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REVIEW ARTICLE

RAS and BRAF genes as biomarkers and target for personalised colorectal cancer therapy: An update

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Abstract

Colorectal cancer (CRC) remains among the most commonly diagnosed cancers and has been on the rise. It is also one of the most lethal diseases globally, being the third leading cause of cancer-related death. Depending on the stages and disease conditions, CRC is treated by surgery, chemo-, radio-therapy, immunotherapy or in combination. However, these therapies have subpar results with unwanted side effects, hence continuous effort is ongoing to explore new type of therapeutic modalities. Among the sub-types of CRC, KRAS, BRAF and NRAS mutated CRC comprise approximately 43%, 10% and 3% of the total cases, respectively. These mutations are associated with tumour progression and anti-epidermal growth factor receptor (EGFR) treatment resistance. Due to their important role in CRC, these genes have thus become targets in the development of novel treatments. In this paper, we discuss the current and upcoming treatment on CRC by targeting these mutated genes, with more focus on KRAS and BRAF due to the higher occurrence of mutations in CRC.

Keywords: colorectal cancer; KRAS mutation; BRAF mutation; personalised therapy; molecular targets; biomarkers

INTRODUCTION

Cancer is one of the disorders that leads to high fatality in human population. Although our understanding on how it occurs and the ways to treat cancer have been readily improved over the years, there is still no cure for cancer. In addition to the discovery of novel anticancer drugs, much effort has been put on identifying the molecular subtypes of the disease and the development of targeted therapies towards these disease subtypes. In this review, we focus on highlighting the role of RAS and BRAF as the biomarkers for colorectal cancer (CRC) as well as their potential uses as therapeutic targets. The KRAS, BRAF and NRAS mutations comprise approximately 43%, 10% and 3% of total mutated CRC cases, respectively. Hence, in this review, more focus will be placed upon KRAS and BRAF mutated CRC, as compared to the NRAS mutations.[1] Through the compilation of various research work conducted not more than five years, we aim to summarise the developments of treatments targeting KRAS and BRAF mutated colorectal cancer (CRC) and the significance of the KRAS and BRAF gene alterations which is highly associated with CRC. The literature search was conducted on PubMed databases, using keywords including 'Colorectal Cancer', 'KRAS', 'BRAF', and 'treatments'. We also further enhanced the search criteria by including words such as screening, prognosis, diagnosis, mutations, and clinical trials in order to obtain more relevant papers.

Colorectal cancer and its biomarkers

CRC has become an increasingly concerning issue, as it now places 3rd as the leading cause of cancer death globally.[2] According to GLOBOCAN 2018, colon cancer is ranked fourth while rectal cancer is ranked eighth, collectively placing colorectal cancer in third, comprising 11% of all cancer diagnoses.[3] The risk of developing CRC is intertwined with a variety of factors, including age, lifestyle, and genetics. According to the American Cancer Society, the risk of developing CRC significantly increases

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with each 5-year age group.[4] Furthermore, recent findings have shown an increased occurrence in young adults.[5] Lifestyle also has an impact as individuals that exercise regularly[6], control their diet[7,8] and refrain from smoking[9] have been found to have lower risk of developing CRC. CRC has been identified as the second most common cancer in women and third in men, while being largely prevalent in developed countries.[10] Increasing cases of CRC are also seen in developing countries[11] which further fuels the need for a proper CRC management including early screening and personalised therapy.

Biomarkers are currently popular to detect certain conditions or diseases, including their subtypes, within an individual. There are two types of biomarkers, predictive biomarkers which allow the prediction of an individual's response to specific treatment, or diagnostic biomarkers that can identify diseases subtypes.[12] The diagnosis of diseases by biomarkers is a critical factor in personalised medicine, which has become increasingly popular due to its generally higher effectiveness.[13] Personalised medicine is the administration of a treatment based on an individual's genetic and epigenetic profile, administering the most suitable medicine to the specific individual, at the appropriate time in suitable dosage at a more cost-effective way.[14] This discovery has shifted the paradigm of cancer therapy in recent years. Current cancer treatment includes chemotherapy, radiotherapy, surgery, immunotherapy, or combination therapy. However, these treatments are often accompanied with unwanted side effects, and resistance towards these therapies have become a major issue.[15] As cancer is considered as one of the genetic diseases, genomic biomarkers have become popular targets for cancer diagnosis. To date, numerous oncogenes and tumour suppressor genes have been targeted as biomarkers for various cancers such as the p21 oncogene and the p53 tumour suppressor gene.[16-19] For CRC, the RAS and BRAF genes, which mutate in certain CRC cases, have shown great potential as biomarkers and, possibly, targets for personalised medicine.[20]

