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REVIEW ARTICLE

Biomarkers for colorectal cancer chemotherapy: Recent updates and future perspective

Pey Yee LEE*, Farina Syazwani MD AZHAN, Teck Yew LOW*

UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia, 56000 Kuala Lumpur, Malaysia

Abstract

During the last few decades, the treatment options available for patients with metastatic colorectal cancer (mCRC) have undergone continuous improvements, transitioning from conventional chemotherapy to targeted therapy. These therapeutic innovations have led to significant improvements in patient clinical outcomes. However, there remains a need to improve the outcome for many CRC patients. Chemotherapy remains a cornerstone of CRC treatment, but the wide variability in tumour response and adverse reactions to chemotherapy poses a challenge to cancer treatment management. As a result, there is an unmet need to identify predictive biomarkers of chemotherapeutic response to guide treatment decisions. In this review, we summarise the conventional biomarkers used to predict chemotherapy responses in CRC and provide an overview of emerging predictive biomarkers based on the current understanding of the molecular biology of treatment response. Finally, we explore the challenges and future prospects of biomarker discovery to improve the prediction of patient response and ensure optimal treatment management for patients with metastatic CRC.

Keywords: Biomarker; chemotherapy; colorectal cancer; prediction; treatment response

INTRODUCTION

Colorectal cancer (CRC) is a prevalent and lethal disease, with more than 1.9 million new cases and an estimated 935,000 deaths worldwide.[1] Approximately 50% of CRC patients develop metastases, resulting in unfavourable prognoses with a 5-year survival rate of only 15%.[1] For clinicians to determine the most suitable treatment plan and the prognosis for a CRC patient, they need to first determine the stage of the disease. The TNM staging system is the most adopted scheme and it classifies CRC into four stages based on the extent of the cancerous cells' spread, taking into account tumour size and extent (T), lymph node involvement (N), and distant metastasis (M). For early-stage nonmetastatic CRC (stages I to II), surgery is typically used to remove the tumour, and for high-risk stage II patients, neoadjuvant and/or adjuvant treatment using chemotherapy or radiotherapy is recommended in some cases. For the more advanced stage and metastatic CRC (mCRC) with unresectable tumours, systemic chemotherapy is usually the primary treatment. The past decade has seen significant advancements in the development of new therapeutics, leading to substantial survival improvements, with median overall survival (OS) for mCRC patients increasing from approximately 12 months in the years 2008 to 2016 to currently more than 30 months.[2] Figure 1 illustrates the timeline of therapeutic drug development for CRC.

Despite the emergence of new therapeutics such as targeted kinase inhibitors and immunotherapies, chemotherapies remain the most adopted therapeutic options due to their efficacy and accessibility, albeit with lower costs. Chemotherapeutic agents can be broadly classified into five major types: alkylating agents, antimetabolites, antitumour antibiotics, mitotic inhibitors, and topoisomerase inhibitors.[3] Since the 1960s, an antimetabolite drug named fluoropyrimidine 5-fluorouracil (5-FU) has been the cornerstone of CRC treatment and is still the most widely used chemotherapeutic drug, alone or in combination with other drugs.[4] Standard first-line chemotherapy for CRC usually includes

**Address for correspondence*: Pey Yee Lee, UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia (UKM), UKM Medical Centre, Jalan Yaacob Latiff, Bandar Tun Razak, 56000 Kuala Lumpur, Malaysia. Phone: +60 (3) 9145 9063. Fax: +60 (3) 9171 7185. Email: *leepeyyee@ukm.edu.my*. Teck Yew Low, UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia (UKM), UKM Medical Centre, Jalan Yaacob Latiff, Bandar Tun Razak, 56000 Kuala Lumpur, Malaysia. Phone: +60 (3) 9145 9081. Fax: +60 (3) 9171 7185. Email: *lowteckyew@ppukm.ukm.edu.my*.

