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REVIEW ARTICLE

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A perspective on emerging therapies in metastatic colorectal cancer: Focusing on molecular medicine and drug resistance

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Abstract

The majority of cancer cases are colorectal cancer, which is also the second largest cause of cancer-related deaths worldwide. Metastasis is the leading cause of death for patients with colorectal cancer. Metastatic colorectal cancer incidence are on the rise due to a tiny percentage of tumors developing resistant to medicines despite advances in treatment tactics. Cutting-edge targeted medications are now the go-to option for customized and all-encompassing CRC care. Specifically, multitarget kinase inhibitors, antivascular endothelial growth factors, and epidermal growth factor receptors are widely used in clinical practice for CRC-targeted treatments. Rare targets in metastatic colorectal cancer are becoming more well-known due to developments in precision diagnostics and the extensive use of second-generation sequencing technology. These targets include the KRAS mutation, the BRAF V600E mutation, the HER2 overexpression/amplification, and the MSI-H/dMMR. Incorporating certain medications into clinical trials has significantly increased patient survival rates, opening new avenues and bringing fresh viewpoints for treating metastatic colorectal cancer. These focused therapies change how cancer is treated, giving patients new hope and better results. These markers can significantly transform and individualize therapy regimens. They could open the door to precisely customized and more effective medicines, improving patient outcomes and quality of life. The fast-growing body of knowledge regarding the molecular biology of colorectal cancer and the latest developments in gene sequencing and molecular diagnostics are directly responsible for this advancement.

KEYWORDS

angiogenesis, CRC, drug resistance, metastasis, personalized medicine, tissue-based biomarkers

1 | INTRODUCTION

The epithelial cells lining the colon or rectum of the digestive system are the starting point for colorectal cancer (CRC). It ranks as the second most fatal cancer worldwide, with a death rate of 9.4%. Thanks to surgery or surgery combined with radiation and chemotherapy as part of adjuvant treatment, only 21% of CRC patients initially present with distant metastases. However, 20%–35% of patients whose stage II–III tumors were removed experienced a recurrence within 5 years, with distant metastases accounting for the majority of these cases.[1,2] The most successful first-line chemotherapy and targeted therapy choices for treating

metastatic colorectal cancer (mCRC) depend on one's knowledge of the disease's resistance mechanisms and gene mutation status. Remarkably, the treatment may, in rare cases, aid in tumor downstaging, hence raising the probability of removal. Lifestyle variables that contribute significantly to the increased incidence of colorectal cancer include alcohol consumption, smoking, poor diet, and inactivity. Furthermore, 10% of cases are explained by genetic mutations and variations, and 30% are due to inheritance and family history. These medications primarily target epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) in colorectal cancer using antibodies.[3,4]

In contrast to anti-VEGF-based therapies, where the predictors of therapeutic efficacy are still largely unknown, anti-EGFR treatments have a variety of resistance mechanisms. Although kirsten rat sarcoma viral oncogene homolog (KRAS) mutations have been extensively validated as selection markers for anti-EGFR therapy, only 35% of KRAS wild-type tumors are believed to respond to treatment.[5] Usually, congenital resistance is discovered in the early phases of clinical trials or the field of medicine. Acquired resistance to medication is unique to that therapy. It can arise through various molecular pathways, even though developed resistance to one treatment might occasionally lead to resistance to other drugs with the same or a different mode of action.[6]

Chemotherapy is associated with damage to healthy tissues and is not exclusive to cancer cells; this phenomenon is called multidrug resistance, leading to various drug cross-resistances. It targets explicitly rapidly replicating cells by impeding tubulin assembly or DNA replication. Over the past 15 years, significant attempts have been made to develop biological or targeted medications that kill cancer cells by interfering with specific pathways assumed to be involved in the growth of tumors. Stopping cell growth and survival are the two main goals of targeted therapy for cancer cells. Monoclonal antibodies (mAbs) bind to small molecules that cross the cell membrane and membrane growth factor receptors or their ligands.[7,8] According to developments in omics and next-generation sequencing (NGS) technology, it is now possible to identify the molecular processes causing resistance and create innovative approaches to combat it.[9] Over the past 10 years, advances in colorectal cancer have led to the introduction of targeted medicines into clinical practice, dramatically increasing therapeutic efficacy and extending lifetime. Preclinical models and clinical studies have examined novel medications that target signaling pathways connected to the origin of CRC, such as the EGFR signaling pathway. Experts have been at odds for years about the ideal ratio of targeted therapy to conventional chemotherapy when treating. Understanding the processes driving acquired drug resistance to targeted therapies is essential for developing novel and effective treatment combinations and future therapeutics.[10–12] This article addresses the issues associated with managing drug-resistant and metastatic colorectal cancer and the efficacy of emerging targeted medicines. We also discuss tactics for overcoming targeted therapy resistance. We emphasize that developing effective, targeted medicines depends on using state-of-the-art preclinical models and the clinical relevance of knowledge gained from molecular research.[13]

Significance statement

- Tissue-based biomarkers are intensely involved in the pathogenesis of metastatic colorectal cancer (mCRC).
- It has been shown that the relationship between new drug resistance mechanisms with signaling pathways, cell surface markers, and oncogenic targets is involved in the pathogenesis of mCRC.
- Tissue-based biomarkers and their involvement in mCRC drug resistance can potentially revolutionize and personalize treatment protocols.

2 | TISSUE-BASED BIOMARKERS

2.1 | Microsatellite instability (MSI) as a prognostic factor

Cancers that originate from the mutator pathway have defective DNA mismatch repair (MMR), which allows mutations to accumulate at a pace that is many times greater than average. MSI, or cell-to-cell variability in DNA microsatellite length, is caused by this faulty MMR process. The explanation states that somatic changes in the size of simple repetitive microsatellite nucleotide sequences seen across the genome imply MSI. Therefore, DNA mutations are more prone to occur in genes harboring MSI.[14–16]

The MSI phenotype found in the majority of tumors is linked to hereditary nonpolyposis colorectal cancer because of epigenetic hypermethylation caused by the MutL homolog 1 (MLH1) mismatch repair gene. Individuals with high-frequency MSI tumors (MSI-H) have different phenotypic features from those that follow the chromosomal instability pathway, albeit not all research has consistently shown every trait. More proximal, mucinous, with a notable infiltration of lymphocytes, weakly differentiated, and with a propensity to maintain the natural diploid state are some distinguishing characteristics.[17,18] Furthermore, behavioral patterns and potential differences exist in how MSI-H carcinomas respond to chemotherapy. Consequently, MSI is among the most promising markers being studied. It has predictive value since, according to most studies, MSI-H was associated with a better prognosis.[19,20]

Yet it is still unclear if this phenomenon results from the fact that MSI-H cancers are less aggressive by nature or tolerate chemotherapy better. Chemotherapy options currently include monotherapy, mainly Fluorouracil (5-FU), and combination therapy, which includes one or more medications such as irinotecan and Oxaliplatin (OX). The standard first-line therapy continues to be the combined therapy regimens XELOX or CAPOX (CAP + OX), FOXFIRI (5-FU + IRI), CAPIRI (CAP + OX), and FOLFOX (5-FU + OX).[21,22]

Meanwhile, studies indicate that combined medicines are not inferior to monotherapy in terms of overall survival (OS); still, monotherapy is advised for patients who exhibit poor performance or low risk of deterioration. Numerous studies demonstrate that CRC patients treated with 5 FU had better MSI disease survival.[23]