The KRAS gene is an oncogene belonging to the RAS family proteins alongside NRAS and HRAS, which are a group of GTP-binding proteins. This paper will focus more on KRAS as compared to NRAS, as it has been observed that KRAS mutations have the highest occurrence (43%) in RAS-mutated cancers and CRC, as compared to the low occurrences of mutated NRAS (3%).[21,22] RAS functions as a mediator for intracellular signaling receptors through the mitogen-activated protein kinase (MAPK) signaling pathway, which mostly involves cell growth and survival.[23-25] Mutations in the RAS gene have been shown to be a key step in the early development of CRC progression.[26] Similarly, BRAF gene is also involved in controlling cell growth, proliferation and senescence through the MAPK signaling pathway, working together with RAS to mediate the intracellular signaling receptors, as the BRAF protein is located downstream of the KRAS protein.[27,28] The RAS and BRAF genes are found to be mutated in some cases of CRC, causing loss of function, and resulting in uncontrolled cellular growth and proliferation.[29-32] The major pathways in which RAS and BRAF are involved, including the MAPK and PI3K pathways[33,34] are summarised in Figure 1.

Detection of RAS and BRAF mutations

Currently, the screening of RAS and BRAF mutations in CRC cases are not considered standard procedure, but recent studies and trials have demonstrated that the presence of these mutations hold significant prognostic and predictive value. Several studies have shown that KRAS and BRAF mutations are associated with Overall Survival (OS), time to recurrence, survival after relapse, and treatment outcomes of CRC cases.[35,36] Hence, the screening of mutations in the RAS and BRAF genes prior to administering treatment is a critical step, as CRC containing either of these mutations have been observed to cause strong resistance against anti-EGFR treatments such as cetuximab or panitumumab, which are now a popular treatment choice. EGFR is a tyrosine kinase receptor protein that carries out its signal transduction through multiple pathways including the MAPK and PI3K pathways (Figure 1). These pathways are activated through the binding of different ligands onto the extracellular domains of EGFR. EGFR is a transmembrane glycoprotein that is involved in regulating cell growth, apoptosis, differentiation, and migration. EGFR mutations are highly prevalent in cancer and is expressed in 60-80% of CRC cases. Mutations in the EGFR gene causes it to be overexpressed, resulting in the promotion of tumourigenesis that involves cell cycle dysregulation and promotion of tumour survival. Mutated EGFR has been observed to promote increased angiogenesis and proliferation through the ligands Transforming

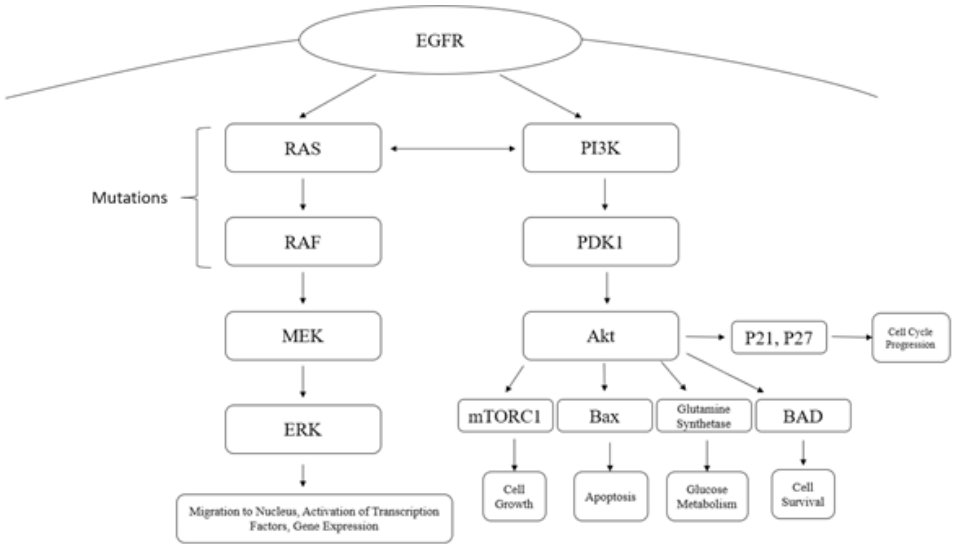

FIG. 1: Summary of pathways involved in EGFR signaling in CRC.

