CLINICAL INVESTIGATION

Personalized medicine in colorectal cancer: the evidence so far

Colorectal cancer is the third most common cancer worldwide and the fourth most common cause of cancer-related death. Current treatment continues to mostly be determined by stratifying patients into fairly broad groups, according to prognosis based on clinicopathological and histopathological staging. Conventional chemotherapy remains the mainstay of systemic treatment, with no established means of predicting response to treatment. However, greater understanding of the molecular biology underlying colorectal cancer, and the manner in which this influences prognosis and response to systemic therapy is increasingly well understood. In the future, key genetic changes in an individual patient's cancer could potentially be used to more accurately predict the risk of relapse following surgery and the likely response to systemic therapy.

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Colorectal cancer is the third most common type of cancer worldwide. In 2008, an estimated 1.23 million new cases of colorectal cancer were diagnosed [1]. Approximately 60% of cases occur in developed countries, with the highest estimated rates in Australia/New Zealand and western Europe and the lowest rates in Africa and south-central Asia. Incidence is greater in men than women, with an overall ratio of 1.4:1. Occurrence is strongly related to age, with 86% of cases in the UK arising in people over the age of 60 [101]. Although the mortality rate for colorectal cancer has decreased overall in the last 30 years, it remains the fourth most common cause of cancer-related death [1].

Current treatment of colorectal cancer is still mostly determined by clinico-pathological and histopathological staging; commonly using the American Joint Committee on Cancer TNM classification [2], along with additional factors such as resection margins, presence or absence of vascular/lymphatic invasion, tumor grade and patient performance status. In the adjuvant setting, these factors are used to determine the risk of relapse and overall prognosis and whether or not to give chemotherapy following surgery. Whilst clinicopathological staging is of prognostic value, it is of little value in predicting response to chemotherapy for individual patients.

Adjuvant chemotherapy has a well-established role to play in reducing the risk of recurrence following surgery, particularly in patients with stage III colorectal cancer. However, for stage II disease, the risks and benefits of adjuvant treatment are less clear. The results of the QUASAR study, published in 2007, demonstrated that, for patients with resected stage II, node-negative colorectal cancer, adjuvant chemotherapy with 5-fluorouracil (5-FU) and folinic acid can improve survival, but only in a minority of cases. The relative risk of recurrence with adjuvant chemotherapy verses observation was 0.78 (95% CI 0.67–0.91; p = 0.001) [3]. However,

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this translated into a 5-year overall survival of 80.3% with chemotherapy versus 77.4% with observation alone. Thus a large number of patients with stage II colorectal cancer who have adjuvant chemotherapy are unlikely to derive any benefit.

Systemic therapy for colorectal cancer, in both the adjuvant and metastatic setting, still consists mainly of four chemotherapy drugs – 5-FU, capecitabine, oxaliplatin and irinotecan, either as monotherapy or in combination [4]. As with other solid tumors, colorectal cancer requires the formation of new blood vessels to survive and grow and would thus potentially be susceptible to anti-angiogenic drugs. The anti-angiogenic agent bevacuzimab (Avastin®) has been shown to increase survival in the metastatic setting, when administered in conjunction with 5-FU-containing regimes [5,6]. However, so far no effective biomarker has been discovered for any of these systemic treatments that can be used to predict for response, and so help select which treatments will be more effective in individual patients.

However, in the last 5 years, our understanding of the molecular biology underlying cancer in general and colorectal cancer in particular has increased significantly. Somatic mutations in cancer cells have become increasingly well characterized and found to predict responsiveness to newer targeted therapies, such as the EGFR inhibitors cetuximab and panitumumab.

