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REVIEW ARTICLE

Precision treatment in colorectal cancer: Now and the future

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Abstract

Until recently, a one-drug-fits-all model was applied to every patient diagnosed with the same condition. But not every condition is the same, and this has led to many cases of ineffective treatment. Pharmacogenetics is increasingly used to stratify patients for precision medicine treatments, for instance, the UGT1A1*28 polymorphism as a dosage indicator for the use of irinotecan as well as epidermal growth factor receptor (EGFR) immunohistochemistry and KRAS Proto-Oncogene (*KRAS*) exon 2 mutation tests for determining the likelihood of treatment response to cetuximab or panitumumab treatment in metastatic colorectal cancer (CRC). The other molecular subtypes, such as *KRAS* exon 3/4, B-Raf Proto-Oncogene, *NRAF*, *PIK3CA*, and *PETN*, were also reported as potential new pharmacogenetic targets for the current and the newly discovered anticancer drugs. In addition to next-generation sequencing (NGS), primary tumor cells for *in vivo* and *in vitro* drug screening, imaging biomarker 3′-Deoxy-3′-18F-fluorothymidine positron emission tomography, and circulating tumor DNA (ctDNA) detection methods are being developed and may represent the future direction of precision medicine. This review will discuss the current environment of precision medicine, including clinically approved targeted therapies, the latest potential therapeutic agents, and the ongoing pharmacogenetic trials for CRC patients.

INTRODUCTION

Colorectal cancer (CRC) is the second most common cancer and cause of death across European countries. In 2012, approximately 447 000 Europeans were diagnosed, and 215 000 died from the disease.[1] Over the past few decades, patients with CRC were treated homogenously and provided with the same "standard" care. In addition to the standard colorectal surgery, the recommendation of standard drug treatment based on the tumor staging has successfully improved the treatment efficacy for CRC patients in both overall survival (OS) and disease-free survival (DFS).[2] However, not every patient's condition is the same, and decisions on treatment options made by relying solely on CRC staging is simplistic. This has likely led to many cases of ineffective treatment, adverse drug reactions, and multiple side effects.

Precision cancer treatment could be one of the possible ways to tackle this problem. Precision medicine, also known as personalized medicine, goes beyond a conventional one-drug-fits-all model to match therapy by using particular environmental, lifestyle, cancer staging, and biological characteristics to identify which approach will be most effective for a particular individual. This thereby increases his or her likelihood of response to treatment and reduces the number of adverse drug effects.[3]

Currently, there are several drugs that have been approved for CRC treatment, and a variety of pharmacogenetic tests involving biomarkers have been accepted to aid the patient selection process (Table 1). The aim of this review is to discuss the current state of precision drug treatments, including clinically approved chemotherapy drugs, molecularly targeted therapies such as anti-VEGF (vascular endothelial growth factor) and anti-EGFR (epidermal growth factor receptor) treatments, and the latest ongoing clinical trials for CRC patients.

Precision treatment and implications for early-stage CRC

There are several methods for staging CRC, including the tumour, node, and metastases (TNM) system, Dukes classification, and Astler-Coller classification. Using the most common TNM staging system, CRC can be broadly subdivided into five phases (Table 2).[4] This staging system is important because it forms the basis for decisions regarding treatment options for CRC. For example, patients with stage I CRC normally receive colonoscopic polypectomy, endoscopic mucosal resection, or endoscopic submucosal dissection as their main form of treatment, whereas those with more advanced stages require surgical resection with or without (neo)adjuvant chemotherapy.[5]

More recent research has, however, suggested that a subset of patients with stage I CRC have lymph node metastasis (LNM) and requires additional surgery.[6] Unfortunately, current best practice lacks relevant risk assessment tools, and there is no clear definition of LNM for patients classified with T1 histopathology. This results in several patients being under- or overtreated,

Table 1 Clinically approved drugs and its approved pharmacogenetic targets in colorectal cancer patients