Contrary to common opinion, most research showed that adjuvant 5-FU treatment alone is not beneficial for patients whose tumors exhibit MSI. Additionally, recent meta-analyses and systematic reviews could not demonstrate that adjuvant therapy is typically less advantageous for individuals with MSI CRC. There is proof that the chemosensitivity of MSI-H tumors and MSS colorectal cancer varies. The most likely mechanisms by which resistance to 5-FU is present are decreased thymidylate synthase activity and the inability of MMR genes to bind 5-FU-modified DNA.[24,25] Despite contradictory clinical data, results from in vitro experiments indicate a strong link between MSI-H and 5-FU resistance. For patients with unresectable MSI-H or MMR-deficient colorectal cancer, the recently updated National Comprehensive Cancer Network (NCCN) guidance mentions pembrolizumab or nivolumab, often known as PD-1 inhibitor antibodies, as treatment choices. As of the time of writing, neither medication has been approved for mCRC by the US Food and Drug Administration. As more data are gathered, response rates frequently rise since immunotherapy can take some time. Although response and stable disease remain for a long time, the primary disease progresses quickly.[26,27] A subpopulation of individuals with MSS tumors who might benefit from checkpoint blocking is being studied. Furthermore, several combination strategies are being investigated to increase intratumoral T-cell growth in MSS tumors to prepare them for immunotherapy, such as the cobimetinib plus atezolizumab trial.[28]

2.2 | EGFR

Entireblastosis oncogene B (ErbB)/human epidermal growth factor receptor (HER) is the family that contains the EGFR, Neu/HER2, ErbB3 (HER3), and ErbB4 (HER4) genes. The family of receptor tyrosine kinases has roles in adhesion, survival, angiogenesis, and cell migration.[29] For over 30 years, it has been known that the EGFR receptor tyrosine kinase (RTK) plays a role in developing colorectal cancer. Through downstream signaling via the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/AKT/Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) pathways, EGFR encourages the survival and proliferation of cancer cells and is acknowledged as an actionable target in mCRC. A series of cycles of hierarchical phosphorylation-activating kinase rings are initiated from the cell surface to the nucleus by the large family of Ser/Tr kinases, of which MAPK is a member.[30,31] Within the three main subfamilies of MAPK, extracellular EGFR is either expressed or upregulated in 80 percent of colorectal cancers. Since this expression is associated with a higher risk of metastasis, blocking EGFR could be a valuable strategy to reduce the rate of cell division. EGFR activation can be prevented by monoclonal antibodies (mAbs) or tyrosine kinase inhibitors (TKIs). The constitutively active signaling pathways for EGFR limit the efficacy of EGFR inhibitors in RAS-mutant cancers. H-Ras, N-Ras, and K-Ras are the three small Ras GTPases. Specific tyrosine residues on homo- or heterodimer-type receptors undergo autophosphorylation because these receptors are visible following ligand engagement. The adaptor protein complex member of the EGFR signaling cascade includes the growth factor receptor-bound protein 2 (Grb2) and the son of seven-less (SOS). By attaching to phosphorylated tyrosine residues, this complex activates Ras-GTP. Following RAS activation, phosphorylation activates the signaling cascade of rapidly accelerated fibrosarcoma (RAF)/mitogen-activated protein kinase kinase (MEK)/extracellular signal-related kinases (ERK)[32–34] (Figure 1).

AKT is a crucial mediator in cell growth and apoptosis in the ErbB-related pathway. Of all the ErbBdimer family members, the ErbB2-3 heterodimer is also the most effective PI3K/AKT pathway activator. Strong associations exist between uncontrolled AKT activity and diabetes and cancer. AKT inhibits cellular apoptosis and controls cell cycle entry and survival by phosphorylating glycogen synthase kinase 3 (GSK-3), forkhead box O (FOXO), a B-cell lymphoma 2 (Bcl-2)-associated agonist of cell death[35,36]; this is accomplished by activating the mammalian target of rapamycin. Cellular biological processes like membrane ruffle formation, cell motility, proliferation, and differentiation are all aided by the enzyme phospholipase C-1 (PLC-1), which is also essential. This 145 kDa protein breaks down Phosphatidylinositol 4,5-bisphosphate to produce inositol-triphosphate (IP3) and diacylglycerol (DAG). It has two pleckstrin homology domains, two SH2 domains, and one SH3 domain, which may interact with EGFR to boost the enzyme's activity. IP3 and DAG increase intracellular Ca2+ release, promoting carcinogenesis and activating protein kinase C.[37,38] Additionally, recent studies suggested that the PLC-1 SH3 domain may be essential for interacting with EGFR. Through the SH3 domain, EGF makes PLC-1/AKT binding easier, which modifies AKT's activity. PLC-1's SH3 domain regulates the action of the PI3K enhancer, dynamin-1, and Racl by acting as guanine nucleotide exchange factors. Dynamin-1 and Racl may facilitate EGF-induced cell migration and proliferation, and PI3K enhancer, a nuclear GTPase, activates nuclear PI3K activity.[39–41]

For the EGFR to dimerize and migrate into the nucleus, controlling the transcription of genes involved in cell division, growth, and death, STATs may directly attach to and phosphorylate the EGFR. Moreover, EGFR is strongly impacted by the nonreceptor tyrosine kinase c-Src, which also indirectly regulates STATs.[42] Numerous cancer cases exhibit upregulation of the proteins EGFR and c-Src, suggesting a possible involvement of both proteins in tumor formation and their tight relationship. The STAT and PI3K pathways are shared by tyrosine kinases in the Src family. Through c-SRC-dependent phosphorylation and the creation of c-SRC-EGFR complexes, SRCs augment EGFR signaling.[23] EGF and other EGFR ligands compete with Cetuximab, a murine-human chimeric monoclonal antibody, for the receptor's binding ability. Because of the potential for immunogenic reactions from completely humanized or

