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.

Key words

chemotherapy, colorectal cancer, epidermal growth factor receptor, personalized medicine, precision treatment, vascular endothelial growth factor.

Accepted for publication 14 January 2019.

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Declaration of conflict of interest: The authors declare no conflict of interest.

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, NRAS, 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.
causing unnecessary treatment side effects and excess morbidity. 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. A panel of five biomarkers—BMI, ETV6, H3F3B, RPS10, and VEGFA—was also shown to outperform clinicopathological prognostic factors for node-negative CRC.

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. 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. The Oncotype DX Colon Cancer assay, which utilizes quantitative polymerase chain reaction (qPCR) to measure 12 biomarkers (seven cancer-related kinases), produces a score from 0 to 100, which represents the predicted recurrence risk to inform decisions regarding adjuvant chemotherapy for CRC patients. It has been shown to predict recurrence risk more accurately than when using T-stage and mismatch repair status alone (NCT01479894). 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%; \( P = 0.006 \)).

### 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. 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. Controversially, negative results were also reported by the other researchers. 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. 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. Further research found that the DYPD 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.

| Class of agent | Name | Biological target | Detection target\(^a\) | U.S. FDA-approved testing kit for CRC (detection method) |
|----------------|------|-------------------|------------------------|----------------------------------------------------------|
| Cytotoxic chemotherapy | 5-FU | TS | DYPD | — |
| | Iринотекан | TOP1 | UGT1A1*28 | — |
| | Оксалиплатин | — | — | — |
| | Ратитрексел | TS | — | — |
| | Лонсуф | TS | — | — |
| | (трифлуронилтипринил) | — | — | — |
| VEGF | Бевакизумаб | VEGF-A | — | — |
| | Зив-афелиберсепт | VEGF-A | — | — |
| | Рамкуримаб | VEGFR-2 | — | — |
| | Регорafenеб | Series of protein kinases\(^b\) | — | — |
| EGFR | Цетрümаб | EGFR | 1. EGFR | 1. DAKO EGFR PharmDx Kit (IHC) |
| | Панитумумаб | EGFR | 2. KRAS exon 23 & 4 | 2. cobas® KRAS Test (qPCR) |
| | — | — | 3. therascreen KRAS Test (qPCR) |

\( ^a\)U.S. FDA-approved pharmacogenomic biomarkers on drug labeling.
\(^b\)NICE UK-approved drug.

\(^c\)Regorafeneb targeted proteins are VEGF receptors 1–3, TIE2, KIT, RET, RAF1, BRAF V600E, PDGFR, and FGFR.

**DPYD**, 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.
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*1/*1 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 ERCC133 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, ESMO 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 RECURSE 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 ([18F]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 panitumumab39,40 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 pharmacogeneic tools that measure a patient’s EGFR expression level43 or detect KRAS Proto-Oncogene (KRAS) exon 2 (codon 12/13) mutations44 (Table 1). However, the effectiveness of these pharmacogeneic tests in detecting and improving treatment response is uncertain. For