Class of agent	Name	Biological target	Detection target[†]	U.S. FDA-approved testing kit for CRC (detection method)
Cytotoxic chemotherapy	5-FU	TS	DYPD	—
	Irinotecan	TOP1	UGT1A1*28	—
	Oxaliplatin	—	—	—
	Raltitrexed[‡]	TS	—	—
	Lonsurf (trifluridine/tipiracil)	TS	—	—
VEGF	Bevacizumab	VEGF-A	—	—
	Ziv-aflibercept	VEGF-A	—	—
	Ramucirumab	VEGFR-2	—	—
	Regorafenib	Series of protein kinases[§]	—	—
EGFR	Cetuximab	EGFR	1. EGFR	1. DAKO EGFR PharmDx Kit (IHC)
	Panitumumab	EGFR	2. *KRAS* exon 2 3 & 4	2. cobas® KRAS Test (qPCR)
				3. *therascreen* KRAS Test (qPCR)

[†]U.S. FDA-approved pharmacogenomic biomarkers on drug labeling.

[‡]NICE UK-approved drug.

[§]Regorafenib targeted proteins are VEGF receptors 1–3, TIE2, KIT, RET, *RAF1*, *BRAF* V600E, PDGFR, and FGFR.

DYPD, Dihydropyrimidine Dehydrogenase [NADP(+)]; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; qPCR, quantitative reverse transcription polymerase chain reaction; TOP1, Topoisomerase 1; TS, thymidylate synthase; VEGF, vascular endothelial growth factor.

causing unnecessary treatment side effects and excess morbidity.[7] The use of biomarkers may aid in the further subclassification of this set of patients. One study has shown that EZR is a potential biomarker for LNM and that this may guide decisions about the need for further surgery.[8] A panel of five biomarkers—BMI, ETV6, H3F3B, RPS10, and VEGFA—was also shown to outperform clinicopathological prognostic factors for node-negative CRC.[9]

Current best practice recommends adjuvant chemotherapy for patients with stage II CRC and high-risk clinicopathological features, but there is also no consensus on how to define the high-risk characteristics.[10] Several molecular assays, such as ColoPrint and Oncotype DX, offer additional means for analyzing patients' risk of recurrence. In the Prospective Analysis of Risk Stratification by ColoPrint (PARSC) study (NCT00903565), relapse rates in stage II CRC were evaluated, and it was demonstrated that ColoPrint may improve the prognostic accuracy beyond the clinical variables and microsatellite instability (MSI) status.[11] The Oncotype DX Colon Cancer assay, which utilizes quantitative polymerase chain reaction (qPCR) to measure 12 biomarkers (seven cancer-related—BGN, C-MYC, FAP, GADD45B, INHBA, Ki-67, MYBL2; 5 reference genes—ATP5E, GPX1, PGK1, UBB, VDAC2), produces a score from 0 to 100, which represents the predicted recurrence risk to inform decisions regarding adjuvant chemotherapy for CRC patients.[12,13] It has been shown to predict recurrence risk more accurately than when using T-stage and mismatch repair status alone (NCT01479894).[13] Studies have also shown that other biomarkers, such as a lack of *CDX2* expression, may offer further insight into the subgroup of patients with high-risk stage II CRC who benefit from receiving adjuvant chemotherapy (5-year DFS: 91% *vs* 56%; $P = 0.006$).[14]

