**Review**

# Prognostic and predictive clinical, pathological, and molecular biomarkers in metastatic colorectal carcinoma – a review

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**160 Character summary:** This article outlines current knowledge of prognostic and predictive biomarkers in metastatic colorectal carcinoma.

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**Abstract**

Ongoing research increasingly reveals that metastatic colorectal carcinoma (mCRC) is a highly heterogeneous entity. Despite extension of the median survival of mCRC patients due to advances in therapeutic options available, further improvement and better rationalisation of resources could be achieved by more accurately predicting individual patient prognoses and responses to specific treatments. It is hence important to further our understanding of prognostic and predictive biomarkers in mCRC to enable accurate estimation of treatment benefit for individual patients and therefore guide patient selection. This information can also be used for improving patient stratification in future studies. The aim of this literature review is to highlight potential prognostic and predictive clinical, pathological and molecular biomarkers in mCRC. Broad categories include patient and tumour markers, protein markers and cell-free DNA, inflammatory markers and genetic markers.

The potential prognostic and predictive values of factors such as performance status, *BRAF* mutational status and neutrophil:lymphocyte ratio (NLR) >5 are supported by consistently strong evidence, but interpretation of the roles of other factors is difficult due to inconsistent findings between studies; however, many studies examine only small cohorts of patients, thereby limiting statistical power and variability in cut-off points may have contributed to different findings between trials. Although existing evidence may be used to select patient treatments and guide stratification in trials, future research with larger patient cohorts and clarification of appropriate cut-off values may prove helpful in elucidating the value of these biomarkers.

## Introduction

In Australia, colorectal carcinoma (CRC) has the second highest incidence and mortality rate of all cancers (excluding non-melanoma skin cancers), with approximately 16,680 diagnoses and 4,110 deaths, annually [1]. Long-term prognosis is largely determined by the presence of distant metastases as part of stage IV metastatic CRC (mCRC), which most commonly involve the liver [2,3]. Up to 20-25% of patients have liver metastases at the time of diagnosis and as many as 50-75% will develop liver metastases over the course of their disease [2-6]. The past two decades have seen the development of multiple novel chemotherapeutic and targeted biologic agents, as well as improved surgical techniques and supportive care. These advancements have substantially improved the prognosis of mCRC; however, individual prognoses and responses to chemotherapy remain highly variable and are not adequately explained or predicted by markers used in clinical practice [2,7-9]. Therefore, in the era of individualised therapy, identification of biomarkers (molecules, genes, or characteristics which can denote prognostic or predictive information) is an important and ongoing area of research and may help to guide and optimise patient treatment decisions and outcomes, and inform future therapeutic targets.

## Materials and Methods

This review aims to summarise current understanding of potential prognostic (relating to survival) and predictive (relating to response) biomarkers in mCRC and highlight relative strengths and deficiencies in this knowledge. It will be limited in scope to patients with metastatic disease. Potential biomarkers will be broadly considered in the categories of patient and tumour characteristics, protein markers and cell-free DNA, inflammatory markers and genetic markers. Literature searches were conducted from March 2016 to September 2017. The biomarkers to be discussed in this report were selected based on the quantity and quality of evidence available regarding their role in prognosis and prediction of treatment benefit.

## Patient and tumour characteristics

*Patient characteristics*

Age has generally been found not to bear prognostic significance in mCRC [4,10-17], although more advanced (≥75 years) and younger (≤40 years) age categories have been independently linked with poorer survival in a small number of studies [5,18-20]. No consistent relationship has been demonstrated between sex and prognosis [4,10-12,14,16-18]. In contrast, the independent prognostic significance of Eastern Cooperative Oncology Group (ECOG) performance status has been well validated in mCRC, with values of ≥1 [8,21-23] or ≥2 [5,16,17,22-24] predicting poorer outcomes in most series.

*Tumour grade*

The influence of primary tumour grade on outcome is unclear. Although some studies report that high tumour grade is an independent negative prognostic factor for survival [10,25], others report that it is not [16,26-28]. The degree of differentiation of colorectal liver metastases has also been investigated, but does not appear to have an independent effect on prognosis [6,27].