| Stage | T (primary tumour) | N (regional lymph nodes) | M (distant metastasis) |
|-------|-------------------|--------------------------|------------------------|
| 0     | Tis               | N0                       | M0                     |
| I     | T1–T2             | N0                       | M0                     |
| II    | T3                | N0                       | M0                     |
| IIa   | T4a               | N0                       | M0                     |
| IIb   | T4b               | N0                       | M0                     |
| III   | T1–T2             | N1 or N1c                | M0                     |
| IIIa  | 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                    |
example, many pathologists have expressed concern about the EGFR detection criteria in the PharmDx immunohistochemistry (IHC) test.\textsuperscript{45,46} Many clinicians also doubt the benefits of anti-EGFR treatment in EGFR-positive CRC patients.\textsuperscript{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.\textsuperscript{48} In fact, up to 35\% of KRAS exon 2 wild-type\textsuperscript{49} and approximately 25\% of EGFR-negative patients responded to EGFR inhibitor treatments.\textsuperscript{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.\textsuperscript{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, \textit{P} < 0.001) and OS (28.4 vs 20.2 months, HR: 0.69, \textit{P} = 0.0024). There was no difference in the other RAS mutant populations (\textit{n} = 63).\textsuperscript{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 NRAS exon 5; 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, \textit{P} = 0.007) compared with the KRAS exon 2 wild-type individuals (PFS: 5.9 vs 3.9 months, HR: 0.73, \textit{P} = 0.004).\textsuperscript{55} Based on the published results of the RAS mutation combination analysis, the ESMO,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{62} Overexpression of VEGF results in tumor progression and metastasis as well as lower patient survival rates.\textsuperscript{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 placentation 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).\textsuperscript{65} Interestingly, the use of FOLFIRI in combination with ziv-aflibercept (VELOUR trial),\textsuperscript{66} bevacizumab (ML18147 trial),\textsuperscript{67} or ramucirumab (PRAISE trial)\textsuperscript{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).\textsuperscript{69} However, the differences in tolerability and the study design in those clinical trials vary.\textsuperscript{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.\textsuperscript{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\textsuperscript{72,73} as planned treatment breaks or discontinuation in antiangiogenic therapy could lead to rapid tumor regrowth.\textsuperscript{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
potential new biomarkers have also been reported for VEGF inhibitors, including KRAS (codons 12 and 13),\textsuperscript{75} VEGF(165)b: VEGF(total) expression ratio,\textsuperscript{76} VEGF-D,\textsuperscript{77} miR-126,\textsuperscript{78} EGFL7,\textsuperscript{79} Ang-2,\textsuperscript{80} NRP-1,\textsuperscript{81} IL-8,\textsuperscript{82} and G12 V and G12A KRAS mutations.\textsuperscript{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]).\textsuperscript{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)\textsuperscript{85} and CONCUR (OS: 8.8 vs 6.3 months, HR = 0.55, P = 0.0016)\textsuperscript{86} trials. Several clinical studies on regorafenib are ongoing to find suitable biomarkers to stratify CRC patients.\textsuperscript{85,87} This includes identifying RAS subtypes (NCT02619435), as well as using imaging biomarkers such as \textsuperscript{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,\textsuperscript{88} in combination with afatinib, an approved EGFR inhibitor for non-small cell lung carcinoma,\textsuperscript{89} 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.\textsuperscript{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.\textsuperscript{90} The next phase of the trial may be conducted in the near future.

More recently, monoclonal antibodies against programmed 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 cytolysis. PD-L1 is frequently upregulated in tumor cells

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

| Drug | Biomarker | ClinicalTrials.gov identifier |
|------|-----------|-----------------------------|
| Bevacizumab + chemotherapy | VEGF-R2 | NCT02623621 |
| Bevacizumab/cetuximab + FOLFIRI | B-RAF & PIK3K in RAS wild-type mCRC | NCT01640444 |
| Bevacizumab, cetuximab + irinotecan | KRAS wild-type, Irinotecan refractory | NCT02292758 |
| Cetuximab + FOLFIRI/FOLFIRX | 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 |

\[^{18}F\] FLT-PET, 3'-deoxy-3'-18F-fluorothymidine positron emission tomography; mCRC, metastatic colorectal cancer.

### 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 | Fluropirimidine Resistant & PIK3CA mutant | II | NCT02861300 |
| HER2 | Ado- Trastuzumab Emtansine | HER2 | II | NCT02465060 |
| PD-1 | Pembrolizumab | KRAS, BRAF & NRAS wild-type | II | NCT02318901 |
| | Nivolumab | MSI status | III | NCT01876511, NCT02563002 |
| MEK | Selumetinib | KRAS mutant & PIK3CA wild-type | II | NCT02450656, NCT02586987 |
| | PD-0325901 | RAS mutant & over-active MET | 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-L1 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 predictive biomarker for lymph node metastasis in colorectal cancer using a proteomic approach. Oncotarget. 2017; 8: 106935–47. Molloy MP, Engel A. Precision medicine beyond medical oncology: using molecular analysis to guide treatments of colorectal neoplasia. Expert Rev. Gastroenterol. Hepatol. 2018; 12: 1179–81.