Growth Factor alpha (TNFα) and Epidermal Growth Factor (EGF). These ligands function as chemoattractant for endothelial cells and Vascular Endothelial Growth Factors (VEGF). Furthermore, cellular apoptosis is inhibited as the proapoptotic protein BAD is inactivated in overexpressed EGFR. The expression of various matrix metalloproteases (MMP) that are involved with tumour progression and metastasis are also affected, such as MMP-9 which is vital in cancer cell invasion and tumour metastasis. Due to this, multiple anti-EGFR drugs were developed as therapeutic treatments, which function through inhibiting the activity of EGFR, either through the binding to extracellular EGFR domains to block ligand-dependent activation or through inhibiting certain pathways. These drugs have displayed some successes in the treatment of CRC cases.[37-39] The activation of EGFR function is linked to RAS and BRAF, which are principal effectors in the MAPK and PI3K pathways. These pathways are deeply associated with EGFR function of regulating cell cycle progression and antiapoptotic signalling, respectively. RAS and BRAF are major proteins that are essential in the signalling cascade of both pathways.[40] Hence, mutations within these proteins may affect EGFR function. It has been observed that KRAS mutations commonly occurs in exons 3 and 4 while NRAS mutations more frequently take place in exon 2 and exon 3. For BRAF mutations, over 90% occur at position V600E which confer strong resistance towards most anti-EGFR treatments. In particular, the KRAS mutations have been observed to cause de novo resistance in tumour cells.[41-43] Presently, RAS mutations can be detected by several methods, which includes DNA sequencing, multiplex immunoassay or polymerase chain reaction (PCR) detection methods such as high-resolution melting or real-time PCR assay.[44-46]

For BRAF mutations, they are commonly detected using formalin fixed paraffin embedded tissue specimens from the CRC patients. These specimens might undergo either immunohistochemistry, utilising BRAF mutant specific monoclonal antibodies or real-time PCR assay methods.[47,48] Besides, direct sequencing and qPCR are other popular choices for the detection of BRAF mutations in CRC.[49,50] Both mutations could also be detected through freely circulating DNA or circulating tumour cells, as the high sensitivity and non-invasive collection method are favourable traits. These diagnosis methods could be used as a complementary platform, as direct sequencing is still considered the gold standard for detecting both RAS and BRAF mutations. Comparatively, direct sequencing is often labourious, cost- and time-consuming.[51]

KRAS, NRAS and BRAF mutations have been observed to affect the prognosis of certain treatments towards CRC such as anti-EGFR treatment. The prognostic value of KRAS, NRAS and BRAF mutations in CRC has been a debatable issue, with controversial results. However, recent studies have managed to improve our