Chemotherapy drugs for precision treatment

Cytotoxic agents such as 5-fluorouracil (5-FU), irinotecan, and oxaliplatin are commonly used as chemotherapy agents for CRC treatment. However, a proportion of CRC patients does not respond to this chemotherapy regimen and/or suffer from severe drug toxicities. 5-FU is a widely used thymidylate synthase (TS) inhibitor that acts as an antimetabolite to block the pyrimidine thymidine synthesis required for DNA replication.[15] In the early years, studies demonstrated that high-frequency microsatellite instability (MSI-H), due to loss of DNA mismatch repair function, is correlated with poor response to 5-FU-based treatment compared to CRC patients with stable microsatellites.[16,17] Controversially, negative results were also reported by the other researchers.[18] The latest systematic review with meta-analysis summarized fourteen 5-FU-based trials and concluded that MSI status has a limited effect on both DFS and OS and is therefore not valuable in guiding 5-FU-based treatment selection.[19] Dihydropyrimidine dehydrogenase ([$NADP^+$], DYPD)—a pyrimidine catabolic enzyme that metabolizes thymine (T) and uracil (U) nucleotides—was later discovered and enables the identification of the 3% of CRC patients who cannot sufficiently metabolize 5-FU. Patients with DYPD deficiency could experience severe 5-FU-related toxicities.[20] Further research found that the DPYD variants DPYD*2A (relative risk: 2.9, $P < 0.0001$), c.1679 T > G (relative risk: 4.4, $P < 0.0001$), c.1236G > A/HapB3 (relative risk: 1.6, $P < 0.0001$), and c.2846A > T (relative risk: 3.0, $P < 0.0001$) are clinically relevant as predictors of fluoropyrimidine-associated intolerance.[21] A prospective trail proved that DPYD*2A-guided 5-FU dosing has significantly reduced the incidence of severe toxicity in DPYD*2A carriers,

Table 2 TNM staging system of colorectal cancer (AJCC 8th edition)

Stage	T (primary tumour)	N (regional lymph nodes)	M (distant metastasis)
0	Tis	N0	M0
I	T1–T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1–T2	N1 or N1c	M0
	T1	N2a	M0
IIIB	T3–T4a	N1 or N1c	M0
	T2–T3	N2a	M0
	T1–T2	N2b	M0
IIIC	T4a	N2a	M0
	T3–T4a	N2b	M0
	T4b	N1–N2	M0
IVA	Any T	Any N	M1a
IVB	Any T	Any N	M1b
IVC	Any T	Any N	M1c

Primary tumour (T): Tx, primary tumour of unknown; T0, no evidence of primary tumour; Tis, carcinoma *in situ*; T1, tumour invades submucosa; T2, tumour invades muscularis propria; T3, tumour invades through the muscularis propria into the peri colorectal tissues; T4a, tumour invades through the visceral peritoneum; T4b: tumour directly invades or adheres to other adjacent organs or structures.
Regional lymph nodes (N): Nx, lymph nodes cannot be assessed; N0, no lymph node metastases; N1, 1–3 lymph node involvement; N1a, 1 lymph node; N1b, 2–3 lymph nodes; N1c, non-nodal tumour deposits without identified lymph node metastases; N2, 4 or more lymph node involvement; N2a: 4–6 lymph nodes; N2b: 7 or more lymph nodes.
Distant metastasis (M): Mx, distant metastasis cannot be assessed; M0, no distant metastasis by imaging; M1, distant metastasis; M1a, metastasis to one organ or site without peritoneal metastasis; M1b, metastasis to two or more organs or sites without peritoneal metastasis; M1c, peritoneal involvement regardless of other organ involvement.

from 73 to 28% ($P < 0.001$).[22] Although DPYD pretreatment screening has been proven to improve drug safety for DPYD*2A carriers by the Food and Drug Administration (FDA) in the United States, the current European Society for Medical Oncology (ESMO) guidelines do not "routinely recommend" upfront genotyping of DPYD*2A before the administration of 5-FU in metastatic CRC (mCRC) patients.[23] This recommendation is now being reviewed.[24]