*Mucinous vs. non-mucinous histology*

Mucinous adenocarcinoma represents 5-15% of primary CRC, and is associated with younger patients, proximal tumour location, more advanced disease at presentation, lower p53 expression, microsatellite instability (MSI), specific *KRAS* mutations, *BRAF* mutation, and a higher index of diploidy [24,29]. It has been independently associated with poor overall survival and resistance to first-line chemotherapy [23,24,29]. Contrasting reports exist [11,30], although one of these projects, Hill *et al.*, [30] studied a paediatric population, which may represent a distinct subgroup of patients with unique tumour biology and behaviour.



*Tumour size*

The size of the primary tumour, Dukes stage, and T stage (of the TNM staging system) do not appear to independently correlate with survival in mCRC [10,11,16,27,28,31]. In assessing the impact of the diameter of the largest liver metastasis, varying cut-off values have been used. Neither ≥3 cm [27,32,33] nor ≥5 cm [6,10,13,14,18] diameter cut-offs significantly impacted on survival rates on multivariate analysis in any previous study. However, Rees *et al.* [25] yielded a significant independent negative association with survival using a cut-off of ≥10 cm diameter. No studies reviewed examined metastasis size as a continuous variable. It is therefore possible that using a larger cut-off for metastasis diameter may be valuable.

*Primary tumour location*

Right-sided colon cancers appear to be a distinct subgroup of CRC, with a different pattern of metastasis and molecular characteristics to left-sided tumours. Right-sided cancers are more likely to be poorly differentiated, be mucinous and harbour *KRAS* and *BRAF* mutations [16,34,35]. Clinically, the pattern of metastatic spread differs, with fewer liver and lung metastases [16]. Recent data suggest that right-sided tumours might be more resistant to epidermal growth factor receptor (EGFR) inhibitors [16]. Right-sided tumours also appear to have an association with synchronous metastatic disease at diagnosis [34], but this may be subject to lead time bias given that right-sided tumours tend to cause fewer symptoms and therefore are commonly diagnosed later. Overall, right-sided cancers seem to be associated with poorer survival on multivariate analysis [5,11,34-36]. However, Brule *et al.* found that right-sided cancer did not confer inferior prognosis in patients who received only supportive care, although it did predict inferior progression-free survival for wild-type (unmutated) *KRAS* patients treated with cetuximab [16]. In light of the above, it is evident that right-sided tumours are clinically different to left-sided tumours and that primary tumour location should be

considered in the prognosis and treatment of all patients. Further research is necessary to understand what drives these differences, particularly at the molecular level.

*Nodal status of the primary tumour*

Involvement of locoregional lymph nodes by metastatic tumour has been variably reported to impact survival. Some studies suggest that the presence [5,11,25] and number ≥4 [28] of nodal metastases both independently impact survival, but the majority of reports suggest that these are not useful markers in mCRC [4,6,10,13,14,16,27]. Minagawa *et al.* [28] specifically identified the presence of hepatic lymph node metastases as an independent prognostic marker, which may offer one promising avenue of future research.

*Synchronous vs. metachronous metastases*

Although synchronous metastases (those diagnosed within six months of the primary tumour) may bear worse prognosis than metachronous metastases on univariable analysis [2,37], an independent prognostic effect has not been consistently demonstrated [2,10,13,14,18].

*Number and location of metastases*

The number of distant metastatic sites (e.g. liver, lungs) is inconsistently reported to impact survival. Although two studies did find an independent prognostic impact of ≥2 sites of metastasis [24,38] and another found that ≥3 had independent impact [16], other studies have stated that the number of sites does not significantly affect prognosis [5,12,15]. Currently, no strong evidence links metastases in any particular location with poorer prognosis. Liver involvement is generally found not to exert an independent influence on survival [22,35,39], although extrahepatic dissemination, in general, may predict poorer survival in patients with known hepatic metastases [2,11,25]. Peritoneal dissemination has been associated with poorer survival [24,31], although this association is inconsistent [5,35] and this relationship may be due to an association with *BRAF* mutations [35].



**Protein markers and cell-free DNA (cfDNA)**

*Carcinoembryonic antigen (CEA)*

CEA is a tumour marker commonly elevated in mCRC. Its prognostic value is historically inconsistent, with cut-off values that vary greatly and studies arriving at opposite conclusions [5,6,10,13-15,25,27,28,32,33,39,40]. This may be because elevated CEA is not specific to CRC, and therefore CEA levels are influenced by other factors such as systemic inflammation and cigarette smoking. Adjusting for these confounding factors may increase the value of CEA in patient stratification [41].