understanding. It has been observed thus far that the presence of KRAS or BRAF mutations are often indications for poor prognosis of anti-EGFR based treatments of CRC. However, the prognosis value is affected by various factors, which includes the progression stage of CRC, the race of the patient and the location of the tumour. KRAS and BRAF mutations are believed to have a much lower prognostic value for Stage I and Stage II CRC regarding Cancer Specific Survival (CSS), Overall Survival (OS), Progression Free Survival (PFS) and Recurrence Free Survival (RFS) of patients after treatment. KRAS mutations have been observed to indicate poor prognosis of CSS in Stage III and Stage IV CRC patients. The more prevalent G12V and G12D KRAS mutations also indicate a worse prognosis of OS and PFS in CRC patients. The prognosis value of KRAS is also more accurate for tumours located in the left colon and the rectum. Similarly, NRAS mutations are also prognostic factors indicating poor OS and PGS in patients. In contrast, BRAF mutations are a strong prognostic factor for poorer OS and DFS. BRAF mutations as a prognostic factor are also more affected by the status of microsatellite instability (MSI) in CRC cases. Although BRAF mutations have been observed to be a reliable prognostic factor in later stage CRC, some studies have shown that BRAF mutations occasionally display prognostic value in some early cases of CRC as well.[52-54] As these genes function to control cell proliferation and survival, mutations in these genes are strongly associated with tumour cell proliferation and prolonged survival. RAS mutations are also known to be highly associated with CRC tumour progression in early stages.[55] Furthermore, due to their association with the MAPK pathway, which is downstream of the EGFR pathway, their mutations can strongly disrupt this pathway. The disruption of the MAPK pathway leads to uncontrolled cell proliferation, ultimately driving the proliferation and survival of tumour cells.[56] Hence, due to their biological significance, the deactivation or inhibition of these genes are expected to result in promising anti-cancer effects.

Current treatments against CRC with RAS and BRAF mutations

Today, there remains no fully recommended treatments towards CRC bearing RAS or BRAF mutations. As these mutations have been observed to confer resistance towards anti-EGFR treatments, the treatment option is not recommended to treat the patients with these mutations. Instead, treatments including combination therapy, involving surgery, chemotherapy or radiotherapy are used. Combination therapy has been observed to improve effectiveness, reducing localised recurrences and OS rate without affecting toxicity too drastically.[57] Standard chemotherapy drugs administered to patients include 5-fluorouracil, folinic acid, irinotecan, and oxaliplatin. Bevacizumab is also administered in combination as it has been observed to improve survival in more malignant tumour cells. Multiple drugs are administered together, as this can improve patient OS rate.[58-60] Unfortunately, although adverse side effects often follow chemotherapy and radiotherapy treatments, they are still commonly used for treating CRC, including those with KRAS and BRAF mutated CRC. In locally advanced rectal cancer, these therapies are used as first-line treatment. Continuous efforts are ongoing to explore new therapeutic modalities, some of which are undergoing clinical trials, these will be discussed in the next sections.

Emerging treatments against CRC with RAS and BRAF mutations

Current treatments against KRAS mutated CRC have focused on two paths - direct and indirect targeting of KRAS. As early studies believed direct targeting of KRAS protein was not practical due to the lack of pocketable hydrophobic spots, compounds targeting the association of RAS with the plasma membrane were focused instead. The small molecule Deltarasin was found to target and bind to the prenyl-binding protein PDEδ, which inhibited the proliferation of KRAS mutant tumour cells. Utilising Deltarasin as a base, the small molecule compound Deltazinone 1 was designed, which exhibited higher selectivity and lower unspecific cytotoxicity, displaying strong potential towards targeting KRAS mutated tumour cells, inhibiting cell proliferation.[61-63] Besides that, the small molecule LY3009120, which is a panRAF inhibitor, displayed strong antitumour activity in both BRAF and KRAS mutants in *in vivo* xenografts. This compound was found to target the RAF dimers, which led to the inhibition of phosphorylation of downstream kinases.[64,65]

Direct targeting of the KRAS protein has become a new venture as understanding towards this protein deepens. Recently, several groups have found a ligand binding pocket on the RAS protein and have found multiple small molecules

that are able to bind to these pockets. The binding of these molecules can inhibit the activation of RAS proteins, through the inhibition of the nucleotide exchange factor son of sevenless (SOS).[66,67] Besides that, the Kobe0065-family compounds can effectively target the pockets on RAS-GTP complexes, eradicating the antitumour and antiproliferative activity on KRAS mutant xenograft models. These compounds could potentially serve as the base for the development of novel RAS inhibitors with high specificity and stronger potency.[68] Other studies have also shown that targeting the RAS-GDP complex is promising, as several small molecules have been found to bind irreversibly to KRAS G12C mutants, causing it to favour the inactive GDP-bound form and promote apoptosis of these cancer cells.[69] Following further screening of these compounds, the ARS-853 compound was found to be among the most potent, selectively inhibiting the growth of mutant cancer cells *in vitro*.[70] However, further studies proved several shortcomings of ARS-853, and improvements and modifications were made. After several changes, three new compounds, AMG510, MRTX849 and ARS3248 were designed by different groups, entering clinical trials.[71] Recently, AZD4785, a 16-mer antisense oligonucleotide (ASO), which is complementary to the untranslated regions of KRAS mRNA had displayed inhibitory effects on KRAS mutant expression and antitumor activity on mice bearing mutant xenografts. As the ASO is complementary to a region outside the mutated region, this ASO targets both wild-type and mutant KRAS but has only displayed inhibitory effects towards the mutant KRAS proteins. The safety profile of this ASO was also well tolerated by mice and monkeys.[72]