Irinotecan is a topoisomerase 1 (TOP1) inhibitor that has a specific pharmacodiagnostic test.[25] Clinical studies demonstrated that the inhibition of TOP1 by irinotecan blocks the DNA ligation process during the cell cycle. However, CRC patients with uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) deficiency cannot sufficiently excrete the active metabolite SN-38, which primarily undergoes glucuronidation in their livers.[26] As a result, a high dose of irinotecan in UGT1A1-deficient CRC patients is associated with severe adverse drug responses such as neutropenia and diarrhea.[27] This has been confirmed by other studies and verified by a meta-analysis.[28] Therefore, the U.S. FDA has recommended a dose reduction of irinotecan for patients with homozygous UGT1A1*28 based on A(TA-6)TAA and A(TA-7)TAA genotyping.[29] Clinical trials focusing on the other UGT1A1 gene polymorphisms, such as UGT1A1*1 (ClinicalTrials.gov Identifier: NCT01639326 and NCT02138617) and UGT1A1*6 (NCT02497157), are still ongoing.

Similar to 5-FU and irinotecan, oxaliplatin is another common antineoplastic agent to which there are varying levels of chemo resistance in CRC patients.[30] The treatment efficacy of this platinum-based regimen can be modulated by excision repair cross-complementing group 1 (ERCC1)—one of the ERCC1-XPF enzyme complexes that play a crucial role in the nucleotide excision and repair (NER) pathway for DNA recombination and DNA repair.[31] In particular, ERCC1-C118T (T/T or T/C) polymorphism[32] or a lower expression of ERCC1[33] has been reported as being associated with unfavorable prognosis in patients undergoing treatment with oxaliplatin. It has therefore been proposed as a surrogate biomarker for oxaliplatin resistance. However, clinical trials have not demonstrated the predictive ability of ERCC1 in oxaliplatin-based treatment.[34] Thus, EMSO has not recommended ERCC1 testing prior to the use of oxaliplatin in routine practice.[23]

More recently, a new cytotoxic drug, lonsurf, was approved by the U.S. FDA, National Institute of Health and Care Excellence (NICE) in England, and the European Medicines Agency (EMA) for refractory mCRC patients. Lonsurf is a combination of trifluridine (thymidine-based nucleoside analogue) and tipiracil (a potent thymidine phosphorylase inhibitor) that suppresses cancer cell proliferation by interfering with DNA synthesis.[35] Based on the RECOURSE group's phase III randomized trial, which included nearly 800 participants from three different geographical areas, lonsurf results in a 1.8-month improvement in median OS compared with the placebo group.[36] Methods for optimizing lonsurf treatment are currently under investigation, including the development of a CRC xenograft experimental model that predicts treatment outcome;[37] the use of 3′-Deoxy-3′-18F-fluorothymidine positron emission tomography ([^{18}F]FLT-PET) as a noninvasive radio-traceable substitute for thymidine; and using the MSI status as an indicator for the use of lonsurf in combination with nivolumab, a PD-1 inhibitor, in refractory mCRC patients (NCT02860546).

EGFR therapies

EGFR is a transmembrane tyrosine kinase receptor that regulates the serine/threonine-specific protein kinase (AKT), JNK, and mitogen-activated protein kinase (MAPK)/ERK signaling pathways responsible for DNA synthesis, cell proliferation, apoptosis, and motility (Fig. 1). Overexpression of EGFR is associated with tumor progression in various cancer types, including CRC.[38] Blocking the EGFR by using monoclonal antibodies such as cetuximab or panitumumab[39,40] with a chemotherapy formula combination with 5-FU, leucovorin plus oxaliplatin (FOLFOX) or a chemotherapy formula combination with 5-FU, leucovorin plus irinotecan (FOLFIRI) results in a better treatment response in mCRC patients.[41,42] Those treatments can be tailored using one of the FDA-approved pharmacogenetic tools that measure a patient's EGFR expression level[43] or detect KRAS Proto-Oncogene (*KRAS*) exon 2 (codon 12/13) mutations[44] (Table 1). However, the effectiveness of these pharmacogenetic tests in detecting and improving treatment response is uncertain. For

