12. Prognostic and predictive factors in colorectal cancer: The importance of reliable markers for effective selection of therapy

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Abstract. In recent years, significant advances have been made in the study of colorectal cancer, (CRC) prognosis and outcome. A better understanding of the molecular basis of this disease, as well as the development of new therapeutic approaches to treat it, has dramatically altered its management. It is essential for physicians to have an effective methodology by which to plan treatment, project prognosis and measure outcome. In this chapter, we discuss predictive and prognostic markers identified in CRC in terms of their utility in assisting the clinician to select the most efficacious and least toxic therapeutic options for each patient.

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Introduction

CRC constitutes one of the leading causes of cancer-related deaths in the Western world. Selection of the most beneficial treatment regimes in CRC remains a challenge and is hindered by a lack of well established prognostic markers (markers that correlate with survival or DFS (disease-free survival)) and predictive markers (markers that predict response to a particular therapy).

The American Joint Committee on Cancer (AJCC) established a Colorectal Working Group to develop a system for the evaluation of prognostic marker values. The goal of this group was to categorise prognostic markers according to their strength and reliability based on data found in the literature, which would allow physicians to predict the aggressiveness of the disease and the likelihood of recurrence after surgery based on analysis of these markers. These factors will be reviewed in the first part of this chapter.

Unfortunately, selection of the most appropriate therapeutic approach has been plagued by the development of drug resistance. In recent years, several studies have attempted to identify critical molecular and/or biochemical markers that can be used to predict response to chemotherapy. The primary aim of such predictive biomarker testing is to allow treatment to be designed according to the molecular phenotype of the tumour. In the second part of this chapter, we will examine some of the potentially clinically important predictive markers for chemotherapy in CRC.

1. Prognostic factors

The AJCC convened a Prognostic Factors Consensus Conference to evaluate the role of biologic, genetic, molecular and other nonanatomic factors in staging cancer (2).

The first edition of this staging manual was published in 1977 (3), and established the use of T (tumour extent), N (lymph node status) and M (the presence or absence of metastases) in an organised staging outline that allowed clinicians to uniformly describe the extent of the disease. Recently, an increasing number of nonanatomic factors have been identified and studied. Some of these factors have been shown to influence outcome predictions and treatment decisions, and have been classified as “prognostic factors”. Under the auspices of the College of American Pathologists, a multidisciplinary group of clinical (medical oncology, surgical oncology and radiation oncology), pathologic and statistical experts reviewed relevant medical literature and stratified prognostic factors into categories that reflect the strength of published evidence demonstrating their prognostic value. The
Prognostic and predictive factors in colorectal cancer

Factors fall into five categories (from the College of American pathologists consensus statement; 2). Category I is comprised of factors definitively proven to be of prognostic importance based on evidence from multiple statistically robust published trials, and are widely used in patient management. Category IIA includes factors that have been extensively studied biologically and/or clinically, and have repeatedly shown to have prognostic value for outcome and/or predictive value for therapy that is of sufficient import to be included in the pathology report, but remains to be validated in statistically robust studies. Factors in Category IIB include those shown to be promising in multiple studies, but lack sufficient data for inclusion in Category I or IIA. Category III includes potential factors not yet sufficiently studied to determine their prognostic value. Category IV includes factors that have been well studied and shown to have no prognostic significance (Table 1).

**Table 1. Classification of prognostic markers in colorectal adenocarcinoma (2).**

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<td>Category I</td>
</tr>
<tr>
<td>Category IIA</td>
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<td>Category IIB</td>
</tr>
<tr>
<td>Category III</td>
</tr>
<tr>
<td>Category IV</td>
</tr>
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</table>

**Category I: Factors well supported by the literature and generally used in patient management**

**a) Pathological assessment of tumour extent (pT)**

**Tumour in situ (Tis).** The designation “Tis” (i.e., carcinoma in situ, a malignancy that has not yet penetrated the basement membrane of the epithelium to invade the underlying lamina propria) is used to refer both to intraepithelial malignancies (“Tie”) and intramucosal carcinomas (“Tim”), tumours that have invaded the mucosal estroma (the lamina propria, up to and including the muscularis mucosae). These designations are of great prognostic
significance in that patients classified with these types of tumours have an extremely good prognosis.