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.

REFERENCES

1 Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur. J. Cancer. 2013; 49: 1374–403.

2 Wilkinson NW, Youthers G, Lopa S, Costantino JP, Petrelli NJ, Wolmark N. Long-term survival results of surgery alone versus surgery plus 5-fluorouracil and leucovorin for stage II and stage III colon cancer: pooled analysis of NSABP C-01 through C-05. A baseline from which to compare modern adjuvant trials. Ann. Surg. Oncol. 2010; 17: 959–66.

3 Verma M. Personalized medicine and cancer. J. Pers. Med. 2012; 2: 1–14.

4 Amin MB, Greene FL, Edge SB et al. The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more “personalized” approach to cancer staging. CA Cancer J. Clin. 2017; 67: 93–9.

5 McQuade RM, Stojanovska V, Bornstine JC, Nurgali K. Colorectal cancer chemotherapy: the evolution of treatment and new approaches. Curr. Med. Chem. 2017; 24: 1537–57.

6 Sohn DK, Chang HJ, Park JW et al. Histopathological risk factors for lymph node metastasis in submucosal invasive colorectal carcinoma of pedunculated or semipedunculated type. J. Clin. Pathol. 2007; 60: 912–5.

7 Glynn-Jones R, Wyrwicz L, Tiret E et al. Rectal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann. Oncol. 2017; 28(Suppl. 4): iv22–40.

8 Morii K, Toiyama Y, Otake K et al. Successful identification of a predictive biomarker for lymph node metastasis in colorectal cancer using a proteomic approach. Oncotarget. 2017; 8: 106935–47.

9 Morgant M, Engel A. Precision medicine beyond medical oncology: using molecular analysis to guide treatments of colorectal neoplasia. Expert Rev. Gastroenterol. Hepatol. 2018; 12: 1179–81.

10 Kannarkatt J, Josep J, Kurniali PC, Al-Janadi A, Hrinczenko B. Adjuvant chemotherapy for stage II colon cancer: a clinical dilemma. J. Oncol. Pract. 2017; 13: 233–41.

11 Kopetz S, Tabernero J, Rosenberg R et al. Genomic classifier ColoPrint predicts recurrence in stage II colorectal cancer patients more accurately than clinical factors. Oncologist. 2015; 20: 127–33.

12 You YN, Rustin RB, Sullivan JD. Oncotype DX® colorectal cancer assay for prediction of recurrence risk in patients with stage II and III colon cancer: a review of the evidence. Surg. Oncol. 2015; 24: 61–6.

13 Bailey H, Turner M, Stoppler MC, Chao C. The 12-gene oncotype DX colorectal cancer recurrence score (RS) test: experience with >20,000 stage 2 patients (pts). J. Clin. Oncol. 2018; 36(4 Suppl): 618–18.

14 Dalerba P, Sahoo D, Paik S et al. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N. Engl. J. Med. 2016; 374: 211–22.

15 Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil, and leucovorin in stage III colon cancer: a clinical dilemma. J. Clin. Oncol. 2009; 27: 1814–21.

16 Des Guet G, Schischmanoff O, Nicolaus P, Perret G-Y, Moreere J-F, Uzzan B. Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis. Eur. J. Cancer. 2009; 45: 1890–6.

17 Sargent DJ, Shi Q, Youthers G et al. Prognostic impact of deficient mismatch repair (dMMR) in 7,803 stage II/III colon cancer (CC) patients (pts): a pooled individual data analysis of 17 adjuvant trials in the ACCENT database. J. Clin. Oncol. 2014; 32 (15 suppl): 3507.