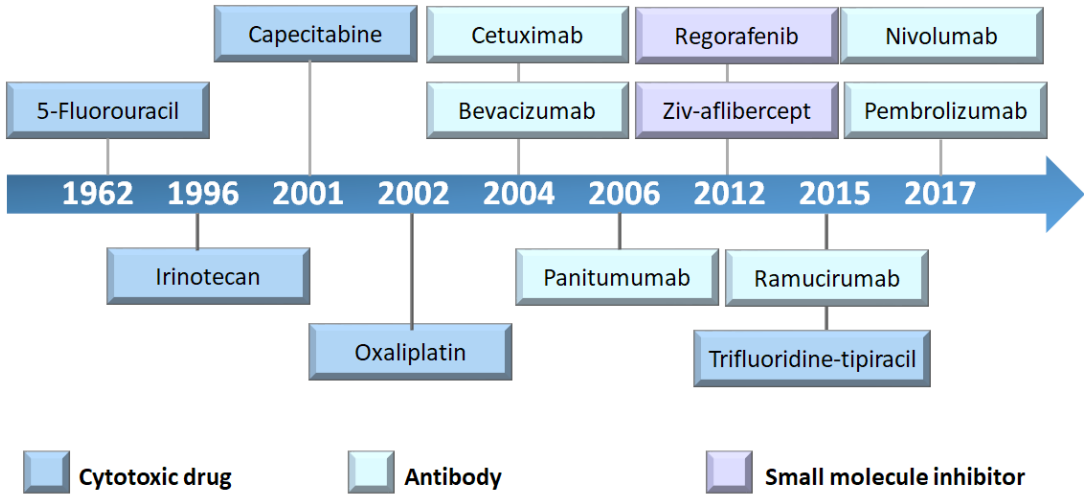


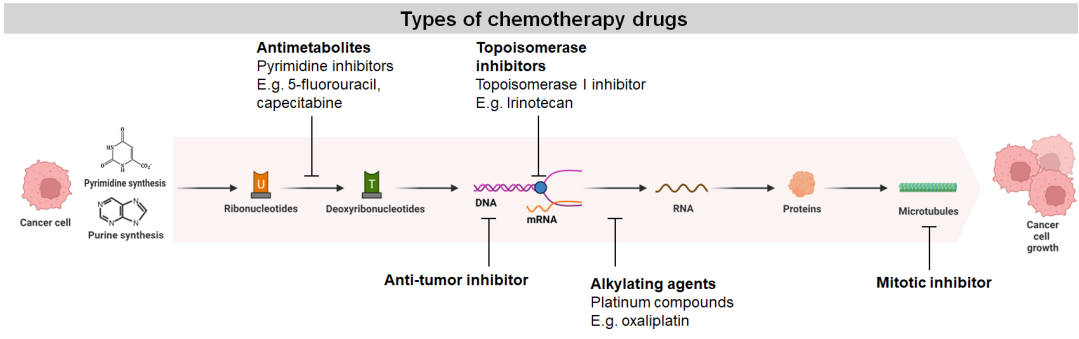
FIG. 1: The development history of treatment for CRC.

a combination of 5-FU and leucovorin. Whereas 5-FU is a nucleoside analogue that inhibits thymidylate synthase, thereby preventing the synthesis of new DNA strands; leucovorin works by providing an excess of building blocks for DNA synthesis and repair, thus boosting the activity of dihydrofolate reductase (DHFR) and enhancing the effectiveness of 5-FU. This combination therapy is widely used for treating colorectal, pancreatic, and gastric cancers.

Further improvement of the 5-FU: leucovorin combination conceived the so-called doublet cytotoxic therapies. For instance, adding oxaliplatin (DNA crosslinking agent, a platinum-based drug) to 5-FU: leucovorin led to the FOLFOX regimen; while adding irinotecan (topoisomerase I inhibitor) led to FOLFIRI.[5] These new combinations offer higher response rates and progression-free survival (PFS) compared to 5-FU alone.[6,7] Later, triplet chemotherapy that combines fluorouracil, leucovorin, oxaliplatin and irinotecan (FOLFOXIRI) was demonstrated to shrink the tumour and improve survival, albeit is only applicable to comorbidity-free patients due to its higher toxicities.[8] Alternatively, capecitabine is used as a 5-FU prodrug as monotherapy[9] or in combination therapy with oxaliplatin (CAPOX or XELOX)[10] to treat mCRC patients. Figure 2 illustrates the different types of chemotherapeutic drugs and the common regimens used for CRC treatment. However, chemotherapy medications are primarily cytotoxic agents that can cause a wide range of toxicities or side effects which can result in severe physiological and psychological stress to the patients. The common side effects of the chemotherapeutic drugs used for CRC treatment are shown in Table 1.

On a separate note, the use of targeted therapeutic agents has significantly improved the treatment outcomes of mCRC patients. Monoclonal antibodies targeting the epidermal growth factor receptor (EGFR), such as cetuximab and panitumumab, and those targeting the vascular endothelial growth factor (VEGF), such as bevacizumab, have been proven effective when used alone or in combination with chemotherapy. In addition, ramucirumab (VEGFR2 inhibitor), and aflibercept (VEGF-A, VEGF-B, and placental growth factor antagonist) have demonstrated their efficacy in combination with chemotherapy as second-line therapy.[11,12] Regorafenib, a multikinase inhibitor; and trifluridine-tipiracil, a chemotherapy drug that combines nucleoside metabolic inhibitor named trifluridine and thymidine phosphorylase inhibitor named tipiracil hydrochloride, have also been recommended as the third or subsequent line of therapy for patients with chemo-refractory mCRC.[13,14] In recent years, immune checkpoint inhibitors such as nivolumab and pembrolizumab have also been approved for the treatment of mCRC patients with microsatellite instability high (MSI-H).[15,16]

Notwithstanding, despite the wide range of treatment options available, the efficacy and adverse effects of these treatments can significantly vary from patient to patient.[17] Therefore, identifying biomarkers that can guide individualized therapy is critical. Specifically, the discovery of predictive biomarkers for cytotoxic



CRC chemotherapy regimens

Regimen	Drug composition
Monotherapy	
5-fluorouracil (5-FU)	5-FU and leucovorin
Capecitabine	Capecitabine
Doublet therapy	
FOLFOX	5-FU, leucovorin and oxaliplatin
FOLFIRI	5-FU, leucovorin and irinotecan
CAPOX or XELOX	Capecitabine and oxaliplatin
Triplet therapy	
FOLFOXIRI	5-FU, leucovorin, oxaliplatin and irinotecan

FIG. 2: The types of chemotherapy drugs and common chemotherapy regimens for CRC.

chemotherapy, which remains the standard of care for CRC, is of great clinical significance. The ultimate goal is to improve patient outcomes by preventing missed treatment opportunities and reducing undesirable side effects.[18] In this article, we present recent advancements in the identification of predictive markers for therapeutic response and toxicity to single chemotherapeutic drugs and their combinations, which can be used to inform treatment decisions. We then discuss the challenges and prospects of biomarker discovery. Table 2 provides a summary of biomarkers for predicting chemotherapy response in CRC.