**Invasion of parietal peritoneum.** The highest category of local extent of colorectal tumour (T4) includes both extension into an adjacent structure or organ and involvement of the parietal peritoneum (serosal involvement), which has been demonstrated to have independent adverse prognostic significance (4). Patients with pT4 tumours that have penetrated the visceral peritoneum have a shorter median survival time after surgical resection compared to patients with pT4 tumours that lack serosal involvement, in either the presence or absence of distal metastases (5). Based on this adverse effect on outcome, it has been suggested that tumours in the T4 category be further classified as T4a (tumours that invade adjacent structures or organs) and T4b (tumours that involve the visceral peritoneum).

b) **Regional lymph node metastases (pN)**

Metastasis to regional lymph nodes as determined by pathologic assessment is among the factors that most strongly predict outcome following surgical resection, second only to distant metastatic disease in importance. It is recommended that all identified lymph nodes be sectioned, and a minimum of 12–15 negative lymph nodes are required to confirm regional node negativity (6). When less than 12 negative lymph nodes are harvested, the dissection is considered insufficient. These patients have a higher incidence of postoperative cancer death than patients with a sufficient dissection (p<0.001) in stage II CRC (7). Therefore, insufficient lymph node dissection constitutes an independent risk factor for postoperative cancer death in patients who undergo CRC surgery.

c) **Presence or absence of blood or lymphatic vessel invasion**

T1 colorectal tumours may invade submucosal vessels, either venous or lymphovascular (i.e., small nonmuscularised vessels that represent either postcapillary venules or lymphatics). The invasion of lymphovascular vessels has been associated with a significantly increased risk of regional lymph node metastasis (8), and invasion of the submucosal venous system has been associated with the development of liver metastasis (9). The presence of one of these factors in a T1 tumour may influence the decision to perform more extensive surgical excision. For these tumours, the T1 category should be further divided into T1a (no evidence of lymphatic or venous invasion) and T1b (the presence of lymphatic or venous invasion).
d) **Residual tumours**

The finding of tumour tissue at a surgical resection margin indicates that the tumour has not been completely removed from the patient at the surgical interface. The surgical margin status should always be examined and reported, as residual tumour tissue is related to a worse prognosis and a higher rate of local or distant recurrence.

e) **Elevated serum carcinoembryonic antigen (CEA)**

CEA is the most widely accepted and frequently used tumour marker for colon cancer. Its detection is relatively inexpensive and easy. The threshold value of CEA varies, but the standard is 2.0–2.5 ng/ml, dependent on the measurement test. Due to frequent false-positive outcomes caused by benign gastro-intestinal disorders and smoking, the generally accepted threshold value in follow-up testing for colorectal carcinoma is 5.0 ng/ml. Preoperative CEA levels >5.0 ngr/ml have been shown to have an adverse impact on prognosis (i.e., survival) that is independent of tumour stage (10, 11). Tumours with elevated CEA levels at presentation should be differentiated from those without CEA elevation by TNM staging (Cx, serum CEA cannot be assessed; C0, serum CEA not elevated; C1, CEA levels elevated ≥5 ng/ml).

II. **Category IIA: Factors shown to have prognostic value for outcome and/or predictive value for therapy but still need to be validated in statistically robust studies**

a) **Presence of residual tumour in the resection specimen following neoadjuvant therapy (ypTNM)**

Any remaining viable tumour found in a resection specimen following neoadjuvant therapy is associated with a worse prognosis. Therefore, the region of resection should always be evaluated.

b) **Radial margins**

The radial margin represents the adventitial soft tissue margin of a non-peritonealized surface. This acquires special relevance in rectal disease (12, 13). By contrast, the colon is encased in segments by a peritonealised (serosal) surface (e.g., the cecum, transverse colon and sigmoid colon) and the only radial margin remaining is the mesenteric resection margin. Due to this, studies examining the relationship between the radial margin and adverse
outcome in colon carcinoma are lacking. However, multivariate analyses of rectal disease have suggested that involvement of this margin in tumour formation may be the most critical factor in predicting local recurrence (14). The radial resection margin is considered to be involved when a tumour is present $\leq 1$ mm from the surface of the specimen (12, 13). In rectal cancer, this is associated with local recurrence and indicates a need for additional therapy. Regardless of whether a colorectal tumour is classified as T3 or T4b, the excision is considered complete only if all surgical margins are negative, including the radial margin. If a radial margin is involved in a tumour, additional therapy, such as local radiation, should be considered.

c) Histologic grade

Traditionally, tumours have been stratified into three or four grades as follows: Grade 1 tumours are well differentiated, Grade 2 tumours are moderately differentiated, Grade 3 tumours are poorly differentiated, and Grade 4 tumours are mostly undifferentiated. Multivariate analyses have shown histologic grade to be of independent prognostic significance, with undifferentiated tumours associated with a worse prognosis (15, 16, and 17). However, in a number of studies, the number of grades has been reduced and tumours are classified as either low or high grade tumours. It is hoped that these new grading systems will reduce inter-observer variability. Therefore, tumours in the low grade include well differentiated or moderately differentiated tumours and poorly differentiated or undifferentiated tumours are considered high grade tumours (18).

d) Tumour border configuration

The growth pattern of the tumour at the advancing edge (tumour border) has been shown to have prognostic significance independent of stage. This pattern may also predict metastasis to the liver. In both univariate (19) and multivariate (20) analyses, an “irregular, infiltrating pattern of growth”, as opposed to a “pushing border”, has been demonstrated to be an independent adverse prognostic factor.