18 Webber EM, Kauffmann TL, O’Connor E, Goddard KA. Systematic review of the predictive effect of MSI status in colorectal cancer patients undergoing 5FU-based chemotherapy. BMC Cancer. 2015; 15: 156.

19 Sanoff HK, McLeod HL. Predictive factors for response and toxicity in chemotheray: pharmacogenomics. Semin. Colon Rectal. Surg. 2008; 19: 226–30.
Meulendijks D, Henricks LM, Sonke GS et al. Clinical relevance of DPYD variants c.1679T>G, c.1236G>A/HapB3, and c.1610G>A as predictors of severe fluoropyrimidine-associated toxicity: a systematic review and meta-analysis of individual patient data. Lancet Oncol. 2015; 16: 1639–50.

Deenen MJ, Meulendijks D, Cats A et al. Upfront genotyping of DPYD*2A to individualize fluoropyrimidine therapy: a safety and cost analysis. J. Clin. Oncol. 2016; 34: 227–34.

Van Cutsem E, Cervantes A, Adam R et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann. Oncol. 2016; 27: 1386–422.

Deenen MJ, Meulendijks D. Recommendation on testing for dihydropyrimidine dehydrogenase deficiency in the ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann. Oncol. 2017; 28: 184.

Ratain MJ. From bedside to bench to bedside to clinical practice: an odyssey with irinotecan. Clin. Cancer Res. 2006; 12: 1658–60.

Iyer L, King CD, Whittington PF et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isofrm 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J. Clin. Invest. 1998; 101: 847–54.

Innocenti F. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J. Clin. Oncol. 2004; 22: 1382–8.

Campbell JM, Stephenson MD, Bateman E, Peters MJD, Keefe DM, Bowen JM. Irinotecan-induced toxicity pharmacogenetics: an umbrella review of systematic reviews and meta-analyses. Pharmacogenomics J. 2017; 17: 21–8.

CAMPTOSAR. CAMPTOSAR-irinotecan hydrochloride injection, solution, 2016.

Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. New Engl. J. Med. 2005; 352: 476–87.

Martin LP, Hamilton TC, Schilder RJ. Platinum resistance: the role of continuous or intermittent fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J. Clin. Oncol. 2010; 28: 4706–13.

Shitara K, Yokota T, Takahari D et al. Cetuximab plus FOLFOX for patients with metastatic colorectal cancer with poor performance status and/or severe tumor-related complications. Case Rep. Oncol. 2010; 3: 282–6.

Peeters M, Price TJ, Cervantes A et al. Final results from a randomized phase 3 study of FOLFOX panitumumab for second-line treatment of metastatic colorectal cancer. Ann. Oncol. 2014; 25: 107–16.

Abd El All HS, Mishriki AM, Mohamed FA. Epidermal growth factor receptor in colorectal carcinoma: correlation with clinicopathological prognostic factors. Color Dis. 2008; 10: 170–78, 70871744921004.

U.S. Food and Drug Administration. List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools) 2015.

Buckley AF, Kakar S. Comparison of the Daco EGFR pharmDX Kit and Zymed EGFR antibody for assessment of EGFR status in colorectal adenocarcinoma. Appl. Immunohistochem. Mol. Morphol. 2007; 15: 305–9.

Shioyama K, Wongsiri T, Mizutani Y, Inada K, Tsutsumi Y. High-sensitivity epidermal growth factor receptor immunostaining for colorectal carcinomas, compared with EGFR PharmDX: a study of diagnostic accuracy. Int. J. Clin. Exp. Pathol. 2013; 6: 24–30.

Hecht JR, Mitchell E, Neubauer MA et al. Lack of correlation between epidermal growth factor receptor status and response to panitumumab monotherapy in metastatic colorectal cancer. Clin. Cancer Res. 2010; 16: 2205–13.