1. Biomarkers for chemotherapy

1.1 Conventional single biomarkers

1.1.1 Dihydropyrimidine dehydrogenase (DPD)

Many patients with mCRC benefit from neoadjuvant and adjuvant chemotherapy with fluoropyrimidine-based regimens and several markers that inform chemotherapeutic sensitivity or toxicity have been proposed.[19] The *DPYD* gene encodes dihydropyrimidine dehydrogenase (DPD), an enzyme that catalyses the inactivation of fluoropyrimidines and is associated with increased chemotherapy-related toxicity.[20,21] *DPYD**2A and A2846T are allelic variants of *DPYD* that are linked to severe toxicity.[22]

TABLE 1: The reported toxicities/side effects associated with the main chemotherapy drugs for CRC treatment

Drugs	Reported toxicities/side effects	Reference
Fluorouracil	Diarrhea, nausea, vomiting, mucositis, myelosuppression, rash, photosensitivity, cardiotoxicity	109
Capecitabine	Diarrhea, nausea, vomiting, stomatitis, hand-foot syndrome, low red and white blood cell count, abdominal pain	110
Oxaliplatin	Peripheral neuropathy, nausea, vomiting, diarrhea, neutropenia	111
Irinotecan	Early-onset diarrhea: abdominal cramping, increased salivation, rhinitis Late-onset diarrhea: nausea and vomiting, low red and white blood cell count	112

Table 2. Summary of biomarkers for chemotherapy in CRC

Biomarkers	Findings	Reference
Conventional biomarkers		
Dihydropyrimidine dehydrogenase (DPD)	*DPYD**2A and A2846T are *DPYD* allelic variants that are linked to severe fluoropyrimidine toxicity	22
Thymidylate synthase	High thymidylate synthase levels and/or its gene polymorphisms (TSER*3/TSER*3) may be involved in 5-FU resistance	29
	Low levels of thymidylate synthase or specific thymidylate synthase promoter polymorphisms (homozygous for the genotype S/S versus S/L or L/L) are associated with favourable responses to capecitabine	30
UDP glucuronosyltransferase 1 family, polypeptide A1 (*UGT1A1*)	*UGT1A1**28 genotype is linked to chemotherapy-dependent toxicity	32
	*UGT1A1**6 and *UGT1A1**28 polymorphisms are linked to irinotecan-induced toxicity	33
Excision repair cross-complementation group 1 (ERCC1)	Combined low expression of ERCC1 and thymidylate synthase was a predictor of response in patients receiving FOLFOX	36
Caudal-related homeobox transcription factor 2 (*CDX2*)	Lack of *CDX2* expression is linked to the therapeutic benefit of adjuvant chemotherapy	39
Microsatellite instability (MSI) status	MSI-H status did not benefit from 5-FU-based treatment	43,44
Genomic biomarkers	Stem-like-subtype were more responsive and associated with clinical benefit to FOLFIRI chemotherapy	54
	B-type tumours showed significant benefit from adjuvant chemotherapy	53
	CMS1 was reported to have a poorer overall survival with FOLFIRI-based regimens	56
	CMS4 subtype was associated with better survival and response rates in CRC patients receiving first-line irinotecan regimens	57,58
	CMS2 and CMS3 subgroups showed significant benefit from postoperative chemotherapy	59
MicroRNA biomarkers	High levels of miR-21 expression were linked to a poor therapeutic response to 5-FU-based adjuvant chemotherapy	63
	Low levels of miR-148a, miR-150, or miR-320e were predictive of poor response to the FOLFOX	64–66
	High expression of miR-625-3p was connected to poor response to XELOX/FOLFOX	67
Proteomics biomarkers	HSPA4, NIPSNAP1, and SPTB proteins are potential biomarkers to predict response to fluorouracil-based neoadjuvant chemoradiotherapy	73
	Integrated proteomic subtypes of CRC that were able to predict the drug sensitivity of cell lines and patients towards 577 drugs or combinations	74

Immune biomarkers	Higher primary tumour-infiltrating lymphocyte density was linked to better response rates to doublet chemotherapy.	82
	The presence of peritumoral tumour-infiltrating lymphocytes had a substantial survival benefit from adjuvant 5-FU chemotherapy.	83
	High Immunoscores had a significantly better clinical outcome to chemotherapy	78, 79
Microbiome biomarkers	*Lactobacillus plantarum* supernatant (LPSN) may contribute to enhancing the chemosensitivity of 5-FU in CRC-resistant cells	95
	Bacteroides fragilis and *Erysipelotrichaceae* enhance the immunogenicity of oxaliplatin therapy	96
	Fusobacterium nucleatum may contribute to 5-FU and oxaliplatin resistance in CRC	90, 91

Although additional variants have been found, their clinical applicability has not yet been established.[23] Although the European Society for Medical Oncology (ESMO) guidelines currently do not suggest routine DPD testing before the administration of 5-FU or capecitabine, some groups have advocated for *DPYD* genotype and/or phenotype-guided personalized dosing to become the new standard of care, given the high incidence of severe toxicity with fluoropyrimidine treatment in up to 30% of patients.[24,25] The Netherlands, France, and Canada, already adopted routine DPD tests.[26]

1.1.2 Thymidylate synthase

The genetic variations in gene encoding thymidylate synthase are potential indicators of fluoropyrimidine response or toxicity.[27,28] 5-FU inhibits thymidylate synthase, an enzyme required for DNA synthesis. A sufficient suppression of thymidylate synthase leads to chemosensitivity to 5-FU, high levels or gene polymorphisms (TSER*3/TSER*3) of this enzyme may confer resistance to 5-FU.[29] Furthermore, low levels of thymidylate synthase or specific promoter polymorphisms (homozygous for the genotype S/S versus S/L or L/L) have been associated with favourable responses to capecitabine, a 5-FU prodrug.[30] Despite this evidence, routine testing of thymidylate synthase in clinical practice is not yet recommended.