It is now well established that combining cetuximab or panitumumab with standard chemotherapy regimes can provide significant benefit to patients with metastatic colorectal cancer, but only those who do not have a somatic mutation in the *KRAS* gene (i.e., their tumor is *KRAS* wild-type). These drugs are generally not beneficial (and can even be detrimental) in cases where the *KRAS* gene is mutated [7–10]. *KRAS* codes for an enzyme called RAS, which is activated by EGFR on the surface of cells, and which in turn activates a number of cell-signaling pathways that drive cell growth and proliferation (Figure 1). Mutations in *KRAS* lead to constitutive activation of the RAS protein (i.e., it is no longer dependent on signals from the EGFR protein, and hence not affected by EGFR inhibitors). The *KRAS* gene is mutated in approximately one-third of colorectal cancers [11], so the discovery that this large subgroup of patients are resistant to EGFR inhibitors clearly has significant implications for treatment. The discovery of other activating mutations in genes such as *BRAF* and *PIK3CA*, which both code for proteins downstream of RAS (namely the RAF and PI3K enzymes, respectively) is likely to further help select patients most likely to be responsive or resistant to specific drugs, as well as providing new targets for treatment, thus opening the door to an era of more personalized medicine, with treatment regimes more tailored to the specific tumor biology of individual patients.

Finally, we now have increasing evidence that determining the molecular biology of colorectal cancer can help predict which patients are most likely to benefit from adjuvant therapy. Molecular markers of chromosomal instability (CIN), such as loss of chromosome 18q, microsatellite instability (MSI), and the development of gene signatures, have the potential to help distinguish poor prognosis cases from good and, thus, help select which patients are most likely to benefit from adjuvant treatment, as well as potentially indicating which treatments they will be most sensitive to.

The aim of this review is to summarize progress so far in these different areas of personalized therapy for colorectal cancer and to consider likely advances in the future.

Molecular biology & genetics

For the majority of patients (~75%) with colorectal cancer, the disease appears to arise sporadically, with no known family history or predisposing genetic factors [12]. Even in cases with a family history of bowel cancer, only approximately 5% are associated with highly penetrant inherited mutations, such as hereditary nonpolyposis colorectal cancer (often referred to as Lynch syndrome) or familial adenomatous polyposis [13]. The rest are likely to be the result of both genetic and environmental factors. We now know that mutations within normal cells lining the bowel epithelium accumulate in a step-wise fashion over time, leading to the change from normal cells to adenomas to cancerous cells, a process which can take years to decades [14–16].

The most common genetic changes that have been found in sporadic colorectal cancers are activating mutations of the oncogene *KRAS* and silencing mutations of the tumor suppressor genes *APC*, *SMAD4* and *p53*, along with multiple alleic losses (*5q, 17q* and *18q*) [14,17]. In a smaller subset of colorectal cancers, mutations in *BRAF* and/or *PIK3CA* have been discovered. More recently, epigenetic changes have been found, which generally cause silencing of tumor suppressor genes, such as hypermethylation of promoter CpG islands (often referred to as the CpG island methylator phenotype [CIMP]) [18]. Mutations in the *KRAS* oncogene and the *APC* tumor suppressor gene often occur early in the development of colorectal cancer. Alleic deletions appear to occur at a later stage of cancer development [17].

There is increasing interest in using genetic changes as biomarkers to help guide systemic therapy.

Biomarkers are biological molecules found in the body that can be assessed (as present or absent) or measured (from low to high level) and indicate something about a particular disease. Prognostic biomarkers indicate overall survival, independent of what treatment is given. Predictive biomarkers indicate the likelihood of responding to a particular treatment. Below (and in Table 1 [11,19–24]) is a summary of the main genetic changes in colorectal cancer that show potential as prognostic and/or predictive biomarkers for treatment.

KRAS mutations

The RAS/RAF/MEK/ERK (or MAP kinase) pathway is now well characterized as playing an important functional role in many solid tumors (Figure 1). Activation of the pathway initiates a number of tumorigenic processes, including gene transcription and cell proliferation [25]. A number of mutations in the *KRAS* gene have been found, which can lead to constitutive activation of RAS [24].

KRAS mutations occur in approximately one-third of colorectal cancers, most commonly within codon 12 or codon 13, and less frequently in codon 61 [26,22]. *KRAS* mutations have also been detected in precancerous early adenomatous polyps, occurring with greater frequency in larger and more dysplastic adenomas, suggesting that mutations in *KRAS* occur at an early stage in the development of colorectal cancer [14,27].