Teigar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. J. Clin. Oncol. 2012; 30: 3570–7.

Allegre CJ, Jessup JM, Somerset MR et al. American Society of Clinical Oncology Provisional Clinical Opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J. Clin. Oncol. 2009; 27: 2091–6.

Chung KY. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J. Clin. Oncol. 2005; 23: 1803–10.

Ciardiello F, Lenz H-J, Kohne C-H et al. Effect of KRAS and NRAS mutational status on first-line treatment with FOLFIRI plus cetuximab in patients with metastatic colorectal cancer (nCRC): new results from the CRYSTAL trial. J. Clin. Oncol. 2014; 32 (3 Suppl.): LBA443.

Peeters M, Oliner K, Price T et al. Updated analysis of KRAS/NRAS and BRAF mutations in study 20050181 of panitumumab (pmab) plus FOLFOXIRI for second-line treatment (tx) of metastatic colorectal cancer (mCRC). Ann. Oncol. 2014; 25(Suppl. 2): ii5–5.

Pentheroudakis G, Kotoula V, De Roock W et al. Biomarkers of benefit from cetuximab-based therapy in metastatic colorectal cancer: interaction of EGFR ligand expression with RAS/RAF, PIK3CA mutation status and/or severe tumor-related complications. Cancer Res. 2008; 68: 7210–30.

Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol. Syst. Biol. 2005; 1: E1–17.

Kim S-Y, Jung JH, Lee HJ et al. [18F]fluorothyminde PET informs the synergistic efficacy of capicitabine and trifluridine/tipiracil in colon cancer. Cancer Res. 2017; 77: 7120–30.

Cervantes A. Biomarkers of benefit from cetuximab-based therapy in metastatic colorectal cancer: interaction of EGFR ligand expression with RAS/RAF, PIK3CA genotypes. BMC Cancer. 2013; 13: 49.

Peeters M, Van Cutsem E, Lenz H-J, Kohne C-H et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J. Clin. Oncol. 2015; 33: 692–700.

Peeters M, Oliner KS, Price TJ et al. Analysis of KRAS/NRAS mutations in a phase III study of panitumumab with FOLFOXIRI compared with FOLFOXIRI alone as second-line treatment for metastatic colorectal cancer. Clin. Cancer Res. 2015; 21: 5469–79.

Van Cutsem E, Cervantes A, Noudlinger B, Arnold D. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann. Oncol. 2014; 25(Suppl. 3): iii1–9.
Precision treatment in colorectal cancer

TO Yau

57 Wong NA, Gonzalez D, Salto-Tellez M et al. RAS testing of colorectal carcinoma—a guidance document from the Association of Clinical Pathologists Molecular Pathology and Diagnostics Group. J. Clin. Pathol. 2014; 67: 751–7.

58 Yang Z-Y, Wu X-Y, Huang Y-F et al. Promising biomarkers for predicting the outcomes of patients with KRAS wild-type metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: a systematic review with meta-analysis. Int. J. Cancer. 2013; 133: 1914–25.

59 De Roock W, Claes B, Bernasconi D et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010; 11: 753–62.

60 Baker JB, Dutta D, Watson D et al. Tumour gene expression predicts response to cetuximab in patients with KRAS wild-type metastatic colorectal cancer. Br. J. Cancer. 2011; 104: 488–95.

61 Martin V, Landi L, Molinari F et al. HER2 gene copy number status may influence clinical efficacy to anti-EGFR monoclonal antibodies in metastatic colorectal cancer patients. Br. J. Cancer. 2013; 108: 668–75.

62 Palmer BF, Clegg DJ. Oxygen sensing and metabolic homeostasis. Mol. Cell Endocrinol. 2014; 397: 51–8.

63 Hashim A, Al-Janabi A, Mahdi L, Al-Toraihi K, Yasseen A. Vascular endothelial growth factor (VEGF) receptor expression correlates with histologic grade and stage of colorectal cancer. Libyan J. Med. 2010; 5: 5059.

