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TOPIC HIGHLIGHT

2016 Colorectal Cancer: Global view

Genomic diversity of colorectal cancer: Changing landscape and emerging targets

Daniel H Ahn, Kristen K Ciombor, Sameh Mikhail, Tanios Bekaii-Saab

Daniel H Ahn, Tanios Bekaii-Saab, Division of Medical Oncology, Mayo Clinic, Phoenix, AZ 85054, United States

Kristen K Ciombor, Sameh Mikhail, Division of Medical Oncology, The Ohio State University Medical Center, Columbus, OH 43210, United States

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Correspondence to: Tanios Bekaii-Saab, MD, Division of Medical Oncology, Mayo Clinic, 5777 E Mayo Blvd, Phoenix, AZ 85054, United States. bekaii-saab.tanios@mayo.edu
Telephone: +1-480-3424800
Fax: +1-480-3014675

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Abstract

Improvements in screening and preventive measures have led to an increased detection of early stage colorectal cancers (CRC) where patients undergo treatment with a curative intent. Despite these efforts, a high proportion of patients are diagnosed with advanced stage disease that is associated with poor outcomes, as CRC remains one of the leading causes of cancer-related deaths in the world. The development of next generation sequencing and collaborative multi-institutional efforts to characterize the cancer genome has afforded us with a comprehensive assessment of the genomic makeup present in CRC. This knowledge has translated into understanding the prognostic role of various tumor somatic variants in this disease. Additionally, the awareness of the genomic alterations present in CRC has resulted in an improvement in patient outcomes, largely due to better selection of personalized therapies based on an individual's tumor genomic makeup. The benefit of various treatments is often limited, where recent studies assessing the genomic diversity in CRC have identified the development of secondary tumor somatic variants that likely contribute to acquired treatment resistance. These studies have begun to alter the landscape of treatment for CRC that include investigating novel targeted therapies, assessing the role of immunotherapy and prospective, dynamic assessment of changes in tumor genomic alterations that occur during the treatment of CRC.

Key words: Colorectal cancer; *KRAS* mutation; *BRAF* mutation; Genomic diversity; Tumor DNA

Core tip: Tumor somatic variants have a prognostic role, in addition to treatment selection in patients with solid tumor malignancies, including colorectal cancer (CRC). The application of this knowledge in the development of novel, targeted therapies has resulted in improved patient outcomes in this disease. Our objective is to provide an overview of the genomic alterations present in CRC and its role in treatment implications, in addition to providing an overview of ongoing and future clinical trials.

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INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cancer in the world, leading to more than 500000 deaths annually[1,2]. An increased awareness of the genomic makeup of CRC has allowed us to understand the prognostic role of certain tumor genomic alterations. This knowledge and its incorporation into the treatment of metastatic CRC has translated to significant improvements in patient outcomes, where patients' median overall survival has approached 3 years[3-5]. The incorporation of our knowledge about the genomic landscape of CRC into treatment decisions with selected targeted agents has led to an improvement in patient outcomes. With this increased understanding, clinical trials are now being designed to assign treatment selection with novel therapies based upon identified specific tumor somatic variants in each individual. Herein we review the genomic landscape of CRC, its current role in treatment selection, and its integration in ongoing and future studies.

GENOMIC ALTERATIONS IN DOWNSTREAM SIGNALING PATHWAYS IN CRC

RAS Mutations in CRC

The Kirsten Ras (*KRAS*) oncogene encodes for a guanosine triphosphate (GTP)/guanosine diphosphate binding protein downstream of the extracellular epidermal growth factor receptor in the RAS/RAF/MAPK signaling pathway. Activating *KRAS* exon 2 mutations occur in up to 45% of all CRC, and are involved in initiation, proliferation and progression of CRC[6-10]. While initial studies suggested a possible clinical benefit from anti-epidermal growth factor receptor (EGFR) therapy for patients whose tumors express *KRAS* codon 13 (G13D) mutations, a meta-analysis comprised of several large phase Ⅲ trials failed to demonstrate benefit from panitumumab, an anti-EGFR monoclonal antibody, in CRC patients whose tumor harbored a *KRAS* codon 13 mutation[11]. In addition to *KRAS* exon 2, an approximate additional 10% of patients with other *RAS* mutations have been identified in CRC, including *NRAS* or non-exon 2 *KRAS* mutations[6]. In patients who exhibited activating non-exon 2 *KRAS* and *NRAS* mutations, an absence of clinical benefit, and perhaps a negative effect, was seen from the addition of anti-EGFR therapy in combination with several chemotherapy regimens in various treatment settings[6,12,13]. On this basis, anti-EGFR therapy should not be given to any patient with CRC exhibiting a *RAS* mutation.