An association between the presence of *KRAS* mutations and poorer prognosis has been suggested, but there is conflicting evidence regarding this. An analysis of 3439 patients in the RASCAL database has reported a statistically significant poorer prognosis in patients with *KRAS*-mutated colorectal cancer. Specifically, the mutation of glycine to valine on codon 12 of the *KRAS* gene (present in 8.6% of all patients with colorectal cancer) was found to increase the risk of recurrence or death by 30% [26,28]. However, other studies suggest patients with a mutation in codon 13 have a poorer prognosis [29,30]. So far, there is insufficient evidence that *KRAS* can be used as a prognostic biomarker in colorectal cancer.

However, as outlined above, *KRAS* mutation status is now firmly established as a predictive biomarker for treatment with EGFR inhibitors. A number of studies have established that the addition of cetuximab to first-line palliative chemotherapy can significantly prolong progression-free survival and overall survival in patients with *KRAS* wild-type colorectal cancer, but not in patients with *KRAS*-mutated disease [7,8,31]. Similarly the addition of panitumumab to first-line and second-line palliative chemotherapy regimes has been shown to significantly improve
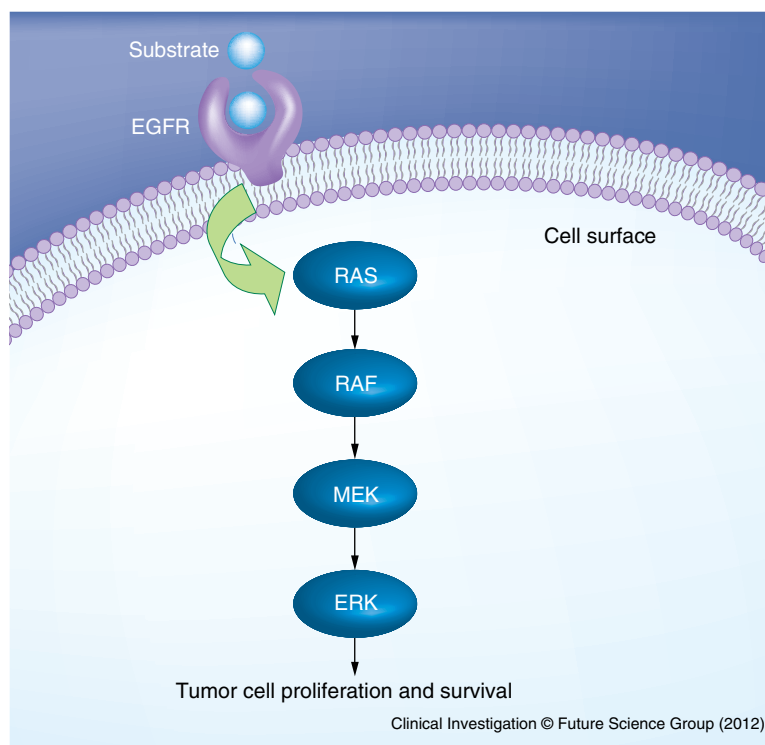

Figure 1. MAP kinase pathway.

survival, but again only in patients without *KRAS*-mutated cancer [9,10]. Monotherapy with cetuximab or panitumumab has also been shown to improve survival compared with best supportive care in patients with *KRAS* wild-type colorectal cancer, but not *KRAS*-mutated disease [32,33]. In 2009, the American Society of Clinical Oncology recommended that *KRAS* mutation testing should be done on all patients with metastatic colorectal carcinoma who may be candidates for anti-EGFR therapy, and if a mutation is detected in codon 12 or 13, EGFR inhibitors should not be given [34]. However, this advice may change in the future as there is emerging evidence that patients with *KRAS* mutations

Table 1. Summary of common mutations in colorectal cancer.		
Mutations	**Frequency (%)**	**Ref.**
Chromosomal instability	65–85	[12]
– Loss of APC	~80	[14]
– Loss of TP53	34–45	[20]
– Chromosome 18q loss	~70	[21]
High microsatellite instability	~15	[12]
KRAS	~35	[22]
BRAF	~8	[22]
PIK3CA	15–20	[23]

in codon 13 may still benefit from EGFR inhibitor therapy [35].

Generally, there appears to be a high concordance of *KRAS* mutation status between primary tumors and corresponding metastases (~95%) [36,37]. However, some heterogeneity can occur, particularly between primary tumor specimens and lymph node metastases [38]. Currently, mutation testing on primary tumor material is generally recommended if possible [38,39].