1.1.3 UDP glucuronosyltransferase 1 family, polypeptide A1 (*UGT1A1*)

Polymorphisms in the UDP glucuronosyltransferase 1 family, polypeptide A1 (*UGT1A1*) gene have also been linked to the efficacy and tolerance to irinotecan-based chemotherapy.[31] This family of enzymes are important for the metabolism of many chemotherapy drugs such as irinotecan, modifying and detoxifying these drugs, making them more water-soluble and easier to excrete from the body. Thus, variations in the *UGT1A* genes can affect an individual's ability to metabolise certain chemotherapy drugs, which can impact the effectiveness and toxicity of the treatment. The *UGT1A1**28 genotype has recently been linked to chemotherapy-dependent toxicity, according to data from the PETACC-3 trial.[32] Furthermore, a meta-analysis revealed that the polymorphisms of *UGT1A1**6 and *UGT1A1**28 are correlated with irinotecan-induced toxicity in Asian patients.[33] Patients who were homozygous for the variants were found to have a greater risk for neutropenia and were also more likely to experience severe diarrhoea.[33] In clinical practice, *UGT1A1* genotyping/phenotyping is an optional testing that is performed when *UGT1A1* deficiency is suspected and when administering more than 180 mg/m^2 of irinotecan.[34] Notably, *UGT1A1**6 is more common in Asian patients than in Caucasian patients, while *UGT1A1**28 is less common in Asian patients.[35] A lower irinotecan threshold dose for genotyping is therefore suggested by the Pan-Asian-adapted ESMO consensus guidelines for the management of patients with mCRC, depending on the frequency of *UGT1A1* polymorphisms in each nation.[35]

1.1.4 Excision repair cross-complementation group 1 (ERCC1)

Excision repair cross-complementation group 1 (ERCC1) protein is a component of the

nucleotide excision repair (NER) pathway that is responsible for repairing DNA lesions caused by a variety of carcinogens. These carcinogens include platinum-based chemotherapy drugs, such as oxaliplatin that creates cross-links in the DNA molecule. ERCC1 helps to repair these cross-links by binding to and stabilising another protein called xeroderma protein F (XPF), which together form a complex that can recognise and remove the damaged DNA. Due to its role, ERCC1 has been proposed as a potential biomarker for predicting the effectiveness of platinum-based chemotherapy of mCRC.[36] This study found that a combination of low expression of ERCC1 and thymidylate synthase was predictive of high response rate in patients receiving FOLFOX, but not FOLFIRI.[36] However, in the MAVERICC trial which is the first prospective study to examine ERCC1 mRNA expression level as a potential biomarker for treatments using oxaliplatin, there was no discernible difference between patients with high versus those low baseline ERCC1 who received bevacizumab plus mFOLFOX6 or FOLFIRI.[37] Therefore, ERCC1 is not currently recommended as a biomarker in clinical practice.

1.1.5 Caudal-related homeobox transcription factor 2 (*CDX2*)

Caudal-related homeobox transcription factor 2 (*CDX2*) is a transcription factor that is only found in the intestines and is considered a "master regulator" of intestinal cell identity as it helps in maintaining normal intestinal tissue architecture and function. Colon tumours lacking *CDX2* expression are associated with *BRAF* mutation and CpG island methylator phenotype (CIMP)-high status; and are known to exhibit aggressive clinical behaviour and poor prognosis.[38] It has been demonstrated that the absence of *CDX2* expression may serve as a marker for the effectiveness of adjuvant treatment in colon cancer. Patients with stage II *CDX2*-negative colon tumours who underwent adjuvant chemotherapy had a longer 5-year DFS than those who did not (91% vs 56%), indicating that the lack of *CDX2* expression is linked to the therapeutic benefit of adjuvant chemotherapy.[39] Although promising, these findings need to be confirmed by prospective randomised trials before *CDX2* expression can be used as a biomarker.

1.1.6 Microsatellite instability (MSI) status

Microsatellite instability (MSI), which is caused by the inactivation of the DNA mismatch repair (MMR) machinery, is identified by the presence of a high frequency of frameshift mutations in microsatellite DNA.[40] MMR deficiency results from the loss of MMR proteins, and these cancers are also known as microsatellite instability-high (MSI-H) tumour. MSI is regarded as the chemical signature of an impaired MMR system. A growing body of data indicates that the presence of MSI predicts a lack of response to 5-FU-based adjuvant chemotherapy in CRC patients.[41] In a pooled analysis of five clinical trials involving patients with stage II and stage III colon cancer who were randomly assigned to either adjuvant 5-FU-based treatment or surgery alone, patients with MSI-H status did not benefit from adjuvant chemotherapy in terms of overall survival.[42] These findings were corroborated by Sargent *et al*. that 5-FU treatment did not improve disease outcomes and was associated with reduced disease-free survival and overall survival in stage II CRC patients with deficient MMR tumours.[43] Similarly, a meta-analysis involving CRC patients at various stages from 16 studies has found that 5-FU-based treatment improved disease-free survival and overall survival in patients with microsatellite stable tumours, but no statistically significant therapeutic benefit was observed for MSI-H CRC.[44] Nevertheless, the predictive value of MSI for combination chemotherapy regimens such as FOLFOX and FOLFIRI is rather unclear. Kim *et al*. reported that MSI status alone did not affect the survival outcomes in patients with stage III CRCs receiving adjuvant treatment with FOLFOX.[45] This ambiguity results from variations in the chemotherapy regimens and MSI tests used in the studies, which make it challenging to compare and draw a solid conclusion regarding the utility of MSI as a predictive marker for conventional chemotherapy. Therefore, more prospective trials involving larger cohorts should be carried out to examine the ability of MSI to predict response to combination chemotherapy regimens.