64 Martins SF, Garcia EA, Luz MAM, Pardal F, Rodrigues M, Filho AL. Chloroaliphatic correlation and prognostic significance of VEGF-A, VEGF-C, VEGF-R2 and VEGF-R3 expression in colorectal cancer. Cancer Genomics Proteomics. 2013; 10: 55–67.

65 Ferrara N, Adamis AP. Ten years of anti-vascular endothelial growth factor therapy. Nat. Rev. Drug Discov. 2016; 15: 385–403.

66 Van Cutsem E, Tabernero J, Lakomy R et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. J. Clin. Oncol. 2012; 30: 3499–506.

67 Bennouna J, Sastre J, Arnold D et al. Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. Lancet Oncol. 2013; 14: 29–37.

68 Tabernero J, Yoshino T, Cohn AL et al. RAISE Study Investigators. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, Lancet Oncol. 2015; 16: 499–508.

69 Goel G, Sun W. Ramucirumab, another anti-angiogenic agent for metastatic colorectal cancer in second-line setting—its impact on clinical practice. J. Hematol. Oncol. 2015; 8: 92.

70 Diaz-Serrano A, Riesco-Martínez MC, García-Carbonero R. The safety and efficacy of ramucirumab for the treatment of metastatic colorectal cancer. Expert Rev. Anticancer Ther. 2016; 16: 585–95.

71 Chiron M, Bagley RG, Pollard J et al. Differential antitumor activity of aflibercept and bevacizumab in patient-derived xenograft models of colorectal cancer. Mol. Cancer Ther. 2014; 13: 1636–44.

72 Grothey A, Sugrue MM, Purdie DM et al. Bevacizumab beyond first progression is associated with prolonged overall survival in metastatic colorectal cancer: results from a large observational Cohort study (BRiTE). J. Clin. Oncol. 2008; 26: 5326–34.

73 Griffioen AW, Mans LA, de Graaf AMA et al. Rapid angiogenesis onset after discontinuation of sunitinib treatment of renal cell carcinoma patients. Clin. Cancer Res. 2012; 18: 3961–71.

74 Desar I, Mulder S, Stillebroer A et al. The reverse side of the victory: flare up of symptoms after discontinuation of sunitinib or sorafenib in renal cell cancer patients. A report of three cases. Acta Oncol. (Madr). 2009; 48: 927–31.

75 Kubicka S, Greil R, Andre T et al. Bevacizumab plus chemotherapy continued beyond first progression in patients with metastatic colorectal cancer previously treated with bevacizumab plus chemotherapy: ML18147 study KRAS subgroup findings. Ann. Oncol. 2013; 24: 2342–9.

76 Bates DO, Catalano PJ, Symonds KE et al. Association between VEGF splice isoforms and progression-free survival in metastatic colorectal cancer patients treated with bevacizumab. Clin. Cancer Res. 2012; 18: 6384–91.

77 Weckhardt AJ, Williams DS, Lee CK et al. Vascular endothelial growth factor D expression is a potential biomarker of bevacizumab benefit in colorectal cancer. Br. J. Cancer. 2015; 113: 37–45.

78 Hansen TF, Carlsen AL, Hegegaard NH, Sorensen FB, Jakobsen A. Changes in circulating microRNA-126 during treatment with chemotherapy and bevacizumab predicts treatment response in patients with metastatic colorectal cancer. Br. J. Cancer. 2015; 112: 624–9.

79 Hansen TF, Nielsen BS, Sorensen FB, Johnsson A, Jakobsen A. Epidermal growth factor-like domain 7 predicts response to first-line chemotherapy and bevacizumab in patients with metastatic colorectal cancer. Mol. Cancer Ther. 2014; 13: 2238–45.