Besides that, targeting the G-Quadruplex structure has been an increasingly promising field in the development of anticancer compounds, and it remains the same for CRC. Three G4 motifs have been found in the KRAS promoter.[73] A recent study has found that the G4 ligand, indolo[3,2-b] quinolines with a 7-carboxylate group and three alkylamine side chains (IQ3A), could target the G4 motifs in KRAS promoter, thereby stabilising the structure and inhibiting transcription, resulting in tumour cell apoptosis.[74] Furthermore, a new chimeric compound, dubbed EMICORON, could selectively target the G4 structure, causing telomere DNA damage through delocalisation of telomeric protein protection of telomeres 1 (POT1), limiting the growth of telomere-positive and negative tumour cells. EMICORON has displayed potent effects towards transformed and tumour cells, but it has not displayed much effect towards normal fibroblasts expressing telomerase, with no adverse effects observed in mice.[75,76] NRAS mutations, despite their rarity, have been observed to be highly similar to KRAS mutations, and combination therapies are being tested for the treatment of these cases. MEK and Kinase inhibitors Binimetinib and Palbociclib are being tested for combinative use for treatment. Besides that, combination of chemotherapy and the selective Wee 1 inhibitor, AZD1775, is also being tested. However, due to the low incidence of NRAS mutations, there are much fewer trials being conducted as compared to KRAS trials.[77,78]

In the effort of targeting mutant CRC, combination therapies utilising various compounds have become the focus. Combination therapies are an emerging strategy in the treatment of cancer, in which multiple therapeutic treatments are utilised to target multiple pathways. Combination therapies are generally more effective compared to monotherapy and, due to multiple pathways being targeted bringing forth an additive effect, lower amounts of each drug are used which results in lower toxicity. This approach is a cornerstone of cancer therapy and potentially reduces resistance while enhancing therapeutic benefits.[79] These therapies include different combinations of RAF inhibitors, MEK inhibitors, EGFR inhibitors or chemotherapy drugs. Following this, the use of Bevacizumab, an antiangiogenic VEGF inhibitor, in combination with double or triple chemotherapy treatments have displayed great success. The addition of Bevacizumab to different treatments have consistently shown favourable results as evidenced by clinical studies and subsequent meta-analysis by several independent studies. In particular, a clinical trial (NCT00354978) using bevacizumab with FOLFIRI (Folinic Acid, Fluorouracil, and Irinotecan) as a first line treatment on participants with mCRC resulted in an OS of 31.3 months[80], favourably higher than the 16.7 months OS of participants treated only with FOLFIRI in another study.[81] Meanwhile, a meta-analysis by Kopetz *et al* reported higher PFS at 12.8 months compared to historical controls, with low rates of toxicity.[80]

Following that, a clinical trial (NCT01437618) utilising FOLFOXIRI (Fluorouracil, Leucovorin, Oxaliplatin, and Irinotecan) with bevacizumab in mCRC patients with BRAF V600E mutations was carried out. This BRAF mutation accounts

for 7 – 10 % of all CRC cases and is associated to poor prognosis. On average, the OS and PFS of BRAF mutant patients on conventional first-line treatment are less than 12 months, and 4 - 6 months, respectively.[82] Analysis of this clinical trial by Loupakis and colleagues described the triplet regime with bevacizumab as extremely encouraging in terms of OS and PFS at 24.1 months and 9.2 months, respectively. As such, this regime should be considered to be used as the first line treatment option for BRAF mutated CRC cases.[82]

