coding microsatellites with no selective advantage)\textsuperscript{10}, others alter elements of pathways which are also targeted in other subtypes of CRC (e.g. CTNNB1 [\(\beta\)-catenin], Tcf4) and, therefore, appear to be genuine. To date, none of these genes have been investigated for prognostic significance. In contrast, APC and k-ras mutations and loss of heterozygosity are seen less frequently in HNPCC tumours than chromosomal instability CRC\textsuperscript{36}.

**Acquired:** Over 80% of sporadic CRCs showing microsatellite instability have been found to have hypermethylation of a CpG island within the promoter region of the hMLH1 gene\textsuperscript{18}. These tumours show a phenotype of global hypomethylation with focal areas of CpG island hypermethylation affecting characteristic genes of which hMLH1 is one, some of the others being p16ink4a/p14arf, ER, MGMT, THBS1 and COX2\textsuperscript{17,20,37}. This is known as the CpG island methylator phenotype (CIMP)\textsuperscript{21}. The gene defect causing this derangement of methylation is yet to be identified. These tumours show a lesser degree of loss of heterozygosity, p53 and APC mutation than chromosomal instability CRCs and a lower frequency of k-ras mutation than even HNPCC microsatellite instability tumours\textsuperscript{17,36}. Studies of these microsatellite instability sporadic tumours have shown varying results with some indicating that these patients have a very much better prognosis\textsuperscript{17,38}. This is an interesting paradox in that these tumours are classically more poorly differentiated than chromosomal instability tumours and is also not our experience when investigating prognosis and response to therapy, with such cases yielding only a small tendency to a better prognosis in unpublished data on 1634 patients from three major randomised clinical trials\textsuperscript{39} with, to date, no significant effect on response to 5-FU.

**Classification by pathway**

**APC/\(\beta\)-catenin/Wnt and Wingless/Tcf4 pathway**

APC has long been recognised as an important tumour suppressor gene involved in the initiation of adenomatous growth\textsuperscript{11}. The great majority of sporadic microsatellite stable and all FAP CRCs carry a mutation in one allele of APC, the other usually being inactivated by loss of heterozygosity. Of these first mutations, 90% result in a stop codon and truncated protein\textsuperscript{40}. More recent work has shown that many microsatellite instability tumours do not carry an APC mutation; however, analysis of the APC pathway found a much higher frequency of mutation of other components of the APC pathway, namely...
CTNNB1 (β-catenin) and Tcf4 within these tumours\textsuperscript{17,40}. This pathway ultimately ends in the transcription factor Tcf4 which is proposed to up-regulate many targets known to be involved in tumourigenesis (e.g. c-myc, cyclin D1, c-jun, fra-1 and matrilysin)\textsuperscript{40} and, therefore, it would seem to be an important target for dysregulation in tumourigenesis. No work on the prognostic/predictive value of these factors has yet been reported.

**DNA mismatch repair pathway**

Inherited and acquired defects of this pathway have been discussed previously. Many elements of this pathway can be mutated (e.g. hMLH1, hMSH2, hMSH6, hPMS1 and hPMS2)\textsuperscript{17}. hMLH1 is also frequently silenced by promoter hypermethylation\textsuperscript{18}. These lesions result in a failure to repair acquired mutations due to slippage of short repetitive DNA sequences. Genes carrying these short repetitive sequences in their coding regions may also acquire functional mutations. Genes affected include other mismatch repair genes (e.g. hMSH3 and hMSH6) which may compound the problem. Some studies on sporadic tumours with microsatellite instability have shown them to have a better prognosis than microsatellite stable cancers\textsuperscript{17}.

**TGF-β/SMAD2 and SMAD4 pathway**

The TGF-β pathway performs many functions within the cell perhaps the most important of which is potent inhibition of proliferation\textsuperscript{40}. TGF-β mediates this effect by binding to TGF-βRII leading to activation of the type I receptor and hence activation of SMAD proteins by phosphorylation. Mutations in TGF-βRII have been found in over 80% of tumours exhibiting microsatellite instability\textsuperscript{10,17,40}. The importance of this pathway was also emphasised by the discovery of an unusual CRC-prone kindred who developed late onset microsatellite stable CRCs and were found to carry a germline mutation in TGF-βRII\textsuperscript{41}. Although TGF-βRII mutations do not appear to play a part in chromosomal instability CRCs, two other components of this pathway SMAD2 and SMAD4 lie on 18q near to DCC and are candidates for the favoured loss of heterozygosity of this region during tumourigenesis\textsuperscript{40}. Bodmer et al\textsuperscript{42} found SMAD4 loss of heterozygosity and/or mutations in nearly half of microsatellite stable CRC cell lines. These lesions appeared to occur after the divergence of the microsatellite instability pathway, but before the development of chromosomal instability. Interestingly, APC-deficient mice which also carry SMAD mutations develop a greater number of malignant colonic tumours\textsuperscript{40}. This would be a very interesting pathway to investigate both in terms of prognostication and therapeutics as loss of 18q appears to predict a failure to respond to 5-FU therapy in the AXIS study\textsuperscript{39}.
Classification by specific cell function

Proliferation/cell cycle
SMAD2 and SMAD4 have been discussed previously. Aneuploidy or gross disruption of chromosome number occurring during defective cell proliferation has been shown in some studies to correlate with more aggressive tumours and a worse prognosis. Cyclin D1 and BUB1 are involved in the cell cycle and its checkpoints, and are known to have dysregulated expression in CRC.