While mutations in *RAS* as a predictive biomarker to anti-EGFR therapy has been recognized, its relevance as a therapeutic target is unknown. Given the high incidence of *RAS* in CRC and its importance as an oncogene, targeting *RAS* represents an ideal and promising strategy. Developing strategies to directly block oncogenic *RAS* activity has remained a challenge due to several factors, including the high binding affinity of the oncoprotein to the GTP-bound "on" state, as well as the lack of accessibility to active sites within *KRAS* to bind[14]. One alternative approach includes targeting pathways and its effectors downstream of *RAS*. The clinical benefit from targeting single pathways is often limited due to mechanisms of resistance including communication between signaling pathways and its resulting downstream effector activation and inhibition through a feedback loop mechanism[15,16]. Alternatively, secondary treatment resistance to anti-EGFR treatment may result from the development of *RAS* mutations during a course of anti-EGFR therapy. Several studies have demonstrated up to 96% of patients who initially had *RAS* and *BRAF* wild-type CRC were later identified to have acquired activating RAS (*KRAS* or *NRAS*) or *BRAF* mutations on repeat tumor genomic assessment at the time of disease progression on anti-EGFR therapy[17-19].

Alternative treatment strategies in *RAS* mutant CRC include the combination of various therapeutic agents targeting several genes involved in the *MAPK* pathway that would cause sufficient suppression of activated *RAS* activity. Combining small molecule inhibitors of *MEK* to anti-EGFR therapies have demonstrated the reversal of acquired anti-EGFR therapy resistance, prompting ongoing clinical trials investigating the clinical utility of the combination of multiple signaling pathway inhibitors in the first-line setting, as well as in salvage therapy for refractory disease (Table 1). Targeting multiple signaling pathways may also be effective in overcoming resistance from secondary activation of parallel signaling pathways[20]. Alternatively, administering anti-EGFR therapies in a pulsatile manner instead of to the point of clinical

Table 1 Ongoing combination targeted therapy trials for colorectal cancer

Agent(s)	Class of agent	Phase	Trial number[1]	Misc
MEK162 + Panitumumab	MEK tyrosine kinase inhibitor, anti-EGFR mAb	Ⅰb/Ⅱ	NCT01927341	mCRC with mutant or wild-type RAS tumors
Dabrafenib + Trametinib + Panitumumab + 5-Fluorouracil	BRAF tyrosine kinase inhibitor, MEK tyrosine kinase inhibitor, anti-EGFR mAb	Ⅰ/Ⅱ	NCT01750918	BRAF-V600E mutant + and in pts with secondary resistance to anti-EGFR mAb
LGX818 + Cetuximab ± BYL719	BRAF tyrosine kinase inhibitor, anti-EGFR mAb, PI3K tyrosine kinase inhibitor	Ⅰ/Ⅱ	NCT01719380	BRAF mutant mCRC
Irinotecan + Cetuximab ± Vemurafenib	anti-EGFR mAb, BRAF tyrosine kinase inhibitor	Ⅱ	NCT02164916	BRAF mutant mCRC
Neratinib + Cetuximab	HER-2 tyrosine kinase inhibitor, anti-EGFR mAb	Ⅰ/Ⅱ	NCT01960023	KRAS, NRAS, BRAF, PIK3CA wild type

[1]Utilizing the NCT number, clinical trial information can be obtained at https://clinicaltrials.gov. mAb: Monoclonal antibody; mCRC: Metastatic colorectal cancer; EGFR: Epidermal growth factor receptor; PIK3CA: Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; BRAF: v-Raf murine sarcoma viral oncogene homolog B.