III. Category IIB: Factors that are well studied but lack sufficient evidence for inclusion in Categories I or IIA

a) Lymphocytic infiltration of tumour or peritumoural tissue

Lymphocytic infiltration of a tumour is indicative of an immunologic response to the invasive malignancy and has been shown by some studies to
be a favorable prognostic factor (21). By contrast, other studies have failed to confirm this prognostic significance (22) or have demonstrated it only by univariate analyses (23).

One type of lymphoid reaction is characterised by direct lymphocytic infiltration of the tumour, also known as “tumour infiltrating lymphocytes”, and is associated with tumours that carry DNA mismatch repair gene mutations and numerous DNA replication errors (RER+) together with microsatellite instability (MSI). Because MSI is associated with improved prognosis, tumour-infiltrating lymphocytes may prove to be a favourable prognostic marker (24). MSI is classified as high (MSI-H) or low (MSI-L) based on the number of markers that exhibit instability. MSI-H is defined as instability in two or more loci, while MSI-L describes a tumour with instability at a single locus (25). Microsatellite stability is defined as 0% unstable loci. Recent studies confirm the association between MSI and a significantly better prognosis for both OS and disease-free survival in CRC patients compared to those with intact mismatch repair (26, 27). Nevertheless, its predictive value for chemosensitivity remains controversial, and further studies are needed (28).

b) Histologic types

Signet cell-type adenocarcinoma and neuroendocrine (small cell) carcinoma: The signet ring cell type of adenocarcinoma and neuroendocrine carcinoma are subtypes of colonic carcinoma that have been demonstrated by multivariate analysis to have an independent adverse impact on prognosis (29). These tumours are respectively assigned grade 3/4 (poorly differentiated) and grade 4/4 (undifferentiated). Therefore, both of these types of tumour are considered high grade and associated with an unfavourable prognosis.

c) Tumour tissue molecular markers

Several molecular markers in colorectal carcinoma have been identified, but their clinical relevance remains unconfirmed. To establish their role as prognostic factors, they must be further evaluated by prospective randomised trials.

18q/DCC. The allelic loss of a region on the long arm of chromosome 18 is commonly observed in CRC; it is also known as “Loss of heterozygosity” (LOH). This region contains several tumour suppressor gene(s), the best known of which is the DCC gene. LOH at this site inhibits expression of the encoded protein, and some previous studies have identified it as an adverse
prognosis factor for Stage II colorectal adenocarcinoma (30). Stage II patients with 18q LOH behave clinically as stage III patients and, by contrast, stage II patients without 18q LOH behave as stage I patients.

A recent meta-analysis revealed that the results from studies investigating the relationship between CRC survival and chromosome 18q allelic imbalance (AI)/loss of DCC expression (LOE) have been inconsistent. Considerable variation exists in the techniques and the assessment methods used in these reports. Nevertheless, it appears that cancers with chromosome 18q loss have a poorer prognosis (31). Further studies are required to determine the clinical utility of this marker, which may allow the molecular staging of stage II patients into two separate categories, good and poor prognosis. However, current data do not support classification of chromosome 18q AI as a marker of survival and it should not be considered outside clinical trials (32).

**K-ras.** Ras mutations occur early in the development of colorectal carcinoma, and are present among 12–75% of colorectal carcinomas. The majority of K-ras mutations in colon adenocarcinoma affect codons 12 and 13. Results regarding the prognostic role of K-ras mutation are controversial. Ras mutations often occur in cancers with other poor prognostic factors. For example, there is a significantly higher mutation rate (65%) observed in tumours with lymphatic or hematogenous metastases compared with tumours lacking these features (33). Likewise, only 28% of carcinomas limited to the muscularis propria (Tis, T1 and T2) contain mutated ras compared with 41% of deeply invasive tumours (T3 and T4) (34). Therefore, there is a trend toward the acceptance of ras mutation as indicative of a worse prognosis. Moreover, the prevalence of kras mutations in codons 12 and 13 occurs in 25% of patients with non-recurrent disease versus 71% in patients with recurrent tumours (33). Nevertheless, no consensus has been reached regarding its prognostic role. Although the large multicentre RASCAL study failed to show an association between the presence or absence of K-ras mutations and Dukes’ stage (35), multivariate analysis suggested that the presence of mutation increased the risk of recurrence and death (35). In addition, the FOCUS study reported that mutation in either KRAS or BRAF was a poor prognostic factor for OS (hazard ratio (HR): 1.40; 95% CI: 1.00 to 1.36; p=0.05) (36). Meanwhile, other studies do not support the prognostic value of KRAS; results from the PETACC-3 showed that KRAS mutations had no major prognostic value regarding DFS or OS (37). Further studies will be required to establish the prognostic role of K-ras mutations in colorectal adenocarcinoma.