Whilst *KRAS*-mutated colorectal cancer excludes the use of EGFR inhibitors currently, the *KRAS*-mutated protein also presents a potential therapeutic target itelf. However, so far effective KRAS inhibitors have not been developed. This may be because the effect of *KRAS*-mutations on tumorigenesis appears to be complex and involves other signaling pathways including Wnt and TGF-β signaling. In approximately 50% of cases, *KRAS* mutations have been found to activate the TGF-β-activated kinase TAK1, which acts to promote cell survival [40]. While KRAS inhibitors have proven difficult to establish, TAK1 inhibition may provide a more specific therapeutic target. TAK1 inhibitors have been shown to induce apoptosis in *KRAS*-mutated cancer cell lines and *in vivo* models [40].

BRAF mutations

Looking further down the MAP kinase pathway, activating mutations in the *BRAF* gene (which codes for a serine-threonine kinase downstream of KRAS) have also been found in a number of different cancers, including colorectal cancer [41]. *BRAF* mutations are not as common as *KRAS* mutations in colorectal cancer, occurring in approximately 8% of cases, and in fact the two mutations appear to be mutually exclusive [38,42]. The most common *BRAF* mutation in colorectal cancer (and other cancers such as melanoma) occurs in codon 600 (typically a V600E mutation, although other activating mutations, such as K601E, have also been reported) [28,43]. *BRAF* mutations are generally found to be closely associated with the CIMP and MSI-high tumors [44–46].

Several studies have reported that *BRAF* mutations correlate with poorer prognosis in colorectal cancer patients, independent of disease stage and therapy [47–49]. However, the association between *BRAF* mutations, CIMP tumors and MSI-high tumors is unclear. Although *BRAF* mutations have been found to be closely associated with CIMP tumors, there is evidence that CIMP-high tumors correlate with improved prognosis, regardless of *BRAF* and MSI status [50]. Thus, like *KRAS* mutation status, the role of *BRAF* as a prognostic biomarker in colorectal cancer

remains unclear.

However, like *KRAS* mutations, the presence of a *BRAF* mutation has also been shown to confer resistance to EGFR inhibitor therapy in colorectal cancer [51,52]. As yet, *BRAF* testing is not routine when deciding whether or not to treat with cetuximab or panitumumab, but this may become standard in the near future [53]. Also, with the development of novel BRAF/MEK inhibitors (MEK is a protein downstream of BRAF), *BRAF* mutations are a potential target for treatment. There is *in vitro* evidence of synergy between cetuximab and sorafenib (which acts as a *BRAF* inhibitor). In colorectal cancer cell lines expressing *BRAF* mutations, the combination of cetuximab and sorafenib has been found to inhibit cell proliferation, and induce a significant apoptotic effect [51]. Thus, resistance to EGFR inhibitors may be overcome by combining them with BRAF/MEK inhibitors. The use of BRAF and MEK inhibitors in the treatment of *KRAS*-or *BRAF*-mutated colorectal cancer is currently being investigated in clinical trials, both as monotherapy and in combination with EGFR inhibitors.

PIK3CA mutations

The PI3K-AKT pathway is another functionally important signaling cascade acting downstream of the EGFR and instrumental in controlling cell proliferation, motility, cell death and cell invasion (Figure 2) [54]. The *PIK3CA* gene, which encodes a catalytic subunit of PI3K, is mutated in various solid tumors, including 15–20% of colorectal cancers, and leads to constitutive activation of the PI3K-AKT pathway [38,55]. Mutations typically occur in exon 9 or exon 20 [50] and can occur concurrently with *KRAS* mutations [38,56].

There is evidence that *PIK3CA* mutations also confer resistance to EGFR inhibitor therapy, both *in vitro* [57] and in the clinical setting [43,58]. PI3K inhibitors are now being developed and are in early-phase clinical trials [59]. There is also preclinical evidence that combining MEK inhibitors and MTOR inhibitors (MTOR is a protein downstream of AKT, involved in regulating cell growth and apoptosis) has a greater effect in suppressing cancer cell proliferation and inducing apoptosis in colorectal cancer cell lines and reducing tumor volume in mice models compared with single-agent therapy [60].