Apoptosis
Bcl-2 is involved in the cell’s decision to undergo or resist apoptosis. Studies have shown conflicting results with some claiming that Bcl-2 protein detectable by immunohistochemistry is correlated with better prognosis, but we have been unable to confirm this. p53 is also deranged in CRC and is suggested to be a prognostic factor. However, there are a very large number of studies and a major meta-analysis is required.

Angiogenesis
VEGF is involved in promoting angiogenesis. Studies show VEGF to be mainly localised to tumour cells and its overexpression can be detected in approximately 50% of tumours. VEGF-positive tumours have been suggested to have a significantly worse prognosis than VEGF-negative tumours. The localisation of this protein to tumour cells makes it an attractive target for therapy. VEGFR-directed antibodies have also been shown to inhibit the growth of peritoneal metastases from colorectal cancer in mice, and this work is now being extended into human subjects.

Cell adhesion recognition
E-cadherin and CD44 both function as adhesion molecules. E-cadherin expression is known to be lost on tumour cells leading to decreased cell-to-cell adhesion and so facilitating metastasis. However, no link between loss of E-cadherin expression and prognosis has been found. Variants of CD44 may be expressed by tumour cells (CD44 is undetectable in normal colonic mucosa). Expression of CD44 variant 6 has been found to be associated with a poorer prognosis.

Lytic enzyme production
Increased matrilysin production by CRC tumour cells has been detected on cDNA microarrays while matrix metalloproteinase (MMP) production is also known to be increased by tumour cells. All of these enzymes degrade the extracellular matrix so allowing tumour cell
invasion. The presence of MMP1 within CRC cells is associated with a poorer prognosis7.

As can be seen from the above, there are many potential targets which may allow the development of a molecular prognostic profile which, if used in conjunction with clinicopathological data, would hopefully give accurate information on an individual patient’s prognosis. Initial studies of some of these genes appear promising, but these studies have many of the faults discussed above and do not answer the basic question of whether these markers add any value when compared with well-performed pathology. Too often, these studies are performed on too small numbers outside of randomised trials and, therefore, markers are never effectively evaluated.

Molecular predictive factors

More important than identifying prognostic molecular factors is the identification of molecular factors linked to treatment response allowing an optimal treatment plan to be designed for each patient. In this area, we have been more successful but, again, many of the studies are flawed. Genomics allows the elucidation of whole pathways: already immunohistochemical analysis of thymidylate synthase levels have been linked to response to treatment to 5-FU therapy5 and we may be better able to predict response by looking at other components of this pathway such as dihydropyrimidine dehydrogenase and thymidine phosphorylase6 either in isolation or as a group. Another intriguing development is the identification of polymorphisms within some of these genes (e.g. the specific number of tandem repeats within TS which influence gene expression levels and, therefore, response to 5-FU)48. In the case of other treatments, their target genes or pathways are now known and these may now be studied to identify relevant predictive factors. Amongst these relatively new agents are irinotecan which is a topoisomerase 1 inhibitor and oxaliplatin which is related to the other ‘platins’ and acts by producing DNA adducts which require repair by excision repair cross-complementing gene 1. Capecitabine acts on the thymidylate synthase pathway (as does 5-FU), but offers the convenience of an oral drug. New technologies may also reveal novel targets for directed treatments.

Identification of new targets and future directions

In the future, work needs to be concentrated on the new technologies of mass analysis such as cDNA microarrays, CGH, SAGE, proteomics, SNP analysis and tissue arrays in the context of large, randomised,
clinical trials. This will detect genomic and proteomic derangements within cancers giving both prognostic and therapeutic information. We also need to profile cancer gene expression fingerprints in response to therapeutic agents to detect likely mechanisms of metabolism and resistance. In the future, targeted antibody and dendritic cell therapies to tumour cell surface antigens will hopefully be developed minimising side-effects and maximising tumour response. Already, potential targets have been identified and some are even at the stage of initial clinical trials (e.g. cerbB2, EGFR and VEGF).

The future for colorectal cancer molecular prognostication and therapeutics is very bright, but we must restrain ourselves from getting carried away by the vast amount of data generated and instead concentrate our minds on optimising current methods such as histopathology and discovering new subtypes of CRC, important new pathways in its development and potential new targets for therapeutics. Importantly, all of these aims must be carried into analysis of large, randomised, controlled trials with collaboration as free sharing of information between all research groups involved. Only in this way can we hope to optimise the advances we make in prognostication and therapeutics for the patients we are ultimately trying to help.

**Key points for clinical practice**

- Prognostic markers give prospective information on patient outcome while predictive factors give information on likely tumour response to a single or group of therapeutic agents
- At present, clinicopathological features, most importantly tumour stage, are the best prognostic markers
- Molecular predictive markers are appearing including enzymes in the thymidylate synthase pathway which correlate with response to 5-fluorouracil
- Development of useful molecular prognostic markers will depend on the use of new high throughput technologies in the context of large, randomised, controlled trials

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