**Microsatellite instability (MSI).** Mutations in one of several mismatch repair (MMR) genes leads to the development of hereditary nonpolyposis CRC (HNPCC) and are responsible for 15–20% sporadic colon carcinomas
(38). Individuals with HNPCC inherit a mutation in one allele of one of the MMR genes and acquire a second somatic mutation in the same gene in tumours with MSI. MSI corresponds to alterations in the length of simple, repetitive microsatellite sequences that occur throughout the genome and appears to predict improved patient survival (39). Results from a recent meta-analysis confirmed an association between MSI and a favourable prognosis based on both the overall and disease-free survival of CRC patients (26).

**Thymidylate synthase.** Thymidylate synthase (TS) converts deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), a step essential for DNA synthesis. TS is also an important target of fluoropyrimidine drugs that are widely used in the treatment of colon carcinoma, a feature that will be discussed later in the chapter. Regarding its prognostic role, a number of studies have investigated the relationship between thymidylate synthase (TS) expression and survival in CRC patients. Most of these studies have reported poorer overall and progression-free survival with high TS expression, but the methodology used in these studies was not consistent (40). Therefore, additional studies are needed to define the precise prognostic value of TS.

**p27.** p27 is a cyclin dependent kinase inhibitor that acts as a cell cycle inhibitor and a potential tumour suppressor. Previous data showed that decreased expression levels of p27 in colorectal carcinoma correlated with poor prognosis (risk ratio for death = 2.9; 41). Recent studies corroborated this theory: Bertagnolli et al showed that patients with p27 negative tumours had a reduced OS [66% 5-year OS (95% CI: 0.59–0.72) versus 75% 5-year OS (95% CI: 0.70–0.79); 42]. Therefore, loss of p27 appears to correlate with reduced survival in stage III colon cancer.

**Bcl-2.** Bcl-2 belongs to a protein family involved in regulating cell death and survival. Bcl-2 suppresses programmed cell death (apoptosis), conferring a survival advantage for these cells. The loss of Bcl-2 expression appears to be correlated with an increased number of relapses in stage II CRC, and could be therefore a potentially useful marker (43). Moreover, the expression of Bcl-2 has been shown to be a valuable indicator of good prognosis in CRC in the distal colorectum (44). Nevertheless, further studies are needed.

**p53.** The gene that encodes p53 is located on the short arm of chromosome 17. p53 is also known as the “guardian of the genome” because it plays a role in controlling cell proliferation, cell differentiation, DNA repair and synthesis and programmed cell death (45). When the normal growth regulatory activity of the protein is lost, genetic instability is promoted. This instability diminishes the probability of apoptosis and contributes to unregulated cell growth (46).
Regarding the prognostic role of p53, a distinction exists between overexpression of the protein and the presence/absence of a mutation. Reports regarding the prognostic significance of p53 overexpression are controversial. Although some studies have shown a significant correlation between high p53 levels and prolonged disease-free survival, especially among stage III cancer patients (47) other studies failed to show a relationship between p53 expression and prognosis (48). The disparity of these results is due to methodological problems with respect to the measurement of p53, which is usually assessed by immunoassay, a technique that has several limitations in the interpretation of the results (49). Regardless, it is agreed that p53 mutation and allelic loss represent useful markers for prognosis, and it is thought that tumours harbouring p53 mutations are at higher risk of metastasis (50).

**Category III: Factors not yet studied sufficiently to meet criteria for inclusion in Categories I, IIA or IIB**

Factors grouped in this category include DNA content, other molecular markers (except loss of heterozygosity 18q/DCC and MSI-H), perineural invasion, microvessel density, tumour cell-associated proteins or carbohydrates, peritumoural fibrosis, peritumoural inflammatory response, focal neuroendocrine differentiation, nuclear organizing regions, and proliferation indices. All of these factors lack sufficient data for specific recommendations.