On the other hand, clinical benefits such as improved OS and PFS stemming from combination therapy with bevacizumab has been demonstrated to be independent of KRAS mutation status in mCRC patients. This is supported by three independent studies. A study by Sharma and colleagues investigated the combination of Oxaliplatin plus leucovorin and 5-fluorouracil (FOLFOX-4) and bevacizumab on KRAS mutated and WT mCRC patients.[83] In the second study, mCRC patients were treated with bevacizumab and oxaliplatin or irinotecan-based therapy.[84] Third, stage IV mCRC patients were treated with bevacizumab in combination with capecitabine and oxaliplatin (XELOX).[85] Collectively, these studies demonstrated that KRAS mutation is not a suitable predictive biomarker for combination therapy involving bevacizumab.

Besides that, among the most prominent of clinical trials conducted against BRAF-mutated CRC, the phase 3 BEACON (Binimetinib, Encorafenib, and Cetuximab Combined to Treat BRAF-Mutant Colorectal Cancer) trials represent a major development. This was a global, open-label phase 3 trial that targeted patients with histological or cytological confirmation of metastatic CRC with BRAF V600E mutations. The trial managed to confirm an overall improvement and significant benefit towards OS and objective response rate of BRAF-mutated CRC patients.[86] Vemurafenib and Encorafenib are RAF inhibitors that can specifically target the BRAF kinase, reducing the signaling pathways in the MAPK pathway and thereby reduce tumour cell proliferation.[87,88] Both drugs have shown significant success in the treatment of BRAF mutated melanoma. However, studies have shown that the monotherapy of vemurafenib or encorafenib have shown rather disappointing results towards the treatment of BRAF mutant CRC, as vemurafenib did not display significant clinical effect, and the more potent encorafenib had only modest effects towards BRAF-mutated CRC tumour cells.[89,90]

Due to the lack of success with monotherapy, more focus has been shifted towards dual and triple combination therapies. Among the potential combinations include utilisation of RAF and EGFR inhibitors, as it was found that the inhibition of EGFR has shown strong synergistic effect with BRAF V600E inhibition. BRAF inhibition was observed to cause a rapid feedback activation of EGFR, promoting tumour cell proliferation. The combined use with anti-EGFR drugs such as cetuximab was able to counter this effect.[91] The combination of vemurafenib (RAF inhibitor) and Panitumumab (EGFR inhibitor) exhibited lower cutaneous toxicity levels and improved antitumor activities, even in particularly aggressive and chemo-resistant subsets of CRC.[92] Besides that, the use of RAF inhibitors in combination with MEK inhibitors have been proven to be highly successful in treating BRAF mutated melanoma, indicating that the inhibition of the MAPK pathway can more efficiently treat BRAF mutated cancers.[93] Furthermore, MEK signalling may potentially dull the effect of various EGFR inhibitors, therefore the use of MEK inhibitors may be advantageous to be used in combination with anti-EGFR treatments.[94] Therefore, the use of Dabrafenib (RAF inhibitor) with Trametinib (MEK inhibitor) is under study to test the effect on BRAF mutated CRC. The MAPK signalling pathway was found to be inhibited in all patients that were evaluated during the clinical trials.[95,96]

Triple combination therapies including the use of EGFR inhibitors, MEK inhibitors, RAF inhibitors and other compounds such as chemotherapy drugs and PI3K inhibitors are also being considered. A study conducted found that the triple combination therapy of Dabrafenib (BRAF inhibitor), Panitumumab (EGFR inhibitor), and Trametinib (MEK inhibitor) could increase the anticancer effect in BRAF mutant CRC patients. The study indicated great suppression of the MAPK pathway, increasing the overall efficacy of the treatment.[97] A separate study by Van Cutsem *et al*. also supports this result.[98] Irinotecan is a chemotherapeutic drug that has been used to treat solid tumours for over 2 decades. The administration of this drug was well tolerated with significant effects[99], prompting studies towards combination therapy of Irinotecan with RAF and EGFR inhibitors. Randomised trials utilising the combination of vemurafenib, cetuximab and irinotecan has