1.2 Genomic biomarkers

Many efforts have been made in recent years to find new molecular markers that could reveal tumour response to cancer therapy. Typically, only one or a few biomarkers such as gene mutations are used for predicting the response to a given chemotherapy treatment, which is insufficient for predicting the complex cellular settings found in cancer.[46] To address the issue of molecular heterogeneity of tumours, omics

profiling technologies have been utilised to identify specific biomarker signatures that can aid in predicting drug response and stratifying patients for treatment.[47,48] The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) are two prominent collaborative initiatives in cancer research that focus on characterising the genomic landscape of various cancer types.[49,50] Both projects aim to improve the understanding of cancer biology, identify potential therapeutic targets, and facilitate the development of personalised cancer treatments.

The discovery of genetic alterations has led to an improved understanding of the response to various anti-cancer therapies in CRC.[51] To understand the heterogeneity of CRC, scientists focus on characterising and classifying it based on its genetic and molecular characteristics. These studies have identified genomic signatures for CRC classification, and demonstrated that each CRC subtype has unique molecular features that are associated with a response to chemotherapy regimens.[52–54] For instance, Sadanandam *et al*. identified six CRC subtypes defined by CRCassigner gene signatures (goblet-like, enterocyte, stem-like, inflammatory, cetuximab-sensitive transit amplifying, and cetuximab-resistant transit amplifying) and revealed that the stem-like-subtype were more responsive to FOLFIRI chemotherapy and associated with better clinical benefit compared to other subtypes.[54] Based on unsupervised classification of whole genome data from CRC patients, Roepman and colleagues described three intrinsic CRC subtypes (A-, B- and C-type) with distinct features of epithelial-to-mesenchymal transition (EMT), deficiency in mismatch repair genes and cellular proliferation. Interestingly, the subtypes were found to be predictive of chemotherapy response, with B-type tumours showing significant benefit from adjuvant chemotherapy, while C-type tumours were non-responsive and did not benefit from chemotherapy.[53]

An international collaborative effort by experts from the CRC Subtyping Consortium later integrated six independent classification systems into consensus molecular subtypes (CMS) for CRC, which allows the classification of CRC into four molecular subtypes with different clinical outcomes.[55] CMS1 (immune) is characterised by microsatellite unstable, hypermutation and strong immune activation; CMS2 (canonical) has epithelial characteristics, high somatic copy number alterations and marked WNT and MYC signalling activation; CMS3 (metabolic) has distinctive *KRAS* mutations and metabolic dysregulation; CMS4 (mesenchymal) is distinguished by mutations in the mesenchymal-epithelial transition, transforming growth factor β activation, stromal invasion, and angiogenesis.[55] This CMS classification has shown the potential to provide a more robust model that can better predict the clinical benefits of chemotherapy. For instance, patients with CMS1 were reported to have a poorer overall survival with FOLFIRI-based regimens than patients with other CMS subtypes in the FIRE-3 trial.[56] Additionally, the CMS4 subtype was associated with better survival and response rates in CRC patients receiving first-line irinotecan regimens.[57,58]

As the CMS classification takes into account the gene expression profiles from the immune, stromal, and tumour cells, a different transcriptomics-based classification that focuses only on the tumour epithelium was developed later. The colorectal cancer intrinsic subtypes (CRIS) categorisation system (CRIS-A, CRIS-B, CRIS-C, CRIS-D, CRIS-E) was designed to describe the biology that specifically drives neoplastic epithelial cells and closely corresponds to the underlying mutations in the tumour. In a retrospective analysis that evaluated the predictive value of CMS and CRIS classifications for response to adjuvant chemotherapy in CRC patients, a significant benefit from postoperative chemotherapy was found in the CMS2 and CMS3 subgroups but not in the CMS1 or CMS4 subgroups for patients with stage III disease.[59] The study also demonstrated that further stratification of CMS2 tumours using the CRIS classification identified CRIS-C and CRIS-D as the patient subgroups within CMS2 that benefit from adjuvant chemotherapy in stage III disease.[59] Interestingly, in stage II CRC patients where the use of chemotherapy is often debatable, there was also a trend for benefit from chemotherapy in the CMS2/CRIS-C subgroup.[59] This highlights the potential clinical utility of CMS and CRIS classifications for predicting the response to adjuvant chemotherapy that warrants further validation.