**Category IV: Factors that are well studied and have been shown to have no prognostic significance**

To our knowledge, no evidence of an association between tumour size and outcome has been reported (51). Regarding histologic type, only a single multivariate analysis has demonstrated that mucinous carcinoma is an independent predictor of adverse outcome (52). Therefore, neither tumour size nor histologic tumour type is of prognostic significance in patients with colorectal carcinoma.

2. **Predictive factors**

In CRC, clear evidence exists that adjuvant chemotherapy improves overall and disease-free survival in patients with resected Dukes’ stage C. 5-Fluorouracil (5-FU)-based chemotherapeutic regimens are the standard
treatment for these patients. However, the response rates for patients given 5-FU as a single-first line treatment in advanced CRC are only 10–15% (53).

Combining 5-FU with newer chemotherapies, such as irinotecan (CPT-11) and oxaliplatin, has improved the response rates of patients with advanced CRC to 40–50% (54, 55). Despite these improvements, new therapeutic strategies are needed.

The use of novel biological agents, such as the monoclonal antibodies, cetuximab (an anti epidermal growth factor receptor (EGFR) inhibitor) and bevacizumab (a Vascular Endotelial Grow Factor Receptor (VEGFR) inhibitor), have recently been shown to provide additional clinical benefit to patients with metastatic CRC (56, 57). These agents are now under intense investigation as adjuvant therapies.

Resistance to chemotherapy limits the effectiveness of current cancer strategies, including those used to treat CRC. Drug resistance is either intrinsic or acquired during treatment, and is believed to be the cause of treatment failure in over 90% of patients with metastatic cancer. Furthermore, drug resistant micrometastatatic tumor cells are likely to reduce the effectiveness of adjuvant chemotherapy following surgery. Therefore, overcoming drug resistance is one of the main challenges of current cancer research.

There are several factors that can affect drug sensitivity: the pharmacokinetic profile of the drug, drug activation and inactivation, alterations in the drug target, the processing of drug-induced damage and the evasion of apoptosis. Currently, several studies are underway that are designed to identify critical molecular and/or biochemical markers that can be used to predict response to chemotherapy. The primary aim of such predictive biomarker testing is to allow treatment to be formulated according to the molecular phenotype of the patient tumour.

3. 5-Fluorouracil

5-FU is converted into several active metabolites, the most important of which include 2’-deoxy-5-fluorouridine-5’monophosphate (FdUMP), 2’-deoxy-5-fluorouridine-5’triphosphate (FdUTP), and 5’-fluorouridine 5’triphosphate (FUTP). 5-FU is converted to 5’fluouridine-5’monophosphate (FUMP) either directly by orotate phosphoribosyl transferase (OPRT) or indirectly via 5-fluorouridine by the sequential action of uridine phosphorylase (UP) and uridine kinase (Figure 1).
Figure 1. Mechanism of action of 5-FU. Abbreviations: 5-FU=5 Fluorouracil; CH₂THF= 5,10-methylenetetrahydrofolate; DPD=dihydropyrimidine dehydrogenase; dUTP= deoxyuridine triphosphate; dUTPase=dUTP pyrophosphatase; 5’DFUR= 5’deoxy-5’fluorouridine; FdUDP=5’-fluoro-2’deoxyuridine-5’monophosphate; FDUR= 2’deoxy-5-fluorouridine; FUMP=5’-fluorouridine-5’triphosphate; LV=leucovorin; TP=thymidine phosphorylase; TS=thymidylate synthase.

Thymidine phosphorylase

Thymidine phosphorylase (TP) catalyses the conversion of 5-FU to 2’-deoxy-5-fluorouridine (FUDR). TP overexpression has been correlated with increased sensitivity to 5-FU, possibly due to enhanced formation of FdUMP (58).

By contrast, results from analysis of TP messenger RNA (mRNA) expression in colorectal tumours showed that tumours with high TP expression were less likely to respond to 5-FU (59). The authors hypothesised that TP acts as an angiogenic factor, based on the finding that it contains a sequence identical to that of platelet-derived endothelial cell growth factor, a well-documented angiogenic factor. Therefore, elevated TP expression might be a marker of a more invasive and malignant tumour phenotype with increased resistance to chemotherapy.
**Thymidylate synthase**

FdUMP is a metabolite of 5-FU and forms a stable ternary complex with the folate-dependent enzyme TS and the reduced folate cofactor 5,10-methylenetetrahydrofolate. The formation of this complex results in inhibition of TS enzyme activity, leading to an imbalance in deoxynucleotide pools and subsequent inhibition of DNA synthesis and repair. Co-administration of Leucovorin increases the intracellular levels of this complex, thereby enhancing and stabilising the ternary complex formation and optimising TS enzyme inhibition. 5-FU can also cause DNA damage through misincorporation of FdUTP into DNA. Furthermore, FUTP can be misincorporated into nuclear and cytoplasmic RNA (Figure 1).