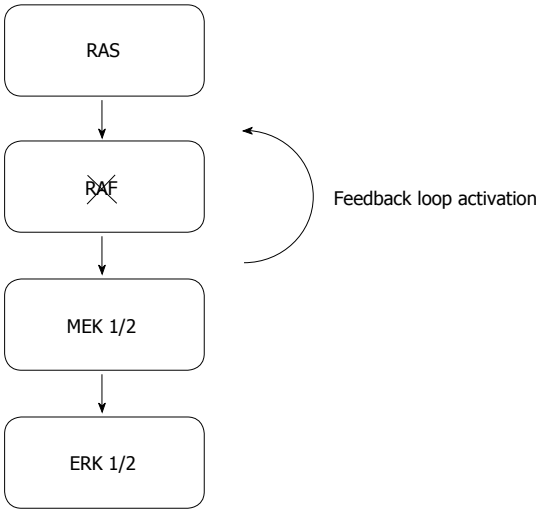

Figure 1 RAS/RAF/MEK/ERK pathway and mechanisms of resistance to *BRAF* inhibition. The figure above shows the MAPK pathway and the compensatory feedback loop activation (arrow) despite *BRAF* inhibition, resulting in the upstream reactivation of the MAPK pathway.

progression may prolong anti-EGFR therapy efficacy. A recent study that performed dynamic monitoring of tumor somatic variants through circulating tumor DNA demonstrated a decrease in drug resistant KRAS clones upon withdraw of cetuximab, allowing for the reemergence of drug sensitivity to anti-EGFR therapy[21]. This suggests a rationale for intermittent EGFR blockade, and explains for the seldom efficacy seen with re-challenging anti-EGFR therapies. Lastly, inhibitors of the MAPK and PI3K/Akt/mTOR pathway are considered to cause G1 cell cycle arrest through the suppression of D-type cyclins and the upregulation of cell cycle inhibitors[22,23]. Pre-clinical studies have demonstrated potent inhibition of G1/S transition and phosphorylation of retinoblastoma (Rb) protein with the inhibition of the MAPK and PI3K/Akt pathway[24]. The combination of MEK and CDK inhibitors may be considered a potential strategy in treating RAS activated CRC or in tumors harboring mutations in CDK.

BRAF mutations in CRC

BRAF V600E mutations occur in up to 5% of metastatic CRC[25,26] and are often associated with a more aggressive phenotype (with the exception in microsatellite instability high CRC, where the effect is attenuated). Patients with *BRAF V600E* mutations tend to respond poorly to conventional chemotherapy and have worse outcomes[6,27-31]. The constitutive activation of *BRAF* evading any upstream inhibition of EGFR may explain the limited clinical benefit seen with anti-EGFR therapies in *BRAF* mutant metastatic CRC[6,32,33].

In metastatic melanoma, inhibition of *BRAF* with small molecule inhibitors has led to improvement in clinical outcomes in patients whose tumors exhibit mutations in *BRAF*[34-37]. However, the clinical efficacy from single agent *BRAF* inhibition has not translated to patients with *BRAF* mutant metastatic CRC[36,38]. The lack of anti-tumor activity may be a result of insufficient inhibition of the MAPK pathway as a result of a feedback loop mechanism, resulting in the persistent activation of the MAPK signaling pathway[16,39] (Figure 1). To overcome this compensatory mechanism, the combination of tyrosine kinase inhibitors aimed at inhibiting the MAPK pathway has resulted in an improvement in treatment efficacy in comparison to single-agent *BRAF* small molecule inhibitor in metastatic melanoma[40]. Based on these results, a phase Ⅱ study investigating the clinical activity from the combination of *BRAF* and *MEK* inhibition with dabrafenib and trametinib was conducted in patients with *BRAF* mutant metastatic CRC[41]. While modest clinical activity was observed with the combination, with 12% of patients experiencing either a partial or complete response, correlative laboratory studies suggest an inhibition in MAPK signaling in patients receiving the combination[37]. The absence of significant anti-tumor activity may be attributed to insufficient suppression of MAPK signaling, which may be due to upstream activation of EGFR, leading to reactivation of MAPK and other integral signaling pathways[15]. Based on this rationale, ongoing studies are investigating the combination of anti-EGFR therapies with *BRAF* tyrosine