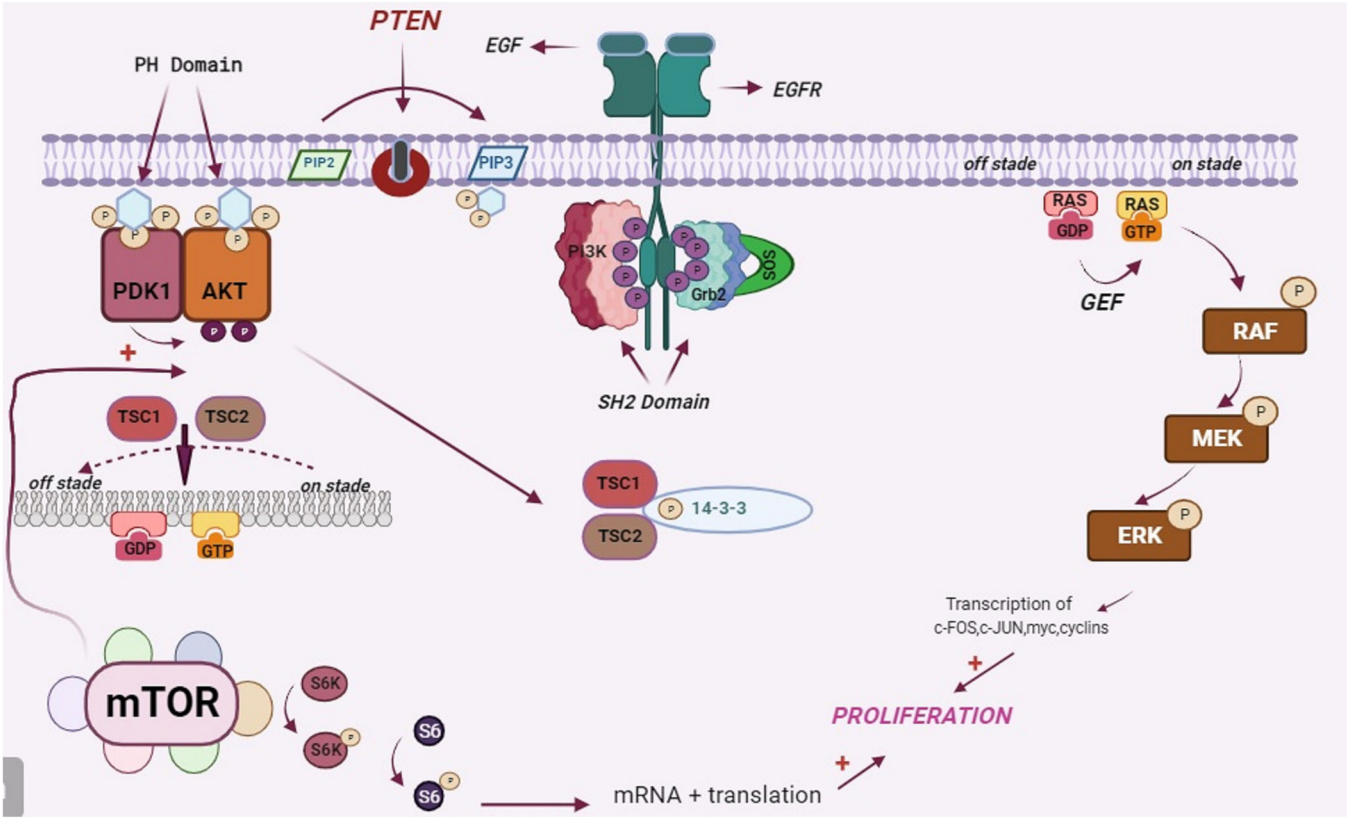

FIGURE 1 Signaling pathways in CRC for EGFR and PI3K. EGF-induced dimerization and the activation of intrinsic kinase activity result from the binding of EGF to the extracellular domain of EGFR. SH2 proteins are among the proteins that are recruited to active EGFR. GRB2, one of the adaptor proteins, attracts SOS to the membrane. SOS activates the GDP/GTP exchange, which attracts RAF to the membrane. ERK is then activated after RAF phosphorylates MEKs. The target genes c-FOS, c-JUN, and myc are expressed as a result of phosphorylated ERK translocating to the nucleus and triggering transcription factors. Another important EGFR signaling pathway mediator, PI3Ks, is attracted by GRB2. PIP2 is changed into PIP3 by PI3Ks. PIP3's binding to its PH domain attracts AKT to the plasma membrane. The activity of numerous proteins involved in cell survival is controlled by the phosphorylation of AKT by PDK1. By phosphorylating TSC2, activated AKT inhibits it. Inactive TSC1/2 cannot bind RHEB, which subsequently permits its activation of mTORC1 at the surface of the lysosome. Through S6K and the eukaryotic translation initiation factor 4E-BP1, mTORC1 regulates many cellular processes after activation, including cell growth, protein synthesis, and autophagy. CRC, colorectal cancer; EGFR, epidermal growth factor receptor.

chimeric murine-human antibodies, the CRYSTAL trial demonstrated that Cetuximab reduced the risk of progression when added to chemotherapy.[43,44]

Multiple studies found that Panitumumab's tolerance and effectiveness, particularly on the left, were similar to cetuximab dot's. EGFR expression is usually more abundant in malignancies than in cancers on the right side. Anti-EGFR treatments differ in their clinical results due to this "sidedness." Research indicates that the position of the tumor plays a crucial role in the efficacy of treatment plans; patients with left-sided tumors react more favorably to anti-EGFR medications than patients with right-sided tumors to anti-VEGF medicines. As previously established, Cetuximab is a human monoclonal IgG1 chimeric antibody that binds to the extracellular domain of EGFR to prevent it from having a pro-cancerous effect on cancer cells. Additionally, in a trial including patients with advanced colorectal cancer who had received irinotecan treatment, it attaches to NK cells and initiates cell-mediated cytotoxicity dependent on antibodies.[45–47] Therapy with Cetuximab alone or combined showed significant clinical activity, improving response rates and median survival times in the combined groups. In first-line treatment for patients with KRAS WT tumors, Cetuximab decreased the risk of mCRC progression by 15% compared to FOLFIRI alone. Complete or partial tumor responses were seen in 46%, 49%, and 38% of patients receiving combination therapy and FOLFIRI (Leucovorin, Fluorouracil, and Irinotecan). FOLFOX4 (5-FU, Leucovorin, OX) and Cetuximab were evaluated in another treatment strategy for mCRC. In this randomized study, the combination-treated group had a higher likelihood of responding and a lower risk of disease progression in KRAS wild-type (WT) individuals than the FOLFOX4 alone group. Besides, it was determined that Cetuximab combined with chemotherapy decreased the risk of progression of cancer.[48–50]

It is known that quinazoline-based EGFR TKIs, which are small chemicals, block the tyrosine kinase domain of numerous receptors, including EGFR. Erlotinib exclusively inhibits EGFR and stops the EGFR receptor from becoming phosphorylated in response to ligands. Gefitinib targets the ERK1/2 pathway in mesothelioma cell lines, just

like Erlotinib does. The effectiveness of EGFR-targeted therapy was tested in various clinical contexts and in vitro investigations that compared chemotherapy regimes with EGFR monotherapy to see how it worked. KRAS status should be considered since it can be utilized as a biomarker to evaluate the efficacy of a treatment.[51,52]

2.3 | KRAS

It has been demonstrated that the KRAS gene was a factor that caused oncogenesis more than 25 years ago. The tyrosine kinase signaling pathways, which include KRAS, are primarily responsible for the therapeutic efficacy of TKIs through its downregulation.[53] The absence of KRAS mutations has been connected to the clinical result of the EGFR inhibitor monoclonal antibody cetuximab. KRAS mutations are most commonly detected in the codons 12, 13, and 62, which are hot sites for KRAS regulation. Mutations in the KRAS oncogene cause constitutive activation linked to uncontrolled proliferation and poor differentiation even without growth factor receptor-ligand interaction.[54] A cell's ability to spread is partly impacted by KRAS overexpression because it produces more proteases, which degrade the extracellular matrix and promote angiogenesis. KRAS mutations, found in 40%–50% of CRC patients, are likely to be early events in the carcinogenesis of colorectal cancer.[55] Patients with mCRC are divided into two groups for first-line therapy based on the status of their KRAS mutation. When treating patients with KRAS WT, most of them typically receive an antibody, such as Bevacizumab, which targets VEGF-A, or Cetuximab and Panitumumab, which target the EGFR.[56]

Patients with KRAS mutations are usually treated with Bevacizumab and chemotherapy. Chemotherapy and Bevacizumab are commonly used as maintenance treatments, regardless of the KRAS status. Because of differences in the KRAS gene mutations being studied, data collection methods, staging procedures, and methodology used to detect KRAS mutations, it would be an obstacle to interpreting the different studies on the predictive function of KRAS.[57,58] Hence, future research on the prognostic role of KRAS mutations in colorectal cancer should be conducted prospectively, utilizing standardized assays. This approach is essential for identifying patients for whom routine mutation analysis could be valuable in determining adjuvant treatment options. Presently, only a few studies have been conducted prospectively in this context. KRAS mutations are robust predictive markers in mCRC patients treated with anti-EGFR mAbs, even though they cannot be used to identify patients who require adjuvant chemotherapy.[59,60]

2.4 | HER2

It is now recognized that the human EGFR2, commonly referred to as ERbB2 or HER2, is vital in colorectal cancer. This pathway employs downstream routes like AKT/PI3K and MEK/RAS/RAF. HER2 has been one of the earliest treatment markers for solid tumors.[61] It affects 11%–51% of patients with mCRC, especially those with KRAS/BRAF mutations. The receptor produces HER2/neu, an oncoprotein with intrinsic tyrosine kinase activity. HER2 does not have an endogenous ligand like other EGFR/HER/ERBB system members.[61] Tyrosine residues in the cytoplasmic domain of HER2, two more EGFR family receptors, are transphosphorylated due to homodimerization or heterodimerization with HER3 and EGFR, activating HER2. HER2-HER3 heterodimers activate the AKT/PI3KAKT pathway, which is connected to the survival and proliferation of cancer cells.[62,63] In CRC, HER2 amplification rates varied, with membranous expression rates ranging from 2 to 11 percent and cytoplasmic expression rates from 47 to 68 percent. Using patient-derived data, a proof-of-concept study was conducted.[64]