*Alkaline phosphatase (ALP)*

ALP is commonly elevated in CRC. Only a small number of studies have yet assessed the prognostic significance of ALP on multivariate analysis, but those that have suggest an independent association with overall survival [16,22,42].

*Lactate dehydrogenase (LDH)*

LDH is important in cancer metabolism and is also a marker of cell lysis, so is commonly raised in malignant disease. Only a limited number of studies investigated serum LDH levels, but all report a significant independent association with survival [16,23,38]. However, despite being prognostic, it is unlikely that ALP or LDH will have a major impact on clinical decision-making given their non-specific nature, similar to CEA.

*C-reactive protein (CRP)*

CRP is a non-specific marker elevated in systemic inflammatory conditions, and is frequently found to be elevated in CRC. Elevated CRP ≥10 mg/L has consistently been reported to be significantly associated with poorer survival on multivariate analysis [14,32,39,43,44]. Read *et al.* [22] did not find a significant association with outcome when analysing CRP as a categorical variable, but an independent prognostic role was discovered when re-analysing as a continuous variable. Only Sharma *et al.* [15] did not define a prognostic role for CRP, and this study was limited in statistical power by a small cohort size. Therefore, current consensus suggests that CRP is a potentially valuable marker in mCRC.

*Cell-free DNA (cfDNA)*

cfDNA refers to small circulating DNA fragments. Recently, cfDNA has been demonstrated to be useful not only as an alternative means of obtaining information about the genetic composition of the tumour in mCRC, but also as a prognostic marker [45-49]. Higher quantitative levels of cfDNA have been linked with poorer survival in a number of small studies [45-48], and qualitative characteristics such as cfDNA methylation and fragmentation have additionally been associated with prognostic roles [48,49]. Further investigation of cfDNA in larger patient cohorts may help to consolidate its role in mainstream clinical use.

**Inflammatory markers**

*Neutrophil:lymphocyte ratio (NLR)*

NLR serves as an indicator of the interaction between the tumour and the patient's immune system, and is associated with a distinct expression profile of cytokines [38]. In particular, elevated NLR values represent a high degree of non-specific systemic inflammation, which can lead to cachexia and may also represent poor lymphocytic cell-mediated immunity against the cancer. Genomic instability and DNA damage induced by chronic inflammation also help to promote carcinogenesis [38]. NLR can be cheaply measured via peripheral blood samples, and although optimum cut-off limits are yet to be defined or standardised [50], NLR >5 has consistently been associated with an independent negative impact on survival [4,8,13,14,38,40]. Its normalisation during treatment has also been shown to predict improved outcomes [8,13]. Therefore, NLR represents an inexpensive, readily available, and reliable biomarker for the prediction of survival in patients with mCRC. Similarly, Okano *et al.* [51] found that a dense lymphocytic infiltrate alone in the tumour independently predicted superior overall survival compared to patients with weak lymphocytic infiltration. This reinforces the benefit of a strong lymphocytic immune response to malignancy.

*Platelet:lymphocyte ratio (PLR) and thrombocytosis*

PLR has been investigated as an alternative to NLR in the assessment of the patient's immune response to the tumour, and as a prognostic marker, but it is considered to be inferior to NLR, because despite correlation with survival on univariate analysis [4,40,52,53], only Neofytou *et al.* [52] found it to be independently predictive. Neofytou *et al.* [52] also unconventionally defined elevated NLR as NLR >2.4 rather than >5.0, possibly confounding this conclusion. Therefore, it is suggested that NLR should be used over PLR for prognostication in mCRC. Absolute thrombocytosis has been more consistently demonstrated to have an independent negative association with survival [42,53,54]. Adams *et al.* [54]specifically identified that a high platelet count predicts worse outcomes in patients receiving intermittent (as opposed to continuous) chemotherapy, suggesting that it may be preferable to avoid this strategy in these patients.