Table 2 Tumor genomic variants and potential targeted therapies of interest

Gene	Agents of Interest	Mechanism of action	Phase	Trial number[1]	Comment
FGFR (*FGFR1, FGFR2, FGFR3, FGFR4*)	Ponatinib	Multi-kinase small molecule inhibitor	II	NCT02272998	
	BGJ398	Pan FGFR small molecule inhibitor	I	NCT01928459	
RET	Cabozantinib	Multi-kinase small molecule inhibitor	I	NCT02008383	Cabozantinib + panitumumab
	Vandetanib	Multi-kinase small molecule inhibitor	I	NCT01582191	
	Apatinib	Multi-kinase small molecule inhibitor			
	Ponatinib	Multi-kinase small molecule inhibitor	II	NCT02272998	
	RXDX-105	RET and BRAF small molecule inhibtor	I	NCT01877811	
	Sunitinib	Multi-kinase small molecule inhibitor			
	Sorafenib	Multi-kinase small molecule inhibitor	I	NCT01531361	
HER-2	AZD8931	Small molecule inhibitor of EGFR, HER-2, HER-3	I / II	NCT01862003	AZD8931 + FOLFIRI
	Neratinib	Small molecule inhibitor of EGFR, HER-2, HER-3	II	NCT01953926	
	HER-2 vaccine	B cell peptide vaccine	I	NCT01376505	
	T-DM1	Antibody-drug conjugate of traztuzumab and DM1	II	HERACLES-RESCUE	At the time of traztumab failure
	Pertuzumab	Anti HER-2 monoclonal antibody	II	HERACLES	Pertuzumab + trastuzumab
	Lapatinib	Anti HER-2 small molecule inhibitor	II	HERACLES	Lapatinib + trastuzumab
c-MET (*MET, HGFR*)	Crizotinib	Multi-kinase small molecule inhibitor		NCT02510001	Crizotinib + PD-0325901
	Tivantinib	c-MET inhibitor		NCT01892527	Tivantinib + cetuximab
	Cabozantinib	c-MET and VEGFR2 inhibitor			
	INC280	Small molecule inhibitor of c-MET	II	NCT2205398	INC280 + cetuximab
	AMG102	HGF inhibitor			
	AV299	HGF inhibitor			

[1]Utilizing the NCT number, clinical trial information can be obtained at https://clinicaltrials.gov. FGFR: Fibroblast growth factor receptor; EGFR: Epidermal growth factor receptor; BRAF: v-Raf murine sarcoma viral oncogene homolog B; HER-2: Human growth factor receptor 2; HGFR: Hepatocyte growth factor receptor; TDM-1: Ado-trastuzumab emtansine; VEGFR: Vascular endothelial growth factor receptor.

kinase inhibitors in patients with *BRAF* mutant mCRC (Table 1)[42-45].

GENOMIC DIVERSITY AND ITS ROLE IN DEVELOPING PERSONALIZED THERAPIES WITH TARGETED AGENTS IN CRC

While *RAS* mutations are the most common genomic alteration in CRC, recent efforts have allowed us to understand the genomic diversity and identify potential therapeutic targets of interest in this disease[46,47]. Through the efforts of The Cancer Genome Atlas (TCGA), 224 CRC cases underwent extensive molecular characterization to describe the genomic landscape present in CRC[46]. 24 genes were significantly mutated, where most were an actionable mutation, including ERBB2 (HER-2/neu) mutations, a therapeutic target in *HER-2* positive gastric and breast cancer, were identified in 19% of tumors[46]. This comprehensive assessment allowed us to have a better understanding of the genomic landscape, in addition to identifying several potential targetable genes of interest that are essential for tumor growth and carcinogenesis that we will discuss in further detail below (Table 2).

Fibroblast growth factor receptor in CRC

The fibroblast growth factor receptors (FGFR) comprise a group of highly conserved tyrosine kinase receptors consisting of four members (FGFR1, 2, 3 and 4). These receptors bind to one of 18 secreted glycoprotein ligands, or fibroblast growth factors (FGFs), to their extracellular domain[48]. Binding of the appropriate ligand results in FGFR dimerization, autophosphorylation and activation of downstream signaling pathways that include the MAPK, PI3K/Akt and signaling transducer and activator of transcription or STAT pathway, inducing cell differentiation, growth and survival[49]. FGFR overexpression has been identified in CRC samples, where the presence of FGFR signaling has been identified as playing an important role in the tumor microenvironment, with a correlation in FGFR overexpression with tumor invasion, advanced stage disease and chemotherapy resistance[50-53]. Pre-clinical studies have demonstrated the reversal of chemotherapy resistance by combining small molecule FGFR inhibitors with chemotherapy in CRC cell lines, confirming the importance of targeting this receptor and representing a potential strategy in overcoming treatment resistance in CRC[54].