1.3 MicroRNA biomarkers

MicroRNAs (miRNAs) are short, non-coding RNAs of about 20–25 nucleotides in length that regulate the expression of genes. Growing data suggest that miRNA expression patterns reflect pathophysiological events, such as carcinogenesis,

metastasis, and treatment responsiveness.[60] Thus, the identification of miRNAs that could act as biomarkers for treatment response has received a lot of attention in recent years.[61] Low tumoural expression of miR-21 was demonstrated to be a predictor of pathological drug response in patients with locally advanced CRC who underwent neoadjuvant chemotherapy based on 5-FU to reduce the tumour size before surgery.[62] In a different study that profiled the miRNA expression of CRC along with their associated non-cancerous tissues, high levels of miR-21 expression in the tumours were linked to a poor therapeutic response to 5-FU-based adjuvant chemotherapy as an additional treatment.[63] Furthermore, low levels of miR-148a, miR-150, or miR-320e in tumour specimens were found to be predictive of poor response to the adjuvant 5-FU/oxaliplatin (FOLFOX) regimen in other studies.[64–66] Interestingly, in mCRC patients receiving the XELOX/FOLFOX regimen, high level of expression of miR-625-3p in the tumour was connected to poor response to the treatment but not disease recurrence.[67] Despite the promise of miRNAs as biomarkers for predicting treatment response, future clinical studies are required to establish the relevance of circulating microRNAs as biomarkers across large patient cohorts.

1.4 Proteomics biomarkers

Proteomics involves the detection and measurement of the entire set of proteins in a cell, tissue, or organism to understand their structure and activities, and it complements other "omics" technologies like genomics and transcriptomics. Different proteomics-based technologies are employed in various research settings to uncover biomarkers and protein expression patterns that can be used to detect the presence of tumour, evaluate tumour prognosis, categorise tumours, and determine therapy response.[68–70] The Clinical Proteomic Tumor Analysis Consortium (CPTAC) is an initiative led by the National Cancer Institute (NCI) that aims to advance our understanding of cancer through comprehensive proteomic analysis. CPTAC employs state-of-the-art proteomic techniques, such as mass spectrometry-based approaches, to analyse tumour samples from CRC patients and aims to gain insights into the molecular mechanisms underlying CRC development, progression, and potential therapeutic targets.[71] The data generated from CPTAC's CRC studies provide a wealth of information on protein expression patterns, protein modifications, signalling pathways, and interactions within the tumour microenvironment.[71] In addition to proteomic profiling, CPTAC integrates multi-omics data, including genomics and transcriptomics, to comprehensively understand the complex molecular alterations occurring in CRC.[71] By combining different layers of molecular information, researchers can uncover potential drivers of CRC development and identify new therapeutic strategies.

Few studies have focused on the characterisation of the proteome for predicting treatment response for CRC. Wang *et al*. performed and compared the proteomic, genomic, and transcriptomic profiles from 44 CRC cell lines, 95 CRC tumour tissues and 60 normal tissue biopsies to predict therapeutic response.[72] When compared to genomic and transcriptomic data, the proteomic profile tended to have a superior potential for predicting sensitivity to 5-fluorouracil, SN-38, erlotinib, regorafenib, and oxaliplatin.[72] This demonstrates how proteome profiling may be valuable in guiding the development of tailored cancer treatment. A recent proteomic study of tumour tissues from patients with locally advanced rectal cancer receiving 5-fluorouracil-based neoadjuvant chemoradiotherapy also revealed distinct protein signatures between total responders and poor responders.[73] Proteins such as HSPA4, NIPSNAP1, and SPTB were found to be differentially expressed and their combination achieved the best predictive performance in both internal and external validation cohorts[73], suggesting they might be potential biomarkers to predict treatment response. Additionally, a multi-omics approach has also been applied to characterise molecular markers that could predict treatment response in CRC. Frejno *et al*. analysed the proteomes of a panel of 65 human colorectal cancer cell lines and merged this data with the transcriptome profiles that matched the proteome profiles of 90 CRC patients.[74] This resulted in the identification of integrated proteomic subtypes of CRC that were able to predict the drug sensitivity of cell lines and patients towards 577 drugs or combinations[74], which may serve as a valuable resource to support future prospective clinical studies.

1.5 Immune biomarkers

The tumour microenvironment (TME) is a very diverse and heterogeneous milieu comprising distinct types of cells and chemicals secreted by different cell types. The functionally crucial

components of the TME typically consist of fibroblasts, myofibroblasts, endothelial cells, mesenchymal cells, adipocytes, immune and inflammatory cells, the vascular networks and the extracellular matrix.[75] The stromal and immune cells that surround cancer cells form the dynamic and intricate TME, which interacts with one another to affect the tumourigenesis and many hallmarks of cancer.[76] Accumulating evidence also indicates that the components in TME are implicated in mediating therapeutic response and drug resistance.[77,78] Thus, the characterisation of TME and its association with clinical features are gaining interest, including the potential use as predictive markers of response to cancer therapy.