Numerous studies have demonstrated that TS is a key determinant of sensitivity to 5-FU. High TS expression correlates with increased resistance to 5-FU (60, 61). A meta-analysis performed by Popat et al. analysed 20 studies and included over 3,000 patients, and concluded that tumours with elevated TS expression had poorer OS compared with tumours that expressed low levels of TS (62). Similar results have been shown in metastatic disease: detection of TS activity in liver metastasis was linked to 5-FU activity and is related to clinical responsiveness to 5-FU (63).

Because TS expression is critical for the efficacy of fluoropyrimidines, identification of the genetic alterations that regulate TS gene expression should be crucial for developing predictive markers. However, the results are contradictory. In some studies, low TS levels were associated with improved survival, which might indicate that these tumours are more sensitive to 5-FU-based therapy (64). By contrast, other larger studies concluded that tumours with elevated TS levels are more likely to benefit from 5-FU-based chemotherapy (65, 66).

The heterogeneity observed between studies of TS expression might reflect differences in the method of assessing TS. The optimal method by which to measure TS expression remains unclear. Although real-time PCR is more specific than immunohistochemistry, as it allows a quantitative determination of TS expression, its widespread use has limited applicability due to the requirement for fresh tissue. Immunohistochemical analysis of TS expression is more efficient and clinically applicable, although it only allows for a semiquantitative determination of TS expression. Therefore, results from studies that use different TS measurement techniques cannot be compared.

**Dihydropyrimidine dehydrogenase (DPD)**

DPD is the rate-limiting enzyme in 5-FU catabolism. More than 80% of an administered dose of 5-FU is normally catabolised by the liver.
Therefore, variation in the expression levels of DPD has a direct effect on the bioavailability of 5-FU, as higher levels result in increased 5-FU metabolism and decreased levels of the drug (67). Conversely, patients who possess inactivating mutations of the DPD gene are deficient in DPD enzyme activity and cannot degrade 5-FU. As a result, these patients experience severe gastrointestinal and hematological toxicity when exposed to 5-FU (68).

Regarding its predictive value, an inverse correlation has been reported between tumour DPD expression and response to 5-FU and capecitabine. Kobayashi et al reported that high DPD activity correlated with low chemosensitivity to 5-FU, while Tsuji et al found that low tumor DPD expression in patients with stage II and III CRC correlated with better response to oral 5-FU chemotherapy; 69, 70). Salonga et al reported that measuring tumour DPD levels in conjunction with TS levels significantly increased the ability to predict responsiveness to 5-FU-based chemotherapy (71).

The results of these studies demonstrate that while the utility of DPD as a marker of toxicity has been firmly established, its role in predicting patient response to 5-FU needs to be further defined (72).

**Capecitabine**

Capecitabine is an oral fluoropyrimidine that is not degraded by DPD in the gut mucosa (73). Instead, capecitabine is absorbed intact through the gastrointestinal wall and is then converted to 5’deoxy-5 fluoridine (DFUR) in the liver by the sequential activity of carboxylesterase and cytidine deaminase (Figure 1). Initially, the conversion of DFUR to 5-FU was attributed solely to TP. However, recent studies suggest that another enzyme, uridine phosphorylase (UP), might play an important role in capecitabine activation (74). Therefore, it would be expected that capecitabine would be most effective in tumours expressing high levels of TP and/or UP. Indeed, among patients with stage II and III CRC treated with oral capecitabine, those with high TP and low DPD expression had the best OS (75).

**4. Irinotecan**

Irinotecan (CPT-11) is a camptothecin analog that improves median survival in advanced CRC compared to 5-FU alone (76). It targets DNA topoisomerase I (Topo I) causing inhibition of DNA replication and subsequent cell death (Figure 2).

To exert its toxic effect, irinotecan must be converted to SN-38 (7 ethyl-10-hydroxy-camptothecan) by carboxylesterase (CES, an enzyme found mainly in serum, the liver, and intestine). SN-38 is detoxified in the liver, primarily
Figure 2. Activation and metabolism of Irinotecan. Abbreviations: β Gluc = beta-glucuronidase; CES = carboxylesterase; SN-38= 7-ethyl-10hydroxy-camptothecan; SN-38G: SN-38 glucuronide; ds = double strand; Topo I= topoisomerase I; ss = single strand; UGT = uridine diphosphate glucuronosyltransferase.

by the 1A1 isoform of uridine diphosphate glucuronosyltransferase (UGT). UGT catalyses the conversion of SN-38 to SN-38G (SN-38 glucuronide), which is excreted in bile and urine (77).