CIN

CIN is the most common type of genomic instability in colorectal cancer, occurring in between 65 and 85% of cases, depending on the definition used [12,61]. CIN is not very clearly defined, but is generally characterized by chromosomal rearrangements that result in

tumor aneuploidy, allelic losses, amplifications and translocations. This can lead to multiple mutations, including loss of tumor suppressor genes such as *APC* or *p53* [14,62].

Until a few years ago, the prognostic significance of CIN was unclear. However, in 2008, a meta-analysis of 63 studies, involving 10,126 patients, unequivocally demonstrated that the presence of CIN in colorectal cancer correlates with a poorer prognosis. CIN was detected in 60% of cases and the overall hazard ratio for death associated with CIN was 1.45 (95% CI: 1.35–1.55; p<0.001) [61].

The same meta-analysis also considered the potential predictive value of CIN in determining response to systemic treatment. There is emerging preclinical data that CIN-mutated cancer cells are intrinsically more resistant to cytotoxic drugs than CIN-negative cancer cells [63]. However, the review concluded that there is insufficient clinical data currently regarding the potential for CIN status as a predictive biomarker and recommends that future clinical trials should stratify patients based on molecular tumor analysis in order to determine the contribution of mutations such as CIN to treatment sensitivity and resistance [61].

Chromosome 18q deletion

A common CIN mutation is the deletion of the long arm of chromosome 18, present in up to 70% of cases [14]. Chromosome 18q contains a number of genes thought to play a role in suppressing tumorigenesis. These include the *DCC* gene, which codes for a netrin-1 cell surface receptor involved in promoting apoptosis (apoptosis being induced in the absence of netrin-1 ligand, rather than in the bound state) [64], and the tumor-suppressing transcription factors SMAD2 and SMAD4, which mediate TGF-β signaling (an important regulator of proliferation, differentiation and apoptosis) [65].

A systematic review and meta-analysis suggests that loss of chromosome 18q correlates with a poorer prognosis [66]. However, whether 18q deletion is an independent prognostic marker, or poorer prognosis is linked to the association between CIN and 18q loss, is unclear.

The role of chromosome 18 deletion as a predictive biomarker for response to systemic therapy is also unclear. A study of 75 patients with Dukes C colorectal cancer found that low SMAD4 levels correlate with poor response to 5-FU-based chemotherapy [67]. In the same study, presence or absence of allelic imbalance in chromosome 18q did not significantly predict for prognosis following surgery and 5-FU-based chemotherapy; however, the numbers involved are small.
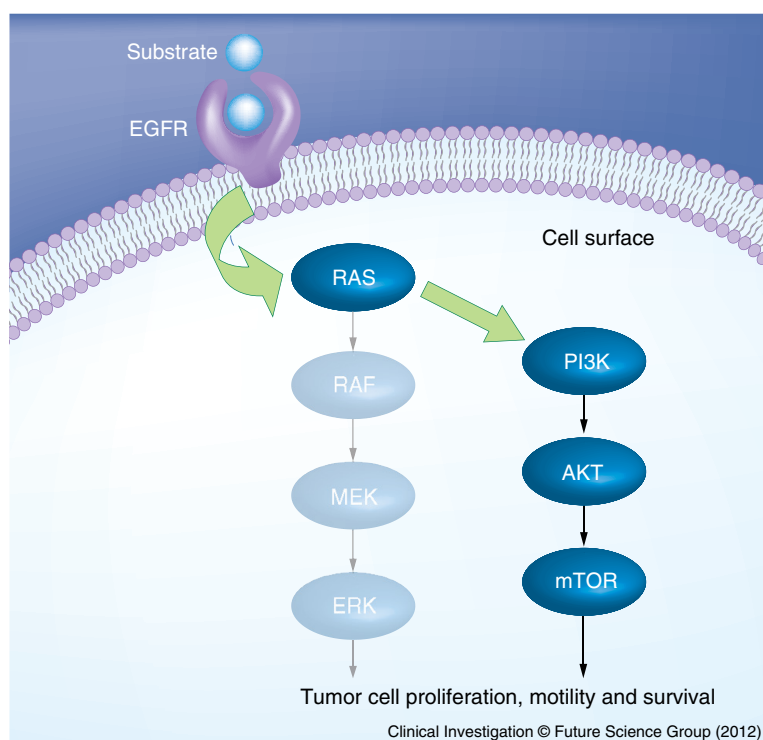

Figure 2. PI3K-AKT pathway.