Table 1: Ongoing clinical trials targeting KRAS mutated CRC

Treatment / Target	Compound	Clinical Trial Phase	Year start	Reference
PDEδ Target	Deltazinone 1	Pre-Clinical	2016	[70]
RAF Dimers	LY3009120	1	2013	[106]
RAS-GTP Complex	Kobe0065-family	Pre-Clinical	2013	[76]
RAS-GDP Complex	ARS3248 / JNJ-74699157	1	2019	[107]
	MRTX849	2	2019	[108]
ASO	AZD4785	1	2017	[109]
G Quadruplex	IQ3A	Pre-Clinical	2015	[110]
	EMICORON	Pre-Clinical	2015	[84]

been observed to prolong PFS, higher disease control rate and higher overall response in patients.[100-102] This was further confirmed by a recent SWOG trial conducted by S. Kopetz, who was involved in the prominent BEACON trials previously. This trial had significant success, as the response rate and corresponding disease control. The circulating tumour DNA of BRAF V600E was also observed to have declined, with overall increased PFS of patients.[103] Besides that, other triple combination studies involving the use of RAF, EGFR and PI3K inhibitors found significantly positive results, with promising clinical activity suppressing the growth of tumour cells and high tolerance levels among patients.[104,105]

Table 1 and 2 summarise the ongoing clinical trials targeting KRAS and BRAF mutated CRC, respectively. These treatments have currently yet to be approved, being in various investigational stages. As more adjustments are made, the treatments are becoming increasingly effective and moving in a positive direction towards approval.

Potential new strategies and challenges

Several studies have also suggested the use of microRNAs as targets, as numerous studies

Table 2: Ongoing trials targeting BRAF mutated CRC

Treatment	Drug name	Clinical Trials Phase	Year start	Reference
Double Combination				
BRAF + EGFR Inhibitor	Vemurafenib + Panitumumab	1	2013	[111]
	Dabrafenib + Panitumumab	2	2012	[112]
	Encorafenib + Cetuximab	2	2016	[105]
BRAF + MEK Inhibitor	Dabrafenib + Trametinib	1	2015	[96]
Triple Combination				
BRAF + MEK + EGFR Inhibitor	Dabrafenib + Trametinib + Panitumumab	2	2012	[112]
BRAF + EGFR Inhibitor + Chemotherapy Drug	Vemurafenib + Cetuximab + Irinotecan	2	2014	[113]
BRAF + EGFR + PI3K Inhibitor	Encorafenib + Cetuximab + Alpelisib	2	2016	[105]

have found microRNAs to be deeply involved in a variety of biological processes, including the regulation of signalling pathways. Several studies have also reported correlation between dysregulated mRNAs and aberrant signalling pathways involving angiogenesis, apoptosis and drug resistance.[114,115] In fact, CRC migration has been found to be highly associated with microRNA-106b, which targets the tumour suppressor DLC1, whereas microRNA-30b was found to act as a tumour suppressor, targeting KRAS to regulate cell proliferation.[116,117] This use of RNAs is considered epigenetic drugs, which also includes drugs that target histones and methylation of DNA. Although epigenetic drugs can be used as monotherapy, there are many advantages in using them as a part of combination therapies, such as increasing the effect of chemotherapeutic drugs through chemo-sensitisation and immunopotentiation of cancer cells. They can also be used to reverse acquired resistance against chemotherapy drugs. These synergistic effects are produced through a variety of methods, which includes the hypermethylation of genes or reactivation of useful genes, such as tumour suppressor genes. Epigenetic drugs have been observed to function more effectively as sensitisers rather than monotherapy agents and have greater potential in combination therapy with other drugs.[118,119]