C-MET (MET)

Hepatocyte growth factor receptor, also known as c-MET, is a proto-oncogene that encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF). Abnormal expression of c-MET through somatic mutations or overexpression has been identified in up to 66.7% of CRC samples and its microenvironment and is a negative prognostic marker related to tumor

oncogenesis, invasiveness, local recurrence, and chemotherapy resistance[55-58]. *MET* activation confers acquired anti-EGFR therapy resistance, through re-activation of anti-apoptotic signaling pathways, including the PI3K and MAPK pathways[59-62]. Thus, inhibiting c-MET represents an emerging target of interest in the development of novel agents in CRC. Pre-clinical studies have demonstrated that the blockade of c-MET inhibits tumor growth in CRC cell lines[63,64], thus agents targeting MET, including small molecule multi-kinase inhibitors (*e.g.,* crizotinib, tivantinib), HGF inhibitors (*e.g.,* AMG102, AV299) and immunotherapeutic agents are of interest and under investigation in the treatment of CRC.

RET

RET is located on chromosome 10q11.2 and encodes a transmembrane receptor tyrosine kinase that has three unique isoforms[65]. Four ligands can bind and activate *RET*, leading to the aberrant activity of several signaling pathways including PI3K/Akt and MAPK pathway[66]. While the aberrant expression of *RET* may function as an oncogene in certain solid tumor malignancies including papillary and medullary thyroid cancers[67], in colorectal cancer *RET* has been identified as a tumor suppressor and as an oncogene. Studies have shown that the hypermethylation and mutational inactivation of *RET*, as well as RET fusions, promote colorectal cancer formation[68-70]. In several studies, RET mutations were identified in up to 7% of mCRC samples[46,71,72]. Regorafenib, an approved multi-target small molecule inhibitor in metastatic CRC[73], has demonstrated tumor growth inhibition in *RET* mutant cancer cells lines[70,74], and may explain part of its efficacy in this disease. Further studies investigating its activity in *RET* mutant CRC is warranted as it may provide further benefit in this specific cohort of CRC patients.

HER-2/Neu

Human growth factor receptor 2, also known as *HER-2* or *HER-2*/neu, is part of the human epidermal growth factor receptor family and has been identified as an oncogene in several solid tumor malignancies, including CRC. Its overexpression has been a poor prognostic biomarker in breast cancer and is an effective therapeutic target in breast and gastric cancer[75-77]. Studies evaluating *HER-2* overexpression in CRC have identified *HER-2* somatic mutations and amplification in 7% of patients with CRC[46], where pre-clinical studies have demonstrated its role in inducing resistance to anti-EGFR therapy, and its inhibition showing durable tumor regression with anti-*HER-2* therapy[78]. Based on these findings, the HERACLES trial, a phase Ⅱ study investigated dual anti-*HER-2* therapy blockade in patients with *HER-2* amplified (immunohistochemistry staining 3+ or 2+ with FISH positive (HER2:CEP17 ratio > 2) in > 50% of tumor cells) mCRC with previous anti-EGFR therapy. Patients received the combination of trastuzumab, an anti *HER-2* monoclonal antibody with either lapatinib, a small molecule inhibitor of *HER-2* or pertuzumab, an anti *HER-2* monoclonal antibody. Early results from the lapatinib and trastuzumab arm demonstrated a 35% response rate and median progression free survival of 5.5 mo, despite being heavily pre-treated after failing multiple lines of therapy[79]. Based on these findings, anti *HER-2* therapy may be an effective treatment option in a pre-selected patient population with mCRC.