Beyond their cytotoxic effects, chemotherapy drugs have been reported to have local and systemic immunomodulatory activities through activation or inhibition of immune players.[79] Hence, apart from the molecular profiles of the tumour cells, the response to chemotherapy also has a significant correlation with the immune phenotypes.[80] There is growing evidence that patients receiving adjuvant chemotherapy with increased tumour inflammatory infiltrates are linked to better overall survival.[81] In stage IV CRC patients, higher primary tumour-infiltrating lymphocyte density was linked to a better response rate to doublet chemotherapy (based on oxaliplatin or irinotecan) (79% vs 48%), suggesting that the local infiltrate at the tumour site could predict the therapy outcome for metastatic disease.[82] In another study, Morris *et al*. reported that stage III patients with the presence of peritumoral tumour-infiltrating lymphocytes had a substantial survival benefit from adjuvant 5-FU chemotherapy than patients without the tumour-infiltrating lymphocytes, implying a potential predictive role of the tumour-infiltrating lymphocytes.[83]

In light of the crucial role of the immune components in cancer, a scoring system based on the immune response, known as Immunoscore was developed. The Immunoscore is an immune-based classification system of cancer patients based on the measurement of the $CD3^+$ and $CD8^+$ T cell density in the tumour and its invasive margin.[84] The assay involves obtaining a small sample of the tumour tissue, typically through a biopsy or surgical resection. Immunohistochemistry (IHC) staining is then performed on these tissue sections to visualise specific immune cell populations and other relevant markers. The predictive significance of the immune cell infiltration measured by Immunoscore assay was evaluated in the SITC and IDEA France trials involving stage III colon cancer patients receiving chemotherapy. In the SITC trial, chemotherapy-treated patients with high Immunoscores had a significantly better clinical outcome than untreated patients.[85] On the other hand, the outcome of the low Immunoscore patients did not differ as compared to the untreated group, indicating that they do not respond to treatment.[85] This subanalysis showed that stage III colon cancer patients with high Immunoscores can strongly predict their response to chemotherapy.[85] In the IDEA phase III trial, which sought to compare the noninferiority of three versus six months of adjuvant therapy with either FOLFOX or CAPOX in patients with resected stage III CC, the ability of the Immunoscore to predict response to the adjuvant chemotherapy was examined.[86] The predictive ability of the Immunoscore for different treatment lengths (three vs. six months) in terms of disease-free survival was found to be statistically significant for patients treated with FOLFOX.[86] A significant benefit of six months of treatment with FOLFOX was predicted by intermediate or high Immunoscore. Patients with low Immunoscores, on the other hand, did not receive significant benefits from the six-month FOLFOX treatment compared to the three-month and had increased recurrence risk.[86] Hence, the Immunoscore may be helpful to identify those individuals who will most likely benefit from chemotherapy that warrants further studies to validate its predictive value.

1.6 Microbiome biomarkers

Within the gastrointestinal tract, the host and the gut microbiota have co-evolved, with the gut microbiota actively participating in maintaining homeostasis, regulating immunity and metabolism as well as influencing nutrient absorption.[87] Recent discoveries indicate that an imbalance in the gut microbiota or dysbiosis in CRC patients is closely linked to colorectal carcinogenesis and this has sparked a new area of interest in CRC research.[88] Furthermore, there is also emerging evidence that the gut microbiota can influence how the host reacts to chemotherapeutic treatments by increasing drug efficacy, promoting chemoresistance, and modulating chemotherapy-induced toxicity and side effects via numerous pathways.[89,90] The TIMER mechanistic framework has been presented as a model to describe the several processes by which the gut microbiota

might influence chemotherapeutic drugs.[90] These mechanisms include translocation, immunomodulation, metabolism, enzymatic degradation, and reduced diversity and ecological variation.[90] These processes play a crucial role in determining the outcome of chemotherapy for various cancer types, particularly CRC.[90]

The significance of host-microbe interactions in increasing the anti-tumour activity of fluoropyrimidine agents that are typically used as a first-line treatment against CRC was underlined by several elegant investigations. Fluoropyrimidines, such as 5-fluorouracil (5-FU), are known to work against cancer by blocking thymidylate synthase, which prevents nucleotide production and ultimately cell proliferation. However, Garca-González and colleagues revealed that 5-FU and 5-fluoro-2'-deoxyuridine (FUDR) function by altering ribonucleotide metabolism rather than DNA metabolism, which is dependent on active bacterial metabolism.[91] They demonstrated that *Escherichia coli* utilises an intrinsic route to convert 5-FU and FUDR into fluorouridine monophosphate (FUMP), an analogue of uridine monophosphate (UMP) that has been shown to block de novo pyrimidine synthesis.[91] Using the *Caenorhabditis elegans* model, Scott *et al*. corroborated this observation and demonstrated that blocking bacterial ribonucleotide metabolism greatly decreased drug efficacy.[92] Moreover, their findings suggest that the diversity of the nematode's microbiome is crucial for the host response to fluoropyrimidines, as the drug's pharmacodynamics can vary up to 256-fold with disruption of bacterial metabolism and up to 40-fold with different bacterial strains.[92]

In line with this, Yuan *et al*. further demonstrated the relationship between host microbiome and fluoropyrimidine efficacy in a mouse model.[93] They evaluated the anti-CRC efficacy of 5-FU in mice treated with a cocktail of antibiotics, including ampicillin, vancomycin, neomycin and metronidazole with those in the absence of these drugs.[93] They reported that the tumour volume was considerably lower in the mice who did not receive antibiotic treatment as opposed to those who had after 35 days.[93] This indicates that the antibiotics disrupted the gut microbiota and decreased 5-FU efficacy, suggesting that microbiome dysbiosis is detrimental to the success of chemotherapy.[93] This observation is further supported by Wang *et al*., which showed that the mouse model of CRC was more likely to be resistant to 5-FU under the conditions of microbiota dysbiosis caused by aberrant TGF-β signalling.[94] Conversely, it has been reported that endemic microbiome bacteria, such as *Lactobacillus plantarum* supernatant (LPSN) may contribute to enhancing the chemosensitivity of 5-FU in CRC-resistant cells through diverse mechanisms such as deactivating the Wnt/B-catenin signalling system and increasing cell death and apoptosis by upregulating caspase 3 activity.[95] In both colorectal cancer patients and mice, Roberti *et al*. showed the significance of the ileal microbiota in determining tolerogenic versus immunogenic ileal intestinal epithelial cell death and the formation of follicular T helper cells.[96] They discovered that the colonisation of ileal intestinal epithelial cells with immunogenic commensals, particularly *Bacteroides fragilis* and *Erysipelotrichaceae* enhance the immunogenicity of oxaliplatin therapy.[96] Interleukin-1R1 and interleukin-12 were generated by the chemotherapy-induced apoptotic ileal crypt cells in the presence of these bacteria, which enhanced the efficacy of chemotherapy by inducing a PD-1+ follicular T helper cell response.[96] Their findings highlight the importance of immunogenic ileal apoptosis in dictating the outcome of chemotherapy, which underscore the crucial role the microbiota play in determining drug response.[96]