HER2 was a valuable therapeutic target in Cetuximab-resistant mCRC in patient-derived xenograft (PDX) models. According to clinical studies in patients with similar clinicopathological traits, Pertuzumab and Lapatinib (a dual TKI EGFR/HER2) increased the response rate and caused tumor regression.[65] The synergistic antiproliferative impact of HER2 and EGFR inhibition was also established in Cetuximab-resistant CRC cell lines. PDX of colorectal cancer had HER2 activating mutations that were responsive to two TKIs, EGFR/HER2 Neratinib and Afatinib, and that resulted in tumor regression when coupled with TKIs and Trastuzumab. The oncogenesis of colon epithelial cells has also been linked to these mutations and resistance to anti-EGFR monotherapy.[66,67]

Several clinical trials have validated the preclinical research findings that address HER2 alterations in conjunction with chemotherapy treatments in patients with metastatic colorectal cancer. Due to excessive toxicity and poor accrual, earlier clinical trials examining the combination of Cetuximab or chemotherapy with HER2 mAbs (Epratuzumab or Trastuzumab) were discontinued.[68] In a phase I study, patients with HER2-positive resistant tumors received Trastuzumab, interleukin (IL)-12, and paclitaxel; however, none of the patients with colorectal cancer exhibited any response. In KRAS codon 12/13 WT, HER2-positive patients, the combination of lapatinib and Trastuzumab resulted in a 30% objective response rate with good tolerability.[69] mCRC patients, according to a more recent study, adhered to the strict HERACLES criteria. Epratuzumab and Trastuzumab emtansine effectiveness was evaluated in the same project's HERACLES-B phase II study; however, it failed to meet its primary endpoint of response rate. Nevertheless, based on the excellent disease control observed, the improved PFS, and minimal toxicity, this combination can be viewed as a possible treatment option for HER2-positive mCRC.[70,71]

2.5 | VEGF

Angiogenesis is a physiological process in which new blood vessels emerge based on existing ones or undergo remodeling. It is essential to a tumor's ability to originate, grow, and spread. Pro- and antiangiogenic substances, including VEGF, fibroblast growth factors (FGFs), transforming growth factors (TGFs), platelet-derived

endothelial cell growth factor (PDGF), and angiopoietins generated by cancerous or stromal cells, are all involved in the intricate regulation of angiogenesis.[72] One of the most critical pathways affecting tumor angiogenesis is the VEGF/VEGFR signaling system, which activates host endothelial cells and promotes tumor growth. Patients with left- or right-sided mCRC who had KRAS/BRAF/NRAS mutations should be considered for VEGF/VEGFR-targeted drug-containing chemotherapy regimens, even though patients with CRC who had or did not have RAS mutations received VEGF/VEGFR-targeted treatment. Many signaling pathways are activated due to the VEGF family of proteins and VEGFRs regulating both pathological and normal tumor angiogenesis.[4,73,74]

VEGF-C and D normally regulate lymphangiogenesis, whereas VEGF-A, B, and PlGF primarily induce angiogenesis. Different biological reactions are triggered when VEGF-A, B, Placental Growth Factor (PlGF), and D are bound to VEGFR-2, VEGFR-1, and VEGFR-3, respectively.[75] Tumor angiogenesis and proliferation increase when VEGFs activate the intrinsic MEK/ERK/RAS/Raf, PKC/PLC, and AKT/PI3K signaling pathways. Among these, the VEGF-A receptors VEGFR-1 and VEGFR-2 are considered attractive targets for cancer treatment in therapeutic settings. The 180 kDa VEGFR-1 belongs to the family of receptor tyrosine kinases, comprising a range of cell types such as cancer, inflammatory, and epithelial cells. While VEGFR-1's affinity for PLGF and VEGF-2 is moderate, it is high for VEGF-1.[76,77] It is noteworthy that VEGFR-1 does not affect vascular cell formation. Instead, it controls the migration and differentiation of cells, particularly those of the epithelium, and stimulates the differentiation of epithelial cells in the initial phases of vascular growth. Additionally, pathological conditions can activate VEGFR-1.[78]

Many downstream pathways, including PI3K, ERK, AKT, and MAPK, are activated by conditions in inflammatory cells; in turn, this causes inflammatory cytokines secretion, such as tumor necrosis factor-alpha (TNF-α) and IL-8, IL-6, and IL-1, as well as the migration of inflammatory cells. VEGFR-1 is hypothesized to regulate angiogenesis, but its exact function is still unknown. PlGF interacts with VEGFR-1, which prefers VEGF-A over VEGFR-2; nevertheless, while VEGFR-1 chooses VEGF-A, VEGF-A may attach to VEGFR-2. Consequently, while angiogenic effects seem to be mediated by VEGF-A/VEGFR-2, VEGFR-1 acts as a decoy regulator to limit the free VEGF-A accessible to activate VEGFR-2.[79,80] Unlike VEGFR-1, VEGFR-2 actively promotes the formation of new blood vessels. Its molecular mass is between 200 and 230 kDa, mainly expressed in lymphatic and blood epithelial cells. VEGFR-2 activation results in the phosphorylation of tyrosine residues. Through their interactions with VEGFR-2, adhesion molecules such as cadherins and -catenin, triggered by the PI3K and MAPK pathways, may also deteriorate the integrity of intercellular connections and reorganize the cytoskeleton of epithelial cells. As a result, vascular permeability would rise. The initiation of several pathways, such as the RAS/RAF/ERK/MAPK and PLC pathways.[81,82]

Additionally, endothelial nitric oxide synthase (eNOS) and nitric oxide (NO), produced by epithelial cells due to AKT protein kinase activation, improve vascular permeability. The results demonstrate that both in healthy and pathological conditions, VEGFR-2 stimulates angiogenesis. Activated VEGFR-2 is critical for developing and spreading cancer angiogenesis, as it profoundly influences the epithelial cells' resistance to apoptosis, differentiation, migration, and proliferation, improving vascular permeability and tubulogenesis.[83] The primary requirement for the formation of lymphatic vessels is the activation of VEGFR-3 through VEGF-C, and D. Activated VEGFR-3 activates the MAPK/RAS/ERK and PKB/PI3K-AKT pathways, which in turn promotes the survival, differentiation, and proliferation of lymphatic endothelial cells. VEGF-C and D have been discovered to be upregulated in tumors with lymphatic metastasis, providing a reasonable explanation for cancer migration through lymphatic capillaries, even though the level of VEGFR-3 expression in tumor cells is still up for debate.[84,85]

The AVF2107, a phase III trial reports regarding metastatic colorectal cancer conducted on Bevacizumab, a humanized IgG monoclonal antibody directed against VEGF-A, showed improved OS and progression-free survival (PFS). As a result, the FDA approved Bevacizumab as the first VEGF-targeted therapy for mCRC; nevertheless, multiple trials comparing it to monotherapy or FOLFOX/FOXFIRI showed only a negligibly significant improvement in OS or PFS.[86,87] According to further research, Bevacizumab may be advantageous for those with KRAS mutations and wild-type genotypes. Aflibercept, also known as ziv-aflibercept, is a soluble molecule of human VEGFR-1 and VEGFR-2 major ligand-binding domains fused to a human IgG1 Fc segment. It binds as a sham receptor to VEGF-A, B, and PlGF.[88] Aflibercept attaches to VEGF-A more quickly and with a greater affinity rate than Bevacizumab. Ramucirumab is a human-only monoclonal IgG1 anti-VEGF-A antibody that inhibits VEGFR-2 and the pathways leading to angiogenesis.[89] The FDA approved it for second-line use with FOLFIRI 90 in patients with mCRC who experienced side effects during or after having treatment with Bevacizumab, Oxaliplatin, and fluoropyrimidines. The multikinase inhibitor regorafenib inhibits several intracellular and membrane-bound receptor tyrosine kinases, such as FGFR and VEGFR, which are assumed to control tumor angiogenesis. Fruquintinib is a potent and selective small-molecule tyrosine kinase inhibitor of VEGFR 1, VEGFR -2, and VEGFR -3. It was licensed in China to treat mCRC and has FDA approval to treat patients who have already received therapy for the ailment.[90] For the treatment of patients with metastatic colorectal cancer who have previously had chemotherapy based on fluoropyrimidine, Oxaliplatin, and irinotecan, as well as EGFR- and VEGF-targeted therapy, the FDA has granted fruquintinib a fast-track designation.[91]