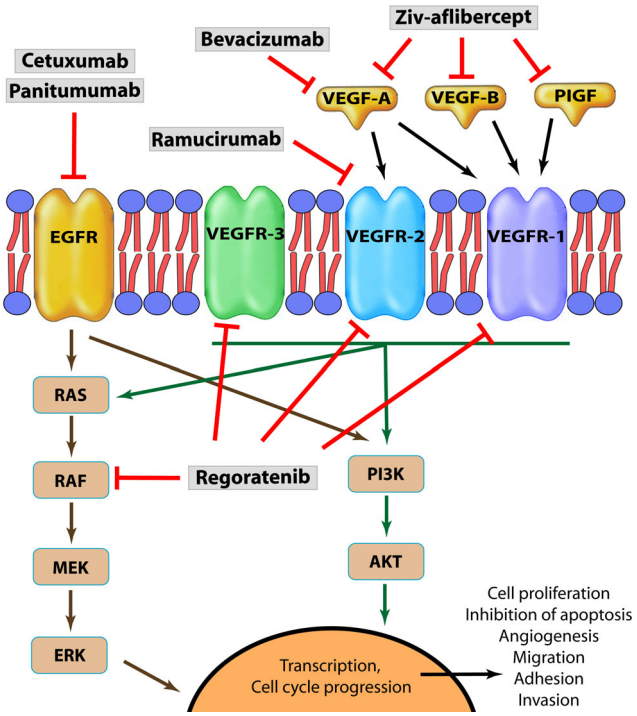

Figure 1 The approved EGFR and VEGF targeted drugs and its receptors in colorectal cancer. AKT, protein kinase B; EGFR, epidermal growth factor receptor; ERK, extracellular-regulated kinase; MEK, mitogen-activated protein/extracellular signal-regulated kinase; PI3K, phosphatadylinositol 3-kinase; PIGF, placental growth factor; *RAF*, rapidly accelerated fibrosarcoma; *RAS*, retrovirus-associated DNA sequences; VEGFR, vascular endothelial growth factor receptor

example, many pathologists have expressed concern about the EGFR detection criteria in the PharmDx™ immunohistochemistry (IHC) test.[45,46] Many clinicians also doubt the benefits of anti-EGFR treatment in EGFR-positive CRC patients.[45,47] The alternative option of *KRAS* exon 2 mutation screening is also problematic because testing is limited to one *KRAS* exon region, and studies have shown that CRC patients with other KRAS mutations will still benefit from anti-EGFR treatment.[48] In fact, up to 35% of *KRAS* exon 2 wild-type[49] and approximately 25% of EGFR-negative patients responded to EGFR inhibitor treatments.[50] Therefore, other *RAS* signaling biomarkers, such as *KRAS* exon 3 (codons 59/61) and 4 (codons 117/146), as well as NRAS proto-oncogene (*NRAS*) exon 2 (codon 12/13), 3 (codons 59/61) and 4 (codons 117/146) mutations, are being investigated for further pharmacodiagnostic development.[51–53]

In a retrospective analysis of the CRYSTAL study, authors assessed the status of other *RAS* mutations (*KRAS* exons 3 and 4; *NRAS* exons 2, 3 and 4). Of the 367 *RAS* wild-type CRC patients, treatment with FOLFIRI plus cetuximab was better than FOLFIRI alone in both PFS (11.4 *vs* 8.4 months, HR: 0.56 $P < 0.001$) and OS (28.4 *vs* 20.2 months, HR: 0.69 $P = 0.0024$). There was no difference in the other *RAS* mutant populations ($n = 63$).[54] Similar results were also reported in another phase III trial for a second-line therapy based on *RAS* mutation status (*KRAS* exons 3, 4; *NRAS* exons 2, 3, 4; and *BRAF* exon 15). The use of FOLFIRI with or without panitumumab in the wild-type *RAS* population improved survival in mCRC patients (PFS: 6.4 *vs* 4.6 months, HR: 0.70, $P = 0.007$) compared with the *KRAS* exon 2 wild-type individuals (PFS: 5.9 *vs* 3.9 months, HR: 0.73, $P = 0.004$).[55] Based on the published results of the *RAS* mutation combination analysis, the ESMO,[56] European Society of Pathology (ESP), and Association of Clinical Pathologists Molecular Pathology and Diagnostics Group in the United Kingdom recommended the *KRAS*/*NRAS* mutation test for mCRC patients.[57]