Numerous studies have also shown that the host microbiota may help to promote chemoresistance towards 5-FU. *Fusobacterium nucleatum* has been previously linked to the pathogenesis of CRC and a recent study by Zhang *et al*. has demonstrated that *F. nucleatum* may also contribute to 5-FU resistance in CRC through activation of the TLR4/NF-kB pathway, which increases the expression of BIRC3, an inhibitor of apoptosis protein.[97] They demonstrated a correlation between *F. nucleatum* abundance and chemoresistance and identified high levels of *F. nucleatum* as a distinct risk factor for recurrence in patients with advanced CRC.[97] A study by Yu *et al*. reported that *F. nucleatum* plays a mechanistic role in promoting resistance to 5-FU and oxaliplatin chemotherapy drugs.[98] They demonstrated that *F. nucleatum* inhibits miRNA-18a and miRNA-4802 expression by activating TLR4 and MYD88 immune signalling.[98] This then prevents the 5-FU and oxaliplatin-induced autophagy process and apoptosis, resulting in chemoresistance.[98] Even though the results of recent studies are encouraging, more research is necessary to delineate the connections between the gut microbiota, the host response and the

outcomes of anti-cancer treatment in order to confirm their utility as potential biomarkers of therapy response.

2. Challenges and future perspectives

While there has been promising progress in cancer therapy, the current treatment paradigm typically takes a "one-size-fits-all" approach, which results in only a small proportion of patients receiving any given chemotherapeutic regimen showing apparent clinical benefit.[99] However, CRC is a heterogeneous disease, both intra- and inter-tumoural[100], which can complicate the identification of predictive biomarkers. It is essential to develop methods that can accurately account for this variability to enable effective personalised treatment. Due to the complicated molecular makeup of these tumours, significant research efforts have been made in recent years to identify potential molecular markers that could provide additional information regarding tumour response to anticancer therapies, which in turn could help enhance efficacy, minimise toxicity, and lower treatment costs for CRC.[101] However, many of the biomarkers mentioned here still need further clinical confirmation. The validation and introduction of new biomarkers into clinical practice, however, is a difficult process with many steps and challenges. Even though many biomarkers are verified retrospectively, these investigations are subject to many types of bias.[102] It is therefore necessary to standardise sample collection, storage, and analysis procedures to ensure reliable and reproducible results. Moreover, large-scale prospective trials are needed to further validate the biomarkers, standardise their use in clinical practice and inform treatment options.[102] Alternative methods for finding biomarkers include employing prospective-retrospective study designs[103] or biobanks from randomised trials.[104]

It is anticipated that further advances in molecular technologies will facilitate the identification of patient-specific biomarkers that can predict treatment response, enabling a more tailored and effective treatment approach. Moreover, integrating data from multiple platforms, including genomics, transcriptomics, and proteomics, can provide a more comprehensive understanding of the mechanisms underlying chemotherapy response.[105] Given that the immune and microbiome components also offer promising avenue for identifying predictive biomarkers of chemotherapy response in CRC, future research should focus on integrating them with other omics data to improve patient.[106] Notably, the interaction between biomarkers is also probably clinically meaningful, and network biomarkers might eventually offer more predictive knowledge.[107] However, these advanced techniques typically generate vast amounts of data, which can be challenging to analyse and interpret. Artificial intelligence and machine learning algorithms offer potential solutions to analyse large, complex datasets and identify patterns that may be challenging to detect with traditional statistical methods.[108] Altogether, the continuously developing methodologies present significant opportunities to derive novel insights and more efficient therapy prediction.

CONCLUSION

Chemotherapy undoubtedly constitutes a vital part of the treatment of CRC patients with metastatic disease. However, the approach to CRC treatment management should shift from one-size-fits-all to selectively targeting certain patient populations in order to optimise the effectiveness of the chemotherapy. Therefore, efficient predictive biomarkers for therapeutic responses are needed to aid patient selection and decision-making. The omic technologies are expected to offer the tools necessary to characterise the high cancer heterogeneity and complex drug response for the discovery of highly specific biomarkers. Furthermore, the biomarker discovery should also take into account the integrated analysis of the tumour microenvironment such as the immune cells as well as the microbiome. Even though many predictive biomarker studies have already been performed or are in progress, the identified biomarkers still need to be clinically validated. We expect that further advancements in molecular profiling and deeper insights into the tumour microenvironment of the cancer cell will aid in uncovering useful biomarkers that define chemotherapy response in CRC patients.

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