2.6 | BRAF V600E mutation

Eighty percent of all documented mutations comprise a thymine-to-adenine transversion at nucleotide 1799, substituting valine with glutamic acid at amino acid residue 600 (V600E). This mutation causes the characteristic u-shaped alteration in BRAF. BRAF belongs to the RAF kinase family, which encodes kinases regulated by RAS

and is responsible for mediating cellular responses to growth factor signals. The most researched BRAF variants are known to be BRAFF V600E mutations, which show a strong correlation with metastatic colorectal malignancies.[92]

The occurrence of MSI-H cancers with germline hMLH1 and hMSH2 mutations is not influenced by BRAF as a prognostic or predictive factor. Additionally, MSS tumors do not have it. BRAF mutation is associated with a lower survival rate in MSS cancers despite high-grade CpG island methylator phenotype (CIMP) appearing to at least partially neutralize the harmful effects of BRAF mutation. It has been found that a BRAF mutation eliminated the excellent prognosis associated with MSI-H malignancies.[93] It has been discovered that BRAF mutations had no impact on patients with MSI tumors, whose forecast was perfect but were significantly associated with worse survival in patients with MSS tumors. Because the effects of the mutation vary depending on the genetic pathway through which it is produced, it has been suggested that the BRAF mutation alone does not always indicate a poor prognosis. It has also been discovered that the predictive potency of patients with MSI tumors was not affected by BRAF mutations, but MSS tumor patients' survival significantly worsened.[93]

It has been proposed that the BRAF mutation does not by itself indicate a poor prognosis because the consequences of the mutation vary depending on the type of genetic pathway through which it is generated. BRAF and RAS mutations cannot coexist and are more frequently found in tumors with high MSI.[94] Additionally, proximal tumor site, elevated T staging, poor differentiation, more excellent rates of distant and peritoneal lymph node metastases, and worse survival outcomes are all associated with BRAF-mt mCRCs. Non-V600E variants in BRAF usually have a better prognosis than V600E mutations and show unique clinicopathological features. EGFR inhibitors have a less beneficial effect on RAS-wt/BRAF-mt cancers because BRAF activates the MAPK pathway downstream of EGFR.[95] The use of BRAF inhibitors alone or in different combinations to treat mCRC with the BRAF V600E mutation has been studied. Thus far, it has been shown that BRAF inhibitor monotherapy is ineffective; nevertheless, a combination approach with a BRAF inhibitor is necessary, and three-drug regimens are marginally more effective than doublets.[23]

The S1406 cooperative group research discovered that adding vemurafenib to irinotecan plus Cetuximab significantly increased PFS (HR 0.42; 95% CI, 0.26–0.66; p .001); this was the first randomized comparison with a standard-of-care regimen.[96] Treatments with targeted inhibitors benefit some patients, but radiographic response is less common than disease stability. Rapidly developing drug resistance is still a problem.[97] Six hundred and fourty-five patients with BRAF V600E-mutated mCRC are being recruited for a phase III trial called BEACON CRC, which is comparing standard treatment with the combination of Binimetinib (a MEK inhibitor) with Enacofenib and Cetuximab. It's been suggested that the BRAF mutation does not necessarily indicate a poor prognosis because its effects differ based on the kind of genetic pathway that causes it.[98,99]

Additionally, neither Cetuximab nor Panitumumab therapy is anticipated to significantly impact individuals whose tumors have the BRAF V600E allele. Due to this, BRAF mutation analysis, similar to KRAS mutation analysis, is currently not used to select patients for adjuvant therapy; however, it might be employed as an additional predictive factor in the metastatic context to identify individuals who would benefit from EGFR-targeted monoclonal antibody therapies.[100] Concurrent mutations are rare despite many cancer forms changing KRAS and BRAF protein codes. Simultaneous mutations are unlikely since both genes have gain-of-function mutations, which indicate distinct ways of stimulating the same pathway. More recently, however, advanced colorectal malignancies and associated lymph node metastases have been linked to a KRAS mutation and the highly aggressive BRAF mutation (V600E).[101] The concurrent existence of BRAF and KRAS mutations appears to hasten the growth of MSS tumors, indicating that the activation of both genes probably works in concert. More independent prospective studies are required to fully understand the impact of these genetic features on the biology and clinical behavior of malignancies. To fully comprehend how CIMP, MSI, KRAS mutation, and BRAF mutation interact, these investigations had to consider a detailed evaluation of each factor.[102]

2.7 | Hepatocyte growth factor (HGF) and c-MET

HGF, which belongs to the cytokine family, specifically binds to the kinase receptor cellular-mesenchymal-epithelial transition factor (c-MET). This pathway is upregulated in many cancers, including CRC, and contributes to their development.[103]

The MET pathway is triggered by mesenchymal lineage cells' hepatocyte growth factor (HGF) secretion. MET, or c-MET, is a proto-oncogene that genes for the tyrosine kinase receptor. In up to 70% of cases, the MET pathway is reported to be overexpressed, indicating that it is commonly improperly active in cancer. Due to its impact on multiple proteins, including survivin and x-linked inhibitors of apoptosis protein, c-MET activation plays a significant role in developing resistance against antiangiogenic therapy. It has to do with how the disease progresses, metastases, and the poor prognosis that cancer patients face.[104,105] MET is triggered when blocking the VEGF pathway. It has been found that tiny compounds with various pharmacological mechanisms or recently produced monoclonal antibodies can inhibit HGF-MET in multiple ways. HGF-targeting drugs bind to MET receptors to hinder the activation and production of HGF. In the latter scenario, chemicals either inhibit intracellular tyrosine kinase activity (MET TKIs) or competitively bind to MET receptors (MET antagonists). While serious adverse effects have not been described concerning these drugs, a small number of individuals have experienced weariness, allergic reactions, reduced appetite, edema, and neutropenia.[106,107]

TKIs like Crizotinib, cMET-inhibitors, or HGF-blocking drugs like Onartuzumab, Emibetuzumab, and JNJ-61186372 (a bispecific antibody targeting both cMET and EGFR) are used in the targeted

treatment of the HGF/cMET pathways.[108] SAR125844 is a Triazolopyridazine derivative that is more effective than Emibetuzumab, Volitinib, Gefitinib, Tepotinib, and Capmatinib and is similar to LY3164530, which targets both c-MET and EGFR. One of the leading causes of CRC mortality is liver metastasis. In this situation, it is found that the HGF/c-Met signaling pathway and forkhead box p3 $(Foxp3)^+$ regulatory T cells (Tregs) are upregulated. Cytotoxic T cells are prevented from spreading and invading by increased HGF/cMET signaling and higher Treg levels.[109,110]

2.8 | Neurotrophic tropomyosin receptor kinase (NTRK)