Mismatch repair/MSI

This form of genomic instability occurs in approximately 15% of sporadic cases of colorectal cancer, as well as in patients with hereditary nonpolyposis colorectal cancer [12]. It is a more clearly defined subtype of genomic instability than CIN, with a typically diploid chromosome component. MSI-high colorectal cancer occurs as a result of defective function of DNA mismatch repair (MMR) proteins [68] and is associated with a better prognosis, when compared with MSI-low and microsatellite-stable (MSS) tumors [69]. The reason that MSI-high colorectal cancer is associated with better prognosis is not clear, but may be because mutations associated with poor prognosis, such as loss of function of *DCC* or *TP53*, or activating mutations in *KRAS*, occur less commonly in MSI-high tumors [70].

There is increasing evidence that the MSI phenotype of colorectal cancer is less responsive to treatment with 5-FU-based chemotherapy. *In vitro* data have shown that MMR protein-deficient cancer cell lines are more resistant to treatment with 5-FU [71,72]. This is backed up by some clinical studies. A prospective study of patients with stage II or III colorectal cancer showed that adjuvant 5-FU chemotherapy improves survival in patients with MMR-competent tumors, but not in patients with MMR-deficient tumors [73]. MMR deficiency was defined as present when either testing with the BAT-26 microsatellite marker showed MSI

or immunohistochemistry showed loss of the MMR proteins MLH1 or MSH2. Retrospective analyses of patients with stage II and stage III colorectal cancer have also shown that patients with high MSI tumors do not benefit from adjuvant 5-FU therapy, whereas those with low MSI or MSS tumors (as determined by various microsatellite markers, including BAT-26) do benefit [74,75].

However, seemingly paradoxically, a study of 244 patients with stage IV colorectal cancer treated with 5-FU chemotherapy in a palliative setting found that patients with high MSI tumors were more responsive to 5-FU chemotherapy, with a statistically significant improvement in survival, compared with those with low MSI or MSS tumors. This study also did not find a statistically significant difference in survival between high-MSI tumors and non-high-MSI tumors in untreated patients with stage IV colorectal cancer. However, numbers in each subgroup were small and the study was nonrandomized as patients could choose whether or not to have palliative chemotherapy [76].

Once again, more robust clinical data is needed to determine if the MSI phenotype will be a useful prognostic and/or predictive biomarker. Overall, the benefit of 5-FU in the adjuvant treatment of stage III colorectal cancer is well established, but for stage II disease this is less clear [69]. In the future, establishing the MSI status of a patient's tumor could play an important role in determining both prognosis and whether or not adjuvant 5-FU chemotherapy is likely to provide significant benefit.

Gene expression signatures

As discussed in the introduction of this review, one of the major goals in colorectal cancer research is establishing more accurate methods of predicting which patients with stage II colorectal cancer are most at risk of relapse following surgery and thus most likely to benefit from adjuvant systemic therapy. As outlined above, understanding the molecular pathology of tumors and the effect on prognosis and response to systemic therapy is complex, and for each individual case often involves establishing the effect of multiple mutations. The development of gene-expression arrays may provide the answer to this complex problem.

The development of DNA microarray technology in the 1990s means that it is now possible to assess the expression of thousands of genes at once. Thus, it is now possible to identify patterns of gene expression that correlate with specific tumor biology and prognosis [77]. In breast cancer the Oncotype DX® assay (which generates a 21-gene signature from paraffin-embedded tumor specimens) has been developed and validated

as both a prognostic and predictive tool for early-stage breast cancer, and is now included in both National Comprehensive Cancer Network and American Society of Clinical Oncology guidelines as a potential risk stratification tool for patients with lymph node-negative, estrogen receptor-positive breast cancer [102,78]. Gene-expression profiling has also been explored as a risk stratification tool in colorectal cancer and two tools for assessing gene-expression signatures have recently been developed.