DNA mismatch repair genes and their therapeutic relevance in advanced CRC

Mismatch repair (*MMR*) genes function to remove erroneous DNA nucleotides during mitosis. With deficient MMR activity, altered DNA nucleotides are incorporated into cells that increase their risk in forming into a neoplastic, hypermutated makeup[80], resulting in microsatellite instability. Deficient MMR activity is found in approximately 15% of CRC, in which 3% is attributed to Lynch Syndrome through germline mutations in MLH1, MSH2, MSH6, PMS2 or EPCAM3 genes[81,82]. The remaining 12% is due to sporadic inactivation of MLH1. While microsatellite instability (MSI) is a driver for tumor formation and proliferation in CRC, recent studies have demonstrated its relevance for potential novel therapeutic agents in this cohort of CRC. Tumors expressing MSI have been characterized by an intense immune infiltration, likely related to a high density of mutations, creating numerous neo-antigens and targets for immune therapies. Based on this rationale, a single-arm phase Ⅱ study was conducted to assess the clinical efficacy of pembrolizumab, a programmed cell death protein (PD)-1 inhibitor, in patients with treatment resistant, metastatic cancer that did or did not express mismatch-repair deficiency[83]. Individuals with deficient MMR colorectal cancer experienced a response rate of 40% and immune-related PFS of 78% at 20 wk. Interestingly, a lack of activity was associated with mismatch repair-proficient CRC patients, confirming that immunotherapeutic agents may be beneficial in only certain cohorts of CRC, unless alternative approaches can be developed to transform tumors into an immune-responsive phenotype[83]. Based on these findings, several ongoing clinical trials are investigating various novel, immunotherapeutic agents in the treatment of CRC that include studies in patients with MSI high tumors and those with positive PDL-1 expression (Table 3). While current trials will substantiate the role of immune therapy in CRC, a better understanding of mechanisms of acquired resistance, optimal duration of necessary treatment and predictive biomarkers associated with treatment efficacy are paramount.

Table 3 Current ongoing immune therapy trials for colorectal cancer

Agent	Class of agent	Phase	Trial number[1]	Comment
MK-3475	Anti-PD-1 mAb	Ⅱ	NCT01876511	MSI-high tumors
MEDI4736	Anti-PD-L1 mAb	Ⅰ / Ⅱ	NCT01693562	
Nivolumab ± ipiliumumab	Anti-PD-1 mAb/anti-CTLA-4 mAb	Ⅰ / Ⅱ	NCT02060188	Recurrent and metastatic CRC
MK-3475 + mFOLFOX6	Anti-PD-1 mAb	Ⅱ	NCT02375672	
Tremelimumab + MEDI4736	Anti-CTLA-4 mAb + anti-PD-L1 mAb	Ⅰ	NCT01975831	

[1]Utilizing the NCT number, clinical trial information can be obtained at https://clinicaltrials.gov. mAb: Monoclonal antibody; CRC: Colorectal cancer; MSI: Microsatellite instability; CTLA: Cytotoxic T associated lymphocyte protein; PD-1: Program cell death protein 1; PD-L1: Programmed cell death protein ligand 1; FOLFOX: Combination of 5-Flurouracil, folinic acid and oxaliplatin chemotherapy regimen.

Tumor genomic assessment at the time of acquired therapy resistance in mCRC

While anti-tumor activity from targeted therapies can lead to dramatic responses, the clinical benefit is often limited due to inherent and acquired resistance through the acquisition of new tumor genomic alternations, including the oncogenic activation of *MET* or acquiring *RAS* mutations, as described above. While tumor genomic alterations are usually assessed through tumor samples, obtaining tissue can be challenging due to insufficient material from tumors only accessible through fine-needle aspirates, as well as the invasive nature of such procedures.

Tumor circulating free DNA can be non-invasively assessed in peripheral blood, a "liquid biopsy," through the assessment of circulating tumor DNA (ctDNA) and tumor cells (CTCs) and are present in advanced malignancies[84,85]. CtDNA and CTCs can provide dynamic assessments of tumor specific mutations that may arise during the course of therapy. Although previous methodologies demonstrate low rates of sensitivity and concordance, the incorporation of new technology, including real time digital PCR has increased sensitivity (87.2%) and specificity (99.2%) in identifying tumor specific mutations responsible for treatment resistance in patients who initially responded to targeted therapies[18]. While there are limitations, including false negative results as well as the inability to identify genomic alterations from central nervous system (CNS) lesions, this non-invasive tool can monitor patients for resistance-conferring mutations as well as assessing all tumors concurrently, as heterogeneity can exist between different foci of disease.

CONCLUSION

Advancements in genomic sequencing have resulted in our increased understanding of the genomic landscape of CRC, allowing us to develop and tailor personalized therapies for patients. Despite these improvements, future studies are needed to characterize and understand the functionality of the various different mutations of each gene, including mutational assessment at the time of treatment failure. This will allow us to improve therapies for patients with CRC by assessing the prognostic and potential therapeutic implication of genes of interest, and to identify predictive biomarkers of response and resistance.

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