Clinical observation revealed that the use of irinotecan is associated with significant toxic effects (irinotecan-related mortality has been reported in up to 7% of patients), the most frequent of which is grade 4 diarrhea, which can be life-threatening (78). The high mortality rate associated with irinotecan use has spurred several efforts to identify the responsible molecular mechanisms. To this end, a polymorphic variant in UGT1A1 has been identified. UGT1A1*28 is associated with a significant decrease in SN-38 glucuronidation, resulting in reduced SN-38 detoxification (79). Patients that exhibit this UGT1A1 polymorphic variant are associated with increased gastrointestinal and bone marrow toxicity (80). Similarly, as CPT-11 requires CES to become active, high levels of CES are associated with increased grade 3/4 neutropenia (81).

Just as is the case with 5-FU, the identities of reliable markers for predicting the efficacy of irinotecan in patients with CRC need to be clearly
defined. The expression of Topo-I, the target enzyme of 5-FU, is currently being investigated as a potential marker for response to irinotecan. Studies in human CRC xenografts have shown that the levels of Topo-I complexes are predictive of response to irinotecan (82). However, the clinical value of Topo-I as a predictive marker for response to irinotecan has yet to be definitively demonstrated, as some studies have shown that Topo-I expression levels in patients receiving 5-FU/irinotecan had no influence on response, time to progression or overall patient survival (83).

5. Oxaliplatin

Oxaliplatin is a third generation platinum analog in which the amine groups of cisplatin are substituted by a 1,2-diaminocyclohexane (DACH) ligand. Cytotoxic platinum compounds form positively charged compounds that block DNA replication and transcription and may initiate apoptosis (84). Once oxaliplatin induces DNA damage, the cell attempts to repair the damage through the Nucleotide Excision Repair (NER) pathway (85) (Figure 3).

![Figure 3. Activation and metabolism of Oxaliplatin. Abbreviations: ERCC1 = excision repair cross complementing protein 1; XRCC1 = x-ray repair cross complementing 1; XPD = xeroderma pigmentosum group D; GSH = glutathione; GST = glutathione-S-transferase; pt = platinum.](image-url)
The protein ERCC1 is a member of the NER pathway. ERCC1 forms a complex with xeroderma pigmentosum group F (XPF), which recognises and splits the damaged DNA strand, repairing damage to the DNA. DNA repair is an important mechanism for resistance to platinum-based chemotherapy. Therefore, several studies have been performed to establish a correlation between the expression of ERCC1 and clinical outcome after oxaliplatin therapy. It has been shown that patients with elevated ERCC1 levels do not respond well to platinum therapy (86). Another study demonstrated that the level of ERCC1 mRNA expression was an independent predictive marker of survival in 5-FU/oxaliplatin chemotherapy (p<0.001; 87). Interestingly, polymorphic variants within the ERCC1 gene are also associated with clinical outcome in patients receiving 5-FU/oxaliplatin (88). Xeroderma pigmentosum group D (XPD) is also important in the NER pathway. A polymorphic variant of this protein has been correlated with lower response rates in patients receiving 5-FU/oxaliplatin (p=0.02; 89). Finally, another family of enzymes important for oxaliplatin metabolism are glutathione-S-transferases (GST), which play a key role in the detoxification of oxaliplatin. Some studies have identified polymorphisms in GST enzymes that show a correlation with response to platinum agents (90).

6. Cetuximab and panitumumab

The EGFR is a member of the human epidermal growth factor receptor (HER)-erbB family of receptor tyrosine kinases. Its activation stimulates a cascade that enhances tumour growth and progression, including proliferation, angiogenesis, invasion and development of metastasis.

Whereas tyrosine kinase inhibitors have little effect in CRC, EGFR-targeted monoclonal antibodies play an important role. Cetuximab, a chimeric mouse-human monoclonal antibody, was the first anti-EGFR therapy approved for CRC treatment (91). Recently, panitumumab, a whole human antibody, has been incorporated into the therapeutic arsenal (92). Both appear to have similar efficacy, though panitumumab is less immunogenic.

Positive EGFR protein expression determined by immunohistochemistry was long considered to be a predictive factor for anti-EGFR treatment. However, subsequent studies obtained objective responses even in patients with low or negative EGFR expression (93). As a result of these findings, intense research was done to identify alternative predictive molecular biomarkers able to help physicians identify which patients are most likely to benefit from EGFR-targeted therapy.