## Genetic markers

*KRAS*

*KRAS* encodes a protein involved in signal transduction downstream from the epidermal growth factor receptor (EGFR), and is a key player in colorectal cancer initiation and progression. *KRAS* mutation has been associated with prognostic and predictive roles in mCRC, being linked with reduced survival [16,21,55-57] and resistance to the anti-EGFR monoclonal antibodies cetuximab and panitumumab [55,56,58-64]. It does not confer resistance to conventional chemotherapeutic agents [57]. The role of KRAS mutation in predicting complete lack of benefit from anti-EGFR antibodies is very well established. However, its prognostic impact on overall survival has been disputed [12,58]. Furthermore, not all studies reporting a negative association performed multivariate analysis of factors influencing survival [56]. Regardless of the impact of *KRAS* mutation on survival, these findings have changed clinical practice so that all mCRC patients are tested for *KRAS* mutations prior to initiating treatment with anti-EGFR-based therapies [65]. Kodaz *et al.* [66] also suggested that primary tumour resection may offer a survival advantage for *KRAS* mutant patients, but not for patients with *KRAS* wild-type tumours. The underlying mechanism accounting for this observation is unclear and there are many factors impacting a decision to remove the primary tumour in mCRC, which must also be considered.

*NRAS*

*NRAS* encodes a signalling protein closely related to *KRAS*. De Roock *et al.* [55] identified that in *KRAS* wild-type patients, carriers of *NRAS* mutations have a significantly lower response rate to cetuximab-based therapy when compared to *NRAS* wild-type patients. A subsequent analysis of the PRIME study by Douillard *et al.* [67] confirmed that mutations in exons 2, 3, and 4 of *KRAS* or *NRAS* all predicted a lack of benefit from anti-EGFR antibodies. Further, Schirripa *et al.* [68] concluded that *NRAS* mutations also confer poorer overall survival in addition to resistance to anti-EGFR-based chemotherapy. Therefore, it is now routine to test for all these mutations in an extended *RAS* panel prior to initiating anti-EGFR therapy [65].



*BRAF*

*BRAF* encodes a protein kinase directly downstream from *KRAS* in the EGFR signalling pathway. *BRAF* mutations have been found to have a substantial and clinically significant prognostic impact on overall survival [21,23,35,55,57,59,63,64,69]. It appears *BRAF* may also have a negative predictive effect for anti-EGFR efficacy [55,59], although not as conclusively as *KRAS* and *NRAS* mutations. Tran *et al.* [35] suggest that differences in survival with *BRAF* mutation may be due to a higher rate of metastases to sites not typically amenable to resection, such as the peritoneum and distant lymph nodes.

*EGFR*

Counterintuitively, evidence supports that detected expression of EGFR by immunohistochemistry may not be a prerequisite for response to anti-EGFR therapy [60-62,64]. Only Chen *et al.* [26] reported a correlation between increased protein expression and improved survival in response to cetuximab treatment. However, while some studies claim that high EGFR gene copy number or amplification is associated with response to cetuximab [58,60,69], others claim that the gene status of EGFR is also not relevant to response or survival [62,64]. It has been suggested that methodological issues such as choice of fixative and storage time may be responsible for these findings [60], and therefore further investigation into the potential role of EGFR is warranted.

*Microsatellite instability (MSI)*

MSI represents approximately 15% of sporadic CRC cases and is an alternate pathway of

tumourigenesis characterised by loss of function of DNA mismatch repair genes [70]. MSI has been found to impart a variable impact on survival in mCRC [71-74] and no apparent impact on benefit from any particular therapeutic agent [70,75]. Tran *et al.* [35]found a negative prognostic effect of MSI on univariate, but not multivariate analysis, and propose this relationship is explained by the association of MSI with *BRAF* mutation demonstrated in this study. This suggests a potentially confounding effect from *BRAF* status that was not accounted for in the other studies above. In contrast, Liang *et al.* [73] suggested an independent positive prognostic role of MSI status, postulating that the superior chemosensitivity of MSI tumours is responsible for their improved survival. Overall, MSI status does not appear to have a major impact on prognosis in mCRC. Of increasing interest is that MSI tumours have a high mutational burden and immune cell infiltrate, and therefore these patients may benefit from treatment with checkpoint inhibitors such as pembrolizumab and nivolumab [76].