The proteins Tropomyosin receptor kinase A (TRKA), TRKB, and TRKC are encoded by three members of the NTRK family, NTRK1, NTRK2, and NTRK3, which are primarily expressed in neural and neuronal tissues. Through homodimerization, these receptors perform biological processes that activate downstream pathways like RAS/Raf/MEK/ERK, PI3K/Akt, and PLC-/PKC. These pathways consequently support gene transcription, cell growth, and survival. The most frequent fusions discovered involved the NTRK gene, with others concerning the BRAF, rearrangement during transfection (RET), FGFR, ROS Proto-Oncogene 1 Receptor Tyrosine Kinase 1 (ROS1), and anaplastic lymphoma kinase (ALK) genes.[111,112] In tumors with high MSI scores and RAS/BRAF wild-type levels, gene fusion rates were higher. Clinical characteristics of patients with these gene fusions included advanced age, right-sided primary tumors, higher lymphatic spread rates, and lower liver metastasis rates. These characteristics are similar to those of patients with BRAF mutations.[113] To improve clinical outcomes for this patient subset, targeting gene fusions is a promising area of research. Investigating new tactics in this context is critical because current evidence suggests that gene fusions may predict resistance to EGFR inhibitors and have a negative prognostic effect. Colorectal cancer also harbors NTRK, ROS1, and ALK fusions.[114]

Furthermore, ALK and ROS1 encode tyrosine kinases that, when fused, become constitutively active. Consequently, these fused proteins activate downstream signaling pathways that promote tumor cell growth and progression. While they are found in only about 2 percent of CRC cases, these fusions are considered oncogenic drivers and potential mCRC treatment targets. Larotrectinib and Entrectinib are first-generation TRK inhibitors. Patients with solid tumors containing NTRK gene fusions are eligible for these inhibitors, as approved by the FDA. Larotrectinib, a selective TRK inhibitor, showed an impressive overall response rate (ORR) of 79 percent and good tolerability in three phase I/II clinical trials involving cancer patients with NTRK fusions (aged 48–67 years).[115,116]

2.9 | Epithelial cellular adhesion molecule (EpCAM)

In colorectal cancer, DNA hypomethylation in the EpCAM promoter region has been commonly reported. It has been reported that over 98% of colorectal cancer cases express EPCAM. The investigation reveals that tumor growth or metastasis does not eliminate this marker. The crucial significance of EpCAM and CSCs in tumor development has also been highlighted by the substantial correlation between the presence of $EpCAM^+/CD44^+$ CSCs and more aggressive and higher tumor grades in CRC patients. Thus, particular targeting or ablation of these CSCs that express EpCAM could be used to create new cancer treatment approaches. In this context, the EpCAM/CD3-bispecific antibody MT110 guided T cells to target the CSCs, showed encouraging outcomes in focusing on colorectal CSCs, and inhibited tumor growth in vivo.[117–119]

Exosomes released from colorectal cancers have been found to express EpCAM, which makes it possible to isolate them using anti-EpCAM-coupled magnetic beads. Furthermore, compared to the blood plasma of their respective healthy controls, colorectal cancer patients had a greater level of EpCAM+ exosome. The process by which EpCAM is present on the surface of exosomes derived from CRC is, nevertheless, poorly understood.[120] However, it has recently been demonstrated that the role of exosome-expressing EpCAM on other cancer-derived CSCs, like glioma, is a promoter of metastasis.[121]

The EpCAM intracellular domain (EpICD) of membrane-bound EpCAM is released into the cytoplasm in colorectal cancer due to proteolytic cleavage. Following its impact on the WNT signaling pathway, this cytoplasmic EpICD upregulates the expression of cell cycle regulators, stimulating the growth and carcinogenesis of cells. It is commonly known that the WNT signaling pathway plays essential roles in differentiation, self-renewal, and stemness maintenance. Additionally, a positive feedback loop occurs between EpCAM and the WNT signaling system. Through the cytoplasmic EpICD, EpCAM activates the ERK1/2 signaling pathway, promoting the migration and proliferation of colorectal cancer cells. All these emphasize even more how crucial the interaction between the WNT signaling pathway and EpCAM is in promoting CRC carcinogenesis.[122,123]

According to another investigation, there was a significant correlation between EPCAM overexpression in colorectal cancer cases and both primary and metastatic tumors. The main tumor's overexpression of EPCAM changed to the metastatic tumors' overexpression in 10% of patients with this malignancy. Additionally, this study showed that 89% of cancer cells in colorectal tumors that metastasize to lymph nodes express EPCAM, and 10% express it less.[124]

3 | DRUG RESISTANCE MECHANISMS

The correlation between the novel drug resistance mechanisms with signaling pathways, cell surface markers, and oncogenic targets has been categorized below, as shown in Figure 2.

3.1 | Cancer stem cells (CSCs)

One of the biggest obstacles to cancer recovery is the ability of CSCs to persist after conventional therapy, thereby regaining their capacity

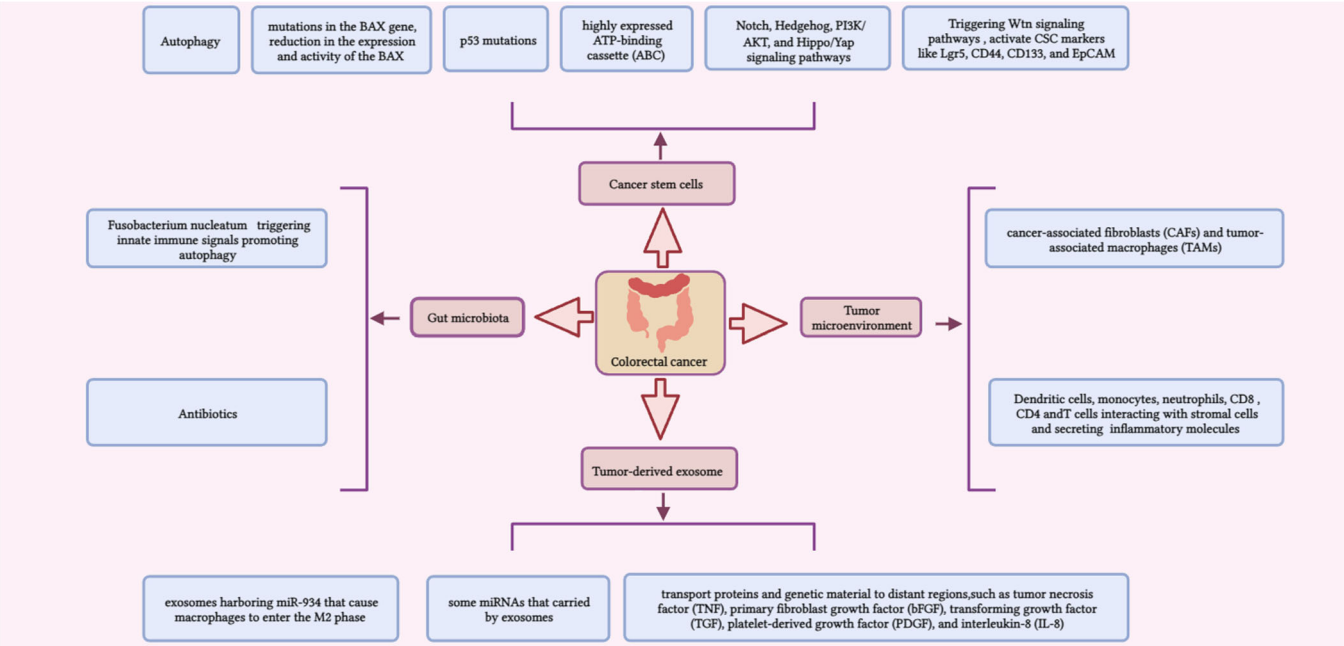

FIGURE 2 The correlation between the novel drug resistance mechanisms with signaling pathways, cell surface markers, and oncogenic targets involved in CRC pathogenesis. CRC, colorectal cancer.