In addition to the *RAS* mutation, other potential biomarkers have been uncovered and may help in the selection of CRC patients suitable for anti-EGFR treatment. These biomarkers include *PIK3CA*, PTEN, Human Epidermal Growth Factor Receptor 2 (HER2), HER3, and the EGFR ligands EREG and AREG.[53,58–61] Although these biomarkers are not yet available for clinical use, the combination of multiple biomarkers may have a stronger predictive power than using one alone.[58] Further prospective studies are needed to substantiate predictive biomarker combinations for EGFR-targeted treatment (Table 3).

VEGF receptor therapies

The VEGF receptor is a transmembrane protein containing a split tyrosine–kinase domain at the intracellular level and seven immunoglobulin-like domains at extracellular levels for angiogenesis and vasculogenesis.[62] Overexpression of VEGF results in tumor progression and metastasis as well as lower patient survival rates.[63,64] Today, three approved biological agents targeting VEGF are available for CRC patients. Ramucirumab targets the VEGF-A receptor activation by modulating VEGFR-2; ziv-aflibercept inhibits placental growth factor (PIGF), VEGF-A, and VEGF-B by using its IgG1 Fc-VEGFR; and bevacizumab blocks VEGF-A to cause ligand sequestering (Fig. 1).[65] Interestingly, the use of FOLFIRI in combination with ziv-aflibercept (VELOUR trial),[66] bevacizumab (ML18147 trial),[67] or ramucirumab (PRAISE trial)[68] in mCRC patients presented similar treatment benefits in median OS (1.4, 1.4, and 1.6 months) and PFS (2.2, 1.6 and 1.2 months). All three antiangiogenic regimens also present with similar types of adverse drug events (e.g. proteinuria, hemorrhage, and hypertension).[69] However, the differences in tolerability and the study design in those clinical trials vary.[70]

Although no obvious difference was found between the approved VEGF-targeted treatments, they also do not directly replace each other due to the different VEGF subtype targets (Fig. 1) and the treatment effectiveness in patient-derived xenograft mouse models.[71] Hence, an ongoing PERMAD phase II trial (NCT02331927) is investigating potential cytokine and/or angiogenic factor(s) as biomarker(s) for a treatment shift from bevacizumab to ziv-aflibercept to increase the treatment effectiveness and limit drug resistance. Furthermore, studies also found that the continuous administration of bevacizumab leads to better OS[67,72] as planned treatment breaks or discontinuation in antiangiogenic therapy could lead to rapid tumor regrowth.[73,74] To monitor the tumor growth and treatment response, the CIRCUS research team is prospectively evaluating circulating VEGFR-2 levels as a predictor of the continuation of bevacizumab treatment in mCRC patients (NCT02623621). Several

Table 3 Ongoing clinical trials for molecular biomarkers in approved CRC drugs

Drug	Biomarker	ClinicalTrials.gov identifier:
Bevacizumab + chemotherapy	VEGFR-2	NCT02623621
Bevacizumab/cetuximab + FOLFIRI	*BRAF* & PIK3K in *RAS* wild-type mCRC	NCT01640444
Bevacizumab, cetuximab + irinotecan	*KRAS* wild-type, Irinotecan refractory	NCT02292758
Cetuximab + FOLFIRI/mFOLFOX6	ERCC1	NCT01703390
Cetuximab or panitumumab	EGFR domain III region	NCT01726309
Panitumumab + FOLFIRI	*RAS* & *BRAF* wild-type mCRC	NCT02508077
Regorafenib	[18F] FLT-PET	NCT02175095
Regorafenib	RAS-mutant advanced CRC	NCT02619435
Ziv-aflibercept	Cytokines & angiogenic factors	NCT02331927