The use of immunotherapy compounds, such as humanised monoclonal antibodies, blocking antibodies, or dendritic cell vaccines has shown significant success towards the treatment for various cancers, including kidney, lung, and prostate cancer respectively.[120-122] The PD-L1 blocking antibody has been approved due to the significant antitumour activity displayed towards various melanoma and renal carcinoma cases.[123-125] Besides that, as tumours can activate cytokines to control regulatory T cells and myeloid derived suppressor cells, which inhibits cytotoxic T cell function, they are able to avoid immune surveillance. Hence, the reactivation of these cytotoxic T cells is believed to be able to bring about antitumour activity. Other approaches of immunotherapy include vaccine therapy and Chimeric Antigen Receptor (CAR)-T therapy. Vaccine therapies aim to illicit strong antitumour response through evoking the tumour-associated antigens by the immune system. CAR-T cell transfer immunotherapy, which involved the transfer of T cells to target the tumour, is currently undergoing early clinical trials for CRC treatment, and has shown success in mice models. Bispecific antibody therapy is another potential approach, as it utilises antibodies that can bind to both T cells and tumour cells to evoke T cell engagement and activity. CEA-TCB is one such bispecific antibody that is currently being studied for CRC treatment. Immunotherapy has also been suggested to be utilised in combination with other forms of therapy, including chemotherapy, radiotherapy, and epigenetic drugs to further improve the efficacy of the treatment. The combinative use of these treatments is expected to have a synergistic effect.[126,127]

Besides that, there are also studies being conducted targeting the metabolic pathways associated with the RAS and BRAF genes. As the continued proliferation of tumour cells require the aid of metabolic reactions, inhibition of these pathways is expected to provide a significant effect. Some gene mutants also hold differing characteristics, such as the KRAS-mutants that have been observed to be more metabolically vulnerable towards glyceraldehyde 3-phosphate dehydrogenase as compared to its wild-type counterpart. Certain compounds such as curcumin have also been observed to possess antitumour effects and are undergoing trials for CRC treatment.[128] The need to consider various approaches are always important, as challenges are faced in every aspect. The development of a proper and effective treatment for CRC is laced with challenges. Various approaches towards treatments must be thoroughly considered, but the diagnosis and post-treatment care must also be improved. As CRC has continued growing as an issue, more effective diagnosis methods must also be developed. The poor prognosis of CRC cases can be attributed to late diagnosis of the disease. More accurate biomarkers for CRC must be explored and studied to help with earlier diagnosis of CRC, as current accurate diagnosis including colonoscopy are rather invasive procedures.[129,130] Furthermore, post-treatment survivor care must also be considered a part of CRC treatment, as it was observed that most treatment survivors face unique problems and risks. The long-term effects of treatments such as anxiety, depression and gastrointestinal problems must be considered carefully and handled appropriately.[131,132]

Besides that, recurrence in CRC is also an issue. Almost 40% of patients that have undergone curative treatment reported recurrences.[133] Cancer tumour resistance is still among the most problematic issues in treating cancer cases, including CRC. More CRC cases are displaying

stronger resistance towards several treatments, which further fuels the need for an alternative.[134] Even among the promising treatments currently undergoing clinical trials, there is still much to be accomplished. The presence of resistance remains a bane towards the development of reliable treatments. As numerous studies have focused on the suppression on the MAPK pathway, new problems arose as some subtypes of aggressive tumour cells have been found to adapt to the suppression, gaining primary resistance through the reactivation of the MAPK pathway.[97] Furthermore, it has been found that under drug exposure, the number of mutant alleles has increased, and the mutations occurred frequently in the KRAS, NRAS, BRAF and PIK3CA genes.[135,136] These alternative mutations cause resistance towards treatment options and drive secondary resistances to develop as well. The EGFR extracellular domain has also been observed to mutate following anti-EGFR treatment, preventing the binding of further anti-EGFR drugs. An example would be the S492R EGFR mutation which has been linked to secondary resistance towards the anti-EGFR drug cetuximab.[137-139] More improvements would also have to be made to each treatment option, as although the aforementioned treatments under clinical trials have shown tremendous potential and inspiring results, there are still various concerns and shortcomings regarding each treatment. As this review focuses on KRAS and BRAF in CRC, the role of other potentially new targets such as PTEN and PIK3A in CRC is not discussed in further details. This is the limitation of current study. Future studies are warranted to explore and discuss the potential use of PTEN and PIK3A as the anticancer targets.

CONCLUSION

In conclusion, CRC remains as a plague that continues to burden our society, and the prevalence of RAS and BRAF mutations in CRC have further limited the treatment options. Numerous clinical trials are ongoing in order to come up with a new drug or therapeutic strategy in treating the CRC subtypes. Although the road to having an effective, efficient and safe treatment towards RAS and BRAF mutated CRC is a long road filled with many challenges, each success represents another step forward on this arduous journey.

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