for dedifferentiation and renewal. While certain factors are known to play a part, the precise mechanisms underlying resistance are still unknown. As CSCs are quiescent and do not enter the cell cycle, they are not targeted by standard therapy, which kills highly proliferating cells. Tumor dormancy also causes recurrent CRC by allowing cells to remain dormant but alive and resume growth when the proper signals are received.[125] Autophagy is necessary for tumor cells to enter the proliferative phase and can promote cancer dormancy by maintaining cancer cell viability. Resistance is brought on by autophagy, which is brought on by various cancer treatments. More research is required to fully comprehend the autophagy process and how it might be applied to prevent medication resistance and recurrence.[126] Characterizing $CD44^+/CD133^+$ colorectal cancer stem cells is highly recommended, as it may lead to the discovery of novel and efficacious treatments for the condition. Specific techniques, like single-cell methods, RNA sequencing, and whole genome sequencing, show promise in identifying $CD44^+/CD133^+$ colorectal cancer stem cells. More specifically, the chemoresistance of colorectal cancer stem cells depends mainly on WNT/B-catenin signaling pathways.[127]

In addition, other essential pathways like Notch, Hedgehog, PI3K/AKT, and Hippo/Yap have been linked to metastasis, increased cellular proliferation, improved survival, chemoresistance, and the maintenance of cancer stem cells. In CSCs, ATP-binding cassette (ABC) transporters are highly expressed and are essential for drug efflux and chemotherapy resistance. The first member of the ABC family, ABCB1 (P-glycoprotein), found in healthy intestinal cells, has been linked to chemotherapy resistance in preclinical and clinical CRC studies. This is because the overexpression of ABCB1 (P-glycoprotein) in healthy intestinal cells has been reported.[128,129] The efficacy of first-, second-, and third-generation ABCB1 inhibitors is highly affinitive, but they may be even more potent. It has been demonstrated that these transporters are upregulated in CRC tumor tissues, indicating that they could be potential targets for reversing drug resistance in CRC. Other ABC members include ABCC6, ABCC11, ABCF1, and ABCF2.[130] A functioning DNA damage response (DDR) is present in all cancers, including colorectal cancer. This damage response, consisting of several kinase-dependent signaling pathways, is necessary to maintain the genome's integrity and stability. Depending on the type of damage, DDR sensors usually send damage signals to downstream molecules and DDR mediators, which either stop the cell cycle, fix DNA damage, or cause apoptosis. The modification of the cell cycle checkpoint and the activation of a strong system for scavenging reactive oxygen species (ROS), which are produced by therapy, are two processes that contribute to CSCs' resilience to DNA damage.[131,132]

Three primary pathways influence the development of CRC. Chromosomal instability, CpG island hypermethylation phenotype, and MSI are among these routes. While spontaneous CRC usually features MSI (caused by the inactivation of the mismatch repair genes, MMR), most CRC cases (80%) have chromosomal instability (CIN). One trait that sets CSCs apart is their innate and acquired resistance to apoptosis. Proapoptotic, antiapoptotic, and pro-survival systems work in concert to regulate apoptosis, which is often disrupted in cancer, particularly colorectal cancer. Chemotherapy-induced DNA damage leads to cell apoptosis, and p53 is essential for this process. However, 5-FU and oxaliplatin resistance are associated with p53 mutations in 85% of CRC patients. Strong antiapoptotic expression is also observed.[133]

Bcl-2 family proteins are prominent in CSCs, which offer protection from apoptosis-induced cell death. Chemoresistance is

brought on by frameshift mutations in the BAX gene, which cause a reduction in the expression and activity of the BAX antiapoptotic protein. Other antiapoptotic proteins connected to chemoresistance besides Bcl-XL and the FADD-like interleukin-1β converting enzyme (FLICE)-inhibitory protein are also known. Furthermore, CRC takes a well-known path by activating several pro-survival signaling pathways.[134] The Wnt/-catenin pathway is essential for stemness and resistance. When the Wnt ligand binds to the Frizzled receptor, catenin, a vital effector for this pathway, is activated. CSCs proliferate and differentiate due to the stimulation of the Wnt pathway, partially mediated by activating molecules known as potential CSC markers like Lgr5, CD44, CD133, and EpCAM. These markers are all linked to CSC resistance to chemotherapy and radiation therapy. The maintenance of CSCs is aided by two additional signaling pathways related to stemness: the Notch and Hedgehog pathways.[134]

3.2 | Tumor-derived exosome (TDE)

Extracellular vesicles, or exosomes, are released by different cells and found in body fluids. A lipid bilayer produced from the plasma membrane encloses these vesicles. These cancer cell vesicles carry genetic material and proteins to far-off locations, which leads to tumor growth, metastasis, and drug resistance. Molecules include TNF, platelet-derived growth factor (PDGF), transforming growth factor (TGF), primary fibroblast growth factor (bFGF), and interleukin-8 (IL-8) can be transported by tumor-derived exosomes (TDEs).[135] Moreover, Many miRNAs transported by exosomes affect the control of angiogenic transcription factors, which encourages angiogenesis and multidrug resistance (MDR) in colorectal cancer. Moreover, one of the deadliest consequences of CRC is liver metastasis, which can be facilitated by exosomes carrying miR-934 and might induce macrophages to enter the M2 phase. Exosomes have a part in the development, resistance, and metastasis of CRC and may serve as biomarkers for the illness.[136,137]

3.3 | Tumor microenvironment

Eepithelial-to-mesenchymal transition (EMT) is a process by which cells take on mesenchymal traits to improve their motility and develop an invasive phenotype. Partial EMT, present in more than 90% of human CRC cell lines, favors the formation of cell clusters during the spread of CRC.[138]

EMT is a promising target to prevent the invasive properties of primary tumors from developing or recurrence after tumor and metastasis resection. The tumor microenvironment (TME), an essential factor in the multi-step process leading from developing adenomatous polyps to invasive CRC, and the resistance of CRC are closely related. The TME comprises signaling molecules, stromal cells, immune cells, endothelial cells, and extracellular matrix (ECM). Dendritic cells, monocytes, neutrophils, $CD8^+$ or $CD4^+$ T cells, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and mesenchymal stem cells all infiltrate solid tumors, including CRC.[139] By interacting with stromal cells and secreting soluble inflammatory molecules during tumor development, tumors can attract immune cells that aid in their survival and ability to metastasize. The most significant cancer-promoting cells in the tumor microenvironment are CAFs and TAMs. These cells actively encourage tumor invasion, angiogenesis, EMT, immunosuppression, and ECM formation. They can have these effects by directly interacting with other cells or secreting cytokines, growth, and angiogenic factors, contributing to cancer development and aggressiveness.[140]

3.4 | Gut microbiota

There is increasing data supporting the importance of the gut microbiota in developing and resisting colorectal cancer. Therapies targeting the gut microbiota can potentially enhance the treatment of the disease. Altering and modifying the microbiota may help treat CRC and make it easier to predict and keep track of therapy's positive and negative side effects. The gut microbiota is one of the most promising avenues for personalized CRC therapy; however, most studies are preliminary and still require clinical confirmation.[141]

Certain gut microbes have been identified to play a crucial role in resistance to 5-FU and Oxaliplatin treatment by modulating autophagy. Studies have demonstrated that Fusobacterium nucleatum increases colorectal cancer chemoresistance by triggering innate immune signals promoting autophagy. Antibiotics have been shown to decrease the anticancer action of Oxaliplatin and promote harmful bacteria like Enterobacter by altering cytokine release and reactive oxygen species generation in the TME.[142] It has been suggested that the gut microbiota serves a role in angiogenesis, which shapes and forms the cancer microenvironment. Probiotics might reduce localized inflammation by inhibiting the VEGF/VEGFR pathway in liver and intestinal cells. Studies have indicated that patients with mCRC undergoing Bevacizumab treatment had a worse prognosis when using antibiotics. This effect could be due to the significant reduction in the variety and density of gut microbes caused by antibiotics. Due to the conflicting reported findings in research based on renal cell carcinoma that claim an ambiguous function for antibiotics in VEGF-blockade treatment, the impact of the microbiota on anti-VEGF medicines is still up for dispute.[143,144]

4 | OUTLOOK AND FUTURE DIRECTIONS

In recent years, the therapeutic paradigm in mCRC has evolved rapidly. The development of innovative methods to combat secondary drug resistance should be one of the major research priorities over the next decade as more and more new targeted drugs are found and tested in patients with mCRC. Targeted treatments combined with chemotherapy have historically been more effective in treating mCRC. However, emerging research suggests that

chemotherapy-free therapies may increase survival in some molecular subgroups, such as patients with BRAF-mt and MSI-H mCRCA range of genetic aberrations identified in CRC, including EGFR, AKT, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), and MAP2K1 mutations, as well as MET and FGFR amplifications, have paved the way for rational treatment options in basket studies and smaller trials. Recent advancements in sequencing technology have enhanced our understanding of the extensive genomic and proteomic abnormalities in mCRC, enabling the selection of more effective treatment strategies.[145,146] Table 1 reveals all molecules involved in drug resistance affected by chemotherapy, targeted therapy, and immunotherapy.