[18F] FLT-PET, 3′-deoxy-3′-18F-fluorothymidine positron emission tomography; mCRC, metastatic colorectal cancer.

potential new biomarkers have also been reported for VEGF inhibitors, including *KRAS* (codons 12 and 13),[75] VEGF(165)b: VEGF(total) expression ratio,[76] VEGF-D,[77] miR-126,[78] EGFL7,[79] Ang-2,[80] NRP-1,[81] IL-8,[82] and G12 V and G12A *KRAS* mutations.[83] However, prospective studies are necessary to verify the results.

In addition to the VEGF single-targeting agents, regorafenib is a dual-targeted VEGFR2-TIE2 tyrosine kinase inhibitor that suppresses a set of protein kinases involved in oncogenesis (B-Raf Proto-Oncogene [*BRAF*], RAF1, RET and KIT) and angiogenesis (tyrosine receptor kinase-2 [TIE2], VEGFR 1–3, fibroblast growth factor receptor [FGFR] and platelet-derived growth factor receptor [PDGFR]).[84] mCRC patients who received regorafenib treatment demonstrated a statistically significant improvement in survival rate when compared with placebo in the CORRECT (OS: 6.4 *vs* 5.0 months, HR = 0.77, $P = 0.0052$; PFS: 1.9 *vs* 1.7 months, HR = 0.49, $P < 0.0001$)[85] and CONCUR (OS: 8.8 *vs* 6.3 months, HR = 0.55, $P = 0.0016$) trials.[86] Several clinical studies on regorafenib are ongoing to find suitable biomarkers to stratify CRC patients.[85,87] This includes identifying *RAS* subtypes (NCT02619435), as well as using imaging biomarkers such as [^{18}F] FLT-PET (NCT02175095) (Table 3). Several clinical trials investigating biomarkers for regorafenib in mCRC patients who failed one prior anticancer treatment are ongoing (NCT01949194, NCT01996969, and NCT02402036).

The development of new molecular targeted therapy in CRC

The development of new molecular targeted therapy in CRC and investigations into their use in combination are ongoing. For instance, selumetinib, a MEK1 and MEK2 inhibitor,[88] in combination with afatinib, an approved EGFR inhibitor for non-small cell lung carcinoma,[88] is currently being tested in an early-stage randomized clinical trial for *KRAS* mutant and *PIK3CA* wild-type CRC patients (NCT02450656) (Table 4). Dual anti-EGFR and anti-VEGF treatments for CRC are also being studied. For example, the use of cetuximab plus regorafenib inhibited AKT and MAPK signaling pathways in *BRAF*-mutated, *KRAS*-mutated, and cetuximab-resistant CRC cell lines and presented a synergistic apoptotic as well as antiproliferative effect in an *in vivo* model.[89] This combination was proven and well tolerated in the phase I clinical trial, and the antitumor effect may greatly benefit MSI-H CRC patients.[90] The next phase of the trial may be conducted in the near future.

More recently, monoclonal antibodies against programed cell death-1 (PD-1) receptor or its ligand PD-L1 have shown promising results in several types of cancers. PD-1 is an immune checkpoint protein expressed on the surface of T-cells and plays a key role in promoting self-tolerance by suppressing T-cell cytokine production. PD-L1 is frequently upregulated in tumor cells

Table 4 Ongoing clinical trials for new CRC drugs and their respective biomarkers