80 Goede V, Coutelle O, Neumeier J et al. Identification of serum angiopoietin-2 as a biomarker for clinical outcome of colorectal cancer patients treated with bevacizumab-containing therapy. Br. J. Cancer. 2010; 103: 1407–14.

81 Benson A, Krivoshik AK, Van Sant C, Gyuris J, Feng B. Abstract A24: Neuroplulin 1 (NR1P1) as a potential biomarker for tivozanib + mFOLFOX6 versus bevacizumab + mFOLFOX6 in metastatic colorectal cancer (mCRC): post-hoc biomarker analysis of BATON-CRC phase 2 trial. Mol. Cancer Ther. 2015; 14(12 Suppl. 1): A24–4.

82 Lambrechts D, Thienpont B, Thuillier V et al. Evaluation of efficacy and safety markers in a phase II study of metastatic colorectal cancer treated with afibreccept in the first-line setting. Br. J. Cancer. 2015; 113: 1027–34.

83 Fiala O, Buchler T, Mohelnikova-Duchonova B et al. GI2V and GI2A KRAS mutations are associated with poor outcome in patients with metastatic colorectal cancer treated with bevacizumab. Tumor Biol. 2016; 37: 6823–30.

84 Wilhelm SM, Dumas J, Adnane L et al. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical anti-tumor activity. Int. J. Cancer. 2011; 129: 245–55.

85 Grothey A, Van Cutsem E, Sobrero A et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013; 381: 303–12.

86 Li J, Qin S, Xu R et al. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2015; 16: 619–29.

87 Goldstein DA, Ahmad BB, Chen Q et al. Cost-effectiveness analysis of regorafenib for metastatic colorectal cancer. J. Clin. Oncol. 2015; 33: 3727–32.

88 Yeh TC, Marsh V, Bernat BA et al. Biological characterization of ARRY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 inhibitor. Clin. Cancer Res. 2007; 13: 1576–83.

89 Napolitano S, Martini G, Rinaldi B et al. Primary and acquired resistance of colorectal cancer to anti-EGFR monoclonal antibody can be overcome by combined treatment of regorafenib with cetuximab. Clin. Cancer Res. 2015; 21: 2975–83.

90 Subbiah V, Khawaja MR, Hong DS et al. First-in-human trial of multikinase VEGF inhibitor regorafenib and anti-EGFR antibody
cetuximab in advanced cancer patients. *JCI Insight*. 2017; 2: e90380.

91 Liu J, Yuan Y, Chen W et al. Immune-checkpoint proteins VISTA and PD-1 nonredundantly regulate murine T-cell responses. *Proc. Natl. Acad. Sci.* 2015; 112: 6682–7.

92 Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol. Rev.* 2010; 236: 219–42.

93 Kim JH, Park HE, Cho N-Y, Lee HS, Kang GH. Characterisation of PD-L1-positive subsets of microsatellite-unstable colorectal cancers. *Br. J. Cancer*. 2016; 115: 490–6.

94 Le DT, Uram JN, Wang H et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* 2015; 372: 2509–20.

95 Sheridan C. Milestone approval lifts Illumina’s NGS from research into clinic. *Nat. Biotechnol.* 2014; 32: 111–2.

96 Hagemann IS, Devarakonda S, Lockwood CM et al. Clinical next-generation sequencing in patients with non-small cell lung cancer. *Cancer*. 2015; 121: 631–9.

97 Diaz LA, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J. Clin. Oncol.* 2014; 32: 579–86.

98 Misale S, Yaeger R, Hobor S et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature*. 2012; 486: 532–6.

99 Murtaza M, Dawson S-J, Tsui DWY et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature*. 2013; 497: 108–12.

100 Pauli C, Hopkins BD, Prandi D et al. Personalized in vitro and in vivo cancer models to guide precision medicine. *Cancer Discov.* 2017; 7: 462–77.