The Oncotype DX® Colon Cancer test (developed by Genomic Health; CA, USA) is a 12-gene signature assay using DNA extracted from fixed, paraffin-embedded tissue samples. The test has been validated using tumor specimens from patients with stage II colon cancer, enrolled in the QUASAR study. Of 711 patients treated with surgery alone, the 12-gene signature score was significantly associated with recurrence risk at 3 years after surgery [79]. The Oncotype DX Colon Cancer test was released for commercial use in January 2010.

The ColoPrint® test (developed by Agendia; CA, USA) is an 18-gene signature assay using DNA extracted from fresh frozen tumor samples. The test has been validated using a set of 206 tumor specimens from patients with stage I–III colorectal cancer and has been shown to be significantly associated with prognosis. In this data set, the ColoPrint test classified 60% of samples as low risk and 40% of samples as high risk for relapse. Relapse-free survival at 5 years was 87.6% in the low-risk group and 67.2% in the high-risk group [80].

Thus, gene-expression profiling shows promise as a prognostic biomarker for colorectal cancer [81–83]. However, neither Colotype DX Colon Cancer nor ColoPrint have shown the potential to be able to predict response to systemic therapy [79]. Both tests have also only been validated retrospectively. Ideally, prospective validation in the context of a clinical trial is needed to more reliably establish the potential of these tests [84]. ColoPrint is currently being tested in this way in a study called PARSC.

Future perspective

For the last 30 years the treatment of colorectal cancer, like most other solid tumors, has been largely determined by clinicopathological and histopathological staging. Currently, of the many patients treated with adjuvant systemic chemotherapy, only a few derive significant benefit. In the metastatic setting, targeted agents are providing increasing options for systemic therapy, but only in a subset of patients. The cetuximab story is a particularly salient example of the importance of understanding the molecular biology of cancer when judging the effectiveness of treatment modalities.

Executive summary

Background
- Current systemic therapy for colorectal cancer mainly consists of 5-fluorouracil, capecitabine, oxaliplatin, irinotecan and bevacuzimab, either as single agents or in combination, with no established means of predicting response to treatment.
- Targeted agents, such as EGFR inhibitors, are only effective in a subpopulation of patients with colorectal cancers, defined by presence or absence of a *KRAS* mutation. Determination of *KRAS* wild-type status is currently required for treatment with anti-EGFR therapy.
- Adjuvant chemotherapy for patients with stage II colorectal cancer provides only modest benefit in a small number of patients treated.

Molecular biology & genetics
- Characterizing mutations in cell signaling genes (e.g., *KRAS*, *BRAF* and *PIK3CA*) and genomic alterations (e.g., chromosomal instability, chromosome 18q deletion and microsatellite instability) can predict both prognosis and response to treatment with systemic agents, both old and new.
- Molecular analysis of tumors provides a way to better stratify patients into those most likely and least likely to benefit from adjuvant systemic therapy.

Future development of targeted therapy
- *BRAF* and *PIK3CA* mutations may further predict likelihood of response to EGFR inhibitors.
- More targeted therapy with BRAF/MEK/PI3K/mTOR inhibitors may be in use soon.
- New potential targets, such as TAK1, are being discovered.
- Gene-expression profiling shows promise as an effective prognostic biomarker and is currently being investigated further via ongoing clinical trials.

Thus, understanding the molecular biology of cancers and establishing the specific mutations driving cancer growth in individual cases will become increasingly important in the coming decade. Powerful new tools such as DNA microarray technology will allow us to build up a more complete picture of the numerous mutations that can exist in one cancer and enable us to better tailor the need for and type of systemic therapy to individual cases.

In the next few years, it is likely that molecular analysis of colorectal cancer specimens at diagnosis will become standard practice, with tests such as gene-expression profiling and somatic mutation analysis helping to predict the risk of relapse, as well as selecting which systemic therapies are most likely to be effective for individual patients. Establishing the specific genetic profile of individual tumors will enable the use of a combination of different targeted therapies, which is likely to reduce resistance and improve response to treatment and control of disease, thus reducing the mortality rate for colorectal cancer even further.

Financial & competing interests

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