Target molecule	Drug name	Biomarker	Trial phase	ClinicalTrials.gov identifier
AKT	Trametinib	*BRAF* mutant	I/II	NCT01902173
	GSK2141795	*BRAF* mutant	I/II	NCT01902173
BRAF	Dabrafenib	*BRAF* mutant	I/II	NCT01902173
cMET	Tivantinib	*KRAS* wild-type	II	NCT01892527
	PF-02341066	*RAS* mutant & over-active MET	I/II	NCT02510001
Glutaminase	CB-839	Fluoropyrimidine Resistant & *PIK3CA* mutant	I/II	NCT02861300
HER2	Ado-Trastuzumab Emtansine	HER2	I/II	NCT02465060
PD-1	Pembrolizumab	*KRAS*, *BRAF* & *NRAS* wild-type	II	NCT02318901
		MSI status	III	NCT01876511, NCT02563002
	Nivolumab	MSI status	II	NCT02860546, NCT03104439
MEK	Selumetinib	*KRAS* mutant & *PIK3CA* wild-type	II	NCT02450656, NCT02586987
	PD-0325901	*RAS* mutant & over-active MET	I/II	NCT02510001
Tyrosine Kinase	Entrectinib	NTRK1/2/3, ROS1, & ALK gene fusion	II	NCT02568267
PI3K	BKM120	*RAS* wild-type	I/II	NCT01304602, NCT01591421

BRAF, B-Raf proto-oncogene; HER2, human epidermal growth factor receptor 2; *NRAS*, NRAS proto-oncogene.

and deactivates antitumor activity in cytotoxic T-cells.[91,92] Research has shown that CRC with MSI highly expresses immune checkpoint molecules, including PD-L1.[93] Thus, in a phase II clinical trial, pembrolizumab, a U.S. FDA-approved PD-1 targeted therapy, was utilized to treat both MSI-H and microsatellite-stable (MSS) CRC patients. The response rate and the 12-week PFS to pembrolizumab in MSI-H mCRC patients (n = 10) were 40 and 78% compared to 0 and 11% in MSS mCRC patients (n = 18), respectively.[94] Combination treatment with pembrolizumab and itacitinib, a JAK1 inhibitor, is also under investigation for use in any patient with MSI instability (NCT02646748). In addition to the clinical trials stratifying treatment based on MSI status (NCT01876511 and NCT02563002), treatment for different molecular subtypes such as pembrolizumab plus trastuzumab treatment for mCRC patients with *KRAS*, *BRAF*, and *NRAS* wild-types (NCT02318901) are under investigation (Table 3).

Future directions and conclusions

Patients with the "same" cancer often respond differently to treatment—this challenge has baffled medical oncologists for decades. Pharmacodiagnostic testing is now becoming an essential tool for selecting the right medication for the right patient. Since the U.S. FDA approved next-generation sequencing (NGS) devices for clinical diagnosis in November 2013,[95] the use of NGS has become a popular tool for the investigation of diseases. For example, NGS was used by Hagemann *et al*. in patients with non-small cell lung cancer to match 11% of their patients with a targeted therapy.[96] NGS can also be applied to noninvasively detect circulating tumor DNA (ctDNA) in CRC patients for real-time monitoring of the disease, facilitating early identification of disease progression.[97] For instance, *KRAS* mutant alleles can be detected in blood plasma from the acquired tumor-resistant patients 10 months before cancer progression is otherwise detected.[98] This is because genetic aberrations coding treatment resistance accumulate during tumor progression and are released from tumor cells into the blood circulation.[99] Another effective method to improve treatment selection was demonstrated by Pauli *et al*.,[100] where tumor tissue collected from a patient was subjected to four separate experiments: (i) NGS for molecular subtype analysis, (ii) primary cell culture, (iii) patient-derived xenograft (PDX) models, and (iv) patient-derived tumor organoids. This cutting-edge screening strategy facilitated precision treatment, but the process itself is costly and therefore may not currently be affordable to the wider public.

In conclusion, the aim of precision medicine is to develop a tailored treatment for each individual and his or her unique condition to maximize potential treatment response and minimize adverse drug reactions. The stratification of patients through the use of biomarkers is thus key. As the use of newer therapeutic agents connected with specific genetic sup-type(s) will increase, ultimately increasing patients' quality of life and life expectancy.

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