While recent therapeutic approaches have significantly benefited patients, the persistent challenges of increasing resistance and the absence of diagnostic biomarkers for current targeted therapies remain significant issues in clinical practice. There is a critical need for

TABLE 1 Chemotherapy-targeted molecules in and function.

Systemic chemotherapy		
Drug	*Targeted molecule*	*Function*
5-FU	_	Inhibits the formation of thymidylate from uracil
Irinotecan hydrochloride	_	Topoisomerase I inhibitor
Oxaliplatin	_	Forms intrastrand DNA adducts
Capecitabine	_	Prodrug of 5-FU; inhibits the formation of thymidylate from uracil
Targeted therapy	Targeted molecule	
Cetuximab	EGFR	In EGFR-mutant RAS/RAF wild-type cancers, any line of therapy in combination with 5-FU, irinotecan, and/or oxaliplatin
Erlotinib	EGFR	Receptor tyrosine kinase inhibitor
Gefitinib	EGFR	Receptor tyrosine kinase inhibitor
Afatinib	EGFR	Receptor tyrosine kinase inhibitor Refractory mCRC
Panitumumab	EGFR	Fully human mAb (IgG1)
Pertuzumab	EGFR/HER2	Monoclonal antibody for Her 2
Lapatinib	EGFR/HER2	Tyrosine kinase inhibitor
Neratinib	EGFR/HER2	RAS-mutated solid tumors KRAS/NRAS/BRAF/PIK3CA-wild-type mCRC
Vemurafenib	EGFR/BRAFV600E	BRAFV600 mutated extended RAS-WT treatment refractory
Encorafenib	EGFR/BRAFV600E	RAF inhibitor
Binimetinib	EGFR/MEK1/2	Previously untreated BRAF-mutant mCRC
Ramucirumab	VEGFR-2	Fully human mAb (IgG1)
Bevacizumab	VEGF-A	Humanized mAb (IgG1)
Regorafenib	VEGFR	Small molecule inhibitor of membrane-bound and intracellular receptor tyrosine kinases
Aflibercept	VEGF-A, VEGF-B, PlGF	Fusion protein which consists of the binding portions of VEGF from VEGF-1 and 2 fused to the Fc portion of immunoglobulin G1 (IgG1)
Entrectinib	TRK, ALK, ROS1	Small molecule of tyrosine kinase inhibitor
Larotrectinib	TRK	Small molecule of tyrosine kinase inhibitor
Nivolumab	PD-1	Fully human mAb (IgG4)
Pembrolizumab	PD-1	Humanized mAb (IgG4)
Immunotherapy		
Pembrolizumab	PD-1 inhibitor	In MSIhigh or dMMR cancers
Nivolumab	PD-1 inhibitor	In MSIhigh or dMMR cancers

Abbreviations: EGFR, epidermal growth factor receptor; RAF, rapidly accelerated fibrosarcoma.

further understanding of resistance mechanisms to advance evidence-based therapies, broaden the scope of available treatments, and achieve personalized medicine in colorectal cancer.[90] Additionally, exploring biomarkers that can predict treatment sensitivity, efficacy, and toxicity is essential for tailoring treatments to individual patients. Treatment of CRC, one of the first diseases to cause deaths worldwide, remains challenging. The stage at which a patient is diagnosed significantly impacts mortality rates, with stage IV diagnoses having no chance of cure and stage I diagnoses having almost 100% chance of cure. Indeed, susceptibility to CRC can be influenced by various factors such as diet, gut microbiota, obesity, smoking, alcohol consumption, chromosomal instability, and the CPG methylation pathway. Individuals at high risk for CRC are advised to undergo regular screening examinations, including stool tests, sigmoidoscopies, colonoscopies, and CT colonoscopies, to detect the disease at its early and more treatable stages.[147]

The significant genetic and proteomic anomalies in mCRC have been better-understood thanks to recent advancements in sequencing technology, which makes treatment plan selection easier. Even though the most recent therapeutic choices have made tremendous progress in treating patients with mCRC, the lack of predictive biomarkers and increasing resistance to current targeted treatments remain significant problems in clinical settings. Expanding treatment options, achieving personalized medicine, and developing new evidence-based therapy strategies for colorectal cancer depend on better knowledge of resistance mechanisms and identifying biomarkers that predict treatment sensitivity, efficacy, and toxicity. The use of novel antibody-based therapy approaches like chimeric antigen receptor T cell therapy, antibody-drug conjugates, radioimmunotherapy, and photoimmunotherapy, in conjunction with advances like colorectal cancer multicellular 3D models, patient-derived xenograft models, and single-cell sequencing strategies, may extend the lives of patients with metastatic colorectal cancer. It is crucial to comprehend the pathophysiology and molecular etiology of mCRC to enhance therapy further.[148,149]

To overcome these obstacles, targeted therapy and nanomedicine have been introduced into the field of cancer therapy. Several areas of cancer biology, such as the tumor microenvironment, DNA repair pathways, cellular signaling pathways, vascular endothelial growth factors, and miRNAs, may be impacted by nano-drugs. Numerous nanomedicine delivery systems, such as hydrogels, bionics, liposomes, exosomes, and theragnostics, have been developed to address these concerns. These developments in nanotechnology present encouraging paths toward creating individualized and tailored cancer treatments. Another promising method with substantial potential for cancer treatment is the single-cell approach. It can aid in our understanding of the cellular signaling, response, and microenvironment of particular tumor cells. There are still several challenges to be solved. For example, the large number of cells required for the study necessitates extensive experimental methods when using single-cell collection. The next barrier is quality control, which could affect the results if ignored. In this context, expensive equipment is yet another problem. Biologists, computer scientists, and material scientists have worked together to create flexible, all-in-one medication delivery systems customized to tumors' characteristics. These developments can lead to future precision colorectal cancer therapy, especially in theragnostic systems. By integrating materials science, computational modeling, and biological understanding, these novel drug delivery systems can improve the targeted and individualized treatments for patients with colorectal cancer.[150,151] It is possible to overcome any potential chemoresistance mediated by the designated biomarkers in addition to identifying prospective predictive biomarkers by precisely understanding the role of CRC-associated indicators in CRC pathogenesis, particularly in metastasis.

AUTHOR CONTRIBUTIONS

Sarce Makaba: Conceptualization; writing—original draft; visualization. **Eyhab Ali**: Conceptualization; writing—original draft; visualization. **Mandeep Singh**: Conceptualization; writing—original draft; visualization. **Mohammed N. Fenjan**: Conceptualization; writing—original draft; visualization. **Irodakhon Rasulova**: Writing—review & editing. **Neeti Misra**: Writing—review & editing. **Sada Ghalib Al-Musawi**: Writing—review & editing. **Ali Alsalamy**: Conceptualization. **Anggraeni E. Kusumaningrum**: Supervision. All authors read and approved the final version of the work to be published.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

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