Currently, we know that the growth of many tumours is secondary to the activation of signalling pathways downstream of the EGFR, regardless of
whether the EGFR is activated or pharmacologically inhibited. The interlinked RAS-MAPK and PI3K signalling pathways play an important role in tumourigenesis via phosphorylation of various proteins and transcription factors that directly control cell growth, differentiation and apoptosis (94). Mutations in KRAS, BRAF and PI3KCA result in constitutive activation of downstream pathways. Although KRAS mutations have been explored as prognostic biomarkers, they do not seem to have a stage-specific prognostic value. Nevertheless, the role of KRAS as a predictive marker has been strongly established. Lievre et al first were the first to report a lack of responsiveness to anti-EGFR therapy in tumours with KRAS mutations (95). A pivotal randomised phase III study of panitumumab in monotherapy was the first to confirm the negative predictive value of KRAS mutations (96). Results from the CRYSTAL and the OPUS studies, in which cetuximab was combined with irinotecan or oxaliplatin, respectively, confirmed that patients carrying KRAS mutations did not benefit from anti-EGFR therapy (97, 98).

BRAF is another downstream molecule in the EGFR signalling pathway. BRAF and KRAS mutations are mutually exclusive. A recently published retrospective analysis of patients who received panitumumab or cetuximab showed that those with tumours that carried BRAF V600E mutations did not respond to EGFR inhibition and had a statistically significant shorter progression-free interval and OS than patients with tumours with wild-type BRAF (99).

PIK3CA mutations and loss of PTEN expression also appear to confer resistance to cetuximab, as revealed by in vitro studies (100). In a clinical analysis, Sartore-Bianchi et al (101) found that PIK3CA mutations and PTEN loss in colorectal cancers were significantly associated with a lack of response to panitumumab or cetuximab. PIK3CA mutations and loss of PTEN expression was also associated with progression-free survival and poorer OS. Nevertheless, further investigation and prospective data are required to confirm these findings before they can be integrated into clinical practice.

The characteristic “acneiform” skin rash observed in patients treated with EGFR inhibitors has also been studied as a potential predictive marker (103). Skin toxicity has been linked with higher response rates and longer survival among patients treated with panitumumab (92) or cetuximab (93). However, there are several limitations on the use of rash as an early marker of efficacy, as there are no criteria for evaluating toxic effects involving skin rash in EGFR-targeted treatment. Some authors highlight the fact that because EGFR is expressed in the skin, the rash may indicate local receptor saturation, although other factors, such as immune status, might alter an individual’s susceptibility to rash.
7. Bevacizumab

The VEGF pathway plays an important role in tumour growth and angiogenesis. The formation of new blood vessels carrying oxygen, nutrients, growth factors and hormones is indispensable for the proliferation of tumour cells.

VEGF has become a common target for therapeutic intervention. Bevacizumab is a recombinant humanised monoclonal antibody that targets VEGF and significantly increases both overall and progression-free survival in metastatic CRC (57).

Therefore, the identification of biomarkers that can help the clinician to identify patients who will benefit from antiangiogenic therapy is of great interest.

Preclinical data suggest that dysregulation of the Ras/Raf/Mek and p53 pathways may have some role in this setting; it has been suggested that tumour cells deficient in p53 experience decreased apoptosis in hypoxic tissue, which might explain their increased responsiveness to antiangiogenic therapy (103). Other studies have focused on the role of potential predictors of response to bevacizumab, such as microvessel density (MVD), thrombospondin (THBS), and VEGF, but no statistical difference in OS was found (104).

Elevated baseline levels of Ca 19.9 have shown to have a predictive value, as patients with increased expression benefited significantly from bevacizumab treatment (105). In addition, other studies have evaluated the role of bevacizumab induced hypertension as a marker of bevacizumab efficacy. A study by Ryanne et al showed that patients who developed any grade of hypertension while on bevacizumab treatment had an adjusted HR for death of 0.32 (p=0.03) compared to those without hypertension (106).

Conclusion

Despite recent efforts to identify individual genes for predicting response to therapy and disease prognosis in colorectal cancer, a definitive list of predictive and prognostic markers still does not exist. As the response to treatment and disease progression is the result of complex pathways and not single molecules, the analysis of individual factors should be supplemented by the identification of gene expression profiles that can predict the response to chemotherapy and help to classify patients according to their predictive outcome.

To date, there are only retrospective analyses, and the design of prospective trials is crucial. Identification of these factors would provide
clinicians the ability to tailor therapeutic intervention to the pharmacogenetic profile of the patient, which would significantly improve the treatment of CRC.

References

Prognostic and predictive factors in colorectal cancer