## Conclusion

Within this review, a number of patient and tumour characteristics, protein markers and cfDNA, inflammatory markers, and genetic markers have been assessed regarding their potential roles as prognostic and predictive biomarkers in mCRC (Table 1). A recurring theme across each of these three broad categories has been a lack of clear consensus on the significance of various factors, driven by issues such as small sample sizes, methodological issues and inadequate statistical analysis. Additionally, the picture may be confounded by associations between multiple poor prognostic factors such as right-sided primary tumour, *BRAF* mutation, and mucinous carcinoma, leading to difficulty assessing the individual impact of each factor. Although the significance of some markers is relatively clear, such as for ECOG performance status, NLR >5, and *KRAS* and *BRAF* mutational statuses, the value of other biomarkers requires clarification by multivariate analysis of data from larger patient cohorts. Further, although some markers appear to carry independent prognostic significance (such as LDH and ALP), their use in clinical practice for guiding treatment decisions may be limited, with a greater importance placed on markers which reflect molecular drivers of the disease process and hence potential targets for cytotoxic or biological agents.

The findings from this study contribute to arguments for and against the use of each of the mentioned biomarkers in prognostication and treatment prediction in mCRC, which will in turn help to guide clinical decision-making and the provision of information to patients. Accurate estimation of prognosis is important to patients as well as clinicians, and better knowledge of relevant prognostic factors in an individual patient may facilitate this discussion. Additionally, predictive factors indicating resistance to particular treatments should be used to guide selection of chemotherapeutic agents, underscoring the need for adequate genetic screening of patients presenting with mCRC early in their disease course. Identification of those factors that confer poorer prognosis or resistance to chemotherapy might also elucidate possible drivers of cancer aggressiveness or resistance, and thereby highlight potential targets for the development of future treatments.

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***Table 1:*** *Summary of markers and their effect on prognosis or prediction of response in mCRC.*

| **Markers** | **Effect** |
|---|---|
| Patient characteristics | • Age and sex are unlikely to have significant role [4,10-18]<br>• Higher ECOG scores appear to correlate with poorer prognosis [5,8,16,17,21-24] |
| Tumour grade | • Unlikely to have significant role [6,16,26-28] |
| Mucinous histology | • Likely associated with poor prognosis and resistance to chemotherapy [23,24,29] |
| Tumour size | • Primary tumour or metastasis size unlikely to have significant role [6,10,11,13,14,16,18,27,28,31-33]<br>• Diameter of largest liver metastasis may have role in very large sizes (>10cm) [25] |
| Primary tumour location | • Right-sided tumours may be more resistant to anti-EGFR therapies and are associated with poorer prognosis [5,11,16,34-36] |
| Nodal status | • Unlikely to have significant role [4,6,10,13,14,16,27] |
| Synchronous vs. metachronous | • No significant independent role [2,10,13,14,18] |
| Number and location of metastases | • Number of sites of metastases inconsistently reported to impact survival [5,12,15,16,24,38]<br>• Location of metastases unlikely to have significant role [5,22,35,39] |
| CEA | • CEA is unlikely to have a significant predictive or prognostic role, but remains a useful marker for monitoring response to treatment [5,6,10,13-15,25,27,28,32,33,39,40] |
| ALP | • High ALP is associated with poorer prognosis [16,22,42] |
| LDH | • High LDH is associated with poorer prognosis [16,23,38] |
| CRP | • High CRP is associated with poorer prognosis [14,32,39,43,44] |
| cfDNA | • Quantitative levels are a marker of poorer prognosis [45-48]<br>• Qualitative features such as methylation and fragmentation also may have prognostic roles [48,49] |
| NLR | • NLR >5 is associated with poorer prognosis [4,8,13,14,38,40]<br>• Normalisation of NLR predicts better prognosis [8,13] |
| PLR and thrombocytosis | • PLR is unlikely to have a significant role [4,40,52,53]<br>• Thrombocytosis is associated with poorer prognosis [42,53,54] |
| KRAS and NRAS mutations | • Currently both assessed using extended RAS screening [65]<br>• Most widely utilised currently for very strong prediction of lack of response to anti-EGFR therapies (e.g. cetuximab) [55,56,58-64,67]<br>• Also probable markers for poorer prognosis [16,21,55-57,68] |

| | |
|---|---|
| BRAF mutation | • Strong marker of poor prognosis [21,23,35,55,57,59,63,64,69]<br>• Possible role in further predicting poor response to anti-EGFR therapies and conventional chemotherapy [55,59] |
| EGFR mutation and copy number | • Unlikely to have major role in predicting response, even to anti-EGFR therapies, or prognosis [60-62,64] |
| Microsatellite instability | • Unlikely to have major role in predicting response or prognosis [70-75] |