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Perspective

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Personalizing adjuvant therapy for patients with colorectal cancer

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Abstract

The current standard-of-care adjuvant treatment for patients with colorectal cancer (CRC) comprises a fluoropyrimidine (5-fluorouracil or capecitabine) as a single agent or in combination with oxaliplatin, for either 3 or 6 months. Selection of therapy depends on conventional histopathological staging procedures, which constitute a blunt tool for patient stratification. Given the relatively marginal survival benefits that patients can derive from adjuvant treatment, improving the safety of chemotherapy regimens and identifying patients most likely to benefit from them is an area of unmet need. Patient stratification should enable distinguishing those at low risk of recurrence and a high chance of cure by surgery from those at higher risk of recurrence who would derive greater absolute benefits from chemotherapy. To this end, genetic analyses have led to the discovery of germline determinants of toxicity from fluoropyrimidines, the identification of patients at high risk of life-threatening toxicity, and enabling dose modulation to improve safety. Thus far, results from analyses of resected tissue to identify mutational or transcriptomic signatures with value as prognostic biomarkers have been rather disappointing. In the past few years, the application of artificial intelligence-driven models to digital images of resected tissue has identified potentially useful algorithms that stratify patients into distinct prognostic groups. Similarly, liquid biopsy approaches involving measurements of circulating tumour DNA after surgery are additionally useful tools to identify patients at high and low risk of tumour recurrence. In this Perspective, we provide an overview of the current landscape of adjuvant therapy for patients with CRC and discuss how new technologies will enable better personalization of therapy in this setting.

Sections

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Introduction

In the absence of any major improvements in adjuvant treatment options for patients with colorectal cancer (CRC) over the past two decades, researchers are exploring how we might better use the currently available drugs by selecting patients who would benefit most from them. Moreover, research is also needed to address how to further improve the therapeutic ratio (also referred to as therapeutic index) by reducing the number of patients who are likely to have life-threatening toxicities. Biomarkers are now available that enable prediction of both the likelihood of clinical benefit and the risk of toxicities from certain treatments.

The principle underpinning adjuvant therapy is the eradication of residual tumour cells, particularly those residing in occult micrometastases, in patients who have undergone apparently curative resection of the primary tumour and whose staging imaging scans show no evidence of disease. Micrometastases are defined by the Union for International Cancer Control in their *TNM Classification of Malignant Tumours*[1] as agglomerations of tumour cells with diameters in the range 0.2–2.0 mm. Clusters of cells with diameters of <0.2 mm or >2.0 mm are referred to as isolated tumour cells and macrometastases, respectively[1]. Considering the long-held assumption that a tumour volume of 1 cm^3 contains approximately 10^9 cells[2], nodules with diameters of 5.8 mm, 2.7 mm and 0.6 mm would contain 10^8 cells, 10^7 cells and 10^5 cells, respectively. The residual micrometastatic tumour burden has a major influence on the time to disease recurrence, and the degree of tumour heterogeneity, and is, therefore, a key determinant of the potential response to adjuvant chemotherapy. The Goldie–Coldman model of resistance to chemotherapy[3] accounts for tumour size and also incorporates the potential for development of acquired resistance. According to this hypothesis, the probability that a cancer contains drug-resistant clones is directly correlated with the mutation rate and size of the tumour. Therefore, for any given mutation rate, size becomes the key determinant in predicting the presence of mutations conferring resistance to treatment and the time to detection of tumour relapse. Studies using multicellular tumour spheroids, which are robust models of prevascularized micrometastases, demonstrated the existence of steep gradients in the concentration of cytotoxic drugs, oxygen and metabolites from the external cell layer to the spheroid centre, all of which could affect chemosensitivity[4–6].

An estimated 80% of colon cancer recurrences occur within the first 3 years after surgical resection, and relatively fewer (<10%) occur beyond 5 years, which is therefore often accepted as the time point at which patients are cured[7]. Currently, the presence of micrometastases is predicted by a range of conventional histopathological criteria, predominantly the Tumour, Node, Metastasis (TNM) classification, tumour grade and morphological evidence of cancer cells in the tumour vascular or lymphatic vessels, which correlate to an extent with recurrence rates and overall survival (OS) outcomes. Although many international experts have reviewed and amended the TNM staging system[8] and/or provided guidelines to aid individual pathologists in its interpretation and implementation, the use of this staging system remains subject to a considerable degree of subjectivity[9]. For example, substantial variability exists in the diagnosis pT4a colon cancer, both at the pathologist and laboratory level. This diagnosis tends to be challenging given the high degree of interobserver variability; therefore, standardization of the assessment of this pathological entity is needed[10] given the different prognostic implications and subsequent potential effects on duration of therapy and consideration of a locoregional treatment approach for T4b disease. Indeed, T4b might be considered more severe owing to invasion of other organs and structures, and several studies have confirmed that patients with T4b disease have worse outcomes than those with T4a[11]. The reporting of vascular invasion in patients with CRC is also highly variable; a study found that the reported incidence of venous invasion can vary between 11% and 90%[12], and is widely under-reported[13,14]. Interobserver variability also poses problems in this regard, with only low to moderate agreement among pathologists when reporting vascular invasion in CRC and concordance rates as low as 50%[15]. Tumour grade is another important variable demonstrated to be a stage-independent prognostic factor on multivariate analysis; however, the dominant drawback of this parameter is, again, that its evaluation is largely subjective[16].

An improved understanding of the biology of CRC and the processes involved in metastasis will lead to the identification of biomarkers of prognosis that can be evaluated in a more objective manner and could help to identify additional therapeutic targets. Single-cell analysis has demonstrated the existence of cells with metastatic and non-metastatic potential co-existing within the same tumour mass[17], suggesting the existence of specific subpopulations that have acquired all the necessary mutations to metastasize. Metastasis is a complex process and its hallmarks, including the conditions, characteristics and steps, have been reviewed in detail elsewhere[17]. Given the range of signalling and metabolic pathways involved in the development, progression and metastasis of CRC, the heterogeneity of primary CRC and the complexity of cancer cell–stromal cell interactions, identifying effective approaches to exploit therapeutic targets remains elusive. To enable comparisons of the different stages of metastasis in order to further clarify the hallmarks of this process and identify potentially actionable targets — and, subsequently, effective medicines — innovative technologies (including single-cell and RNA sequencing) must be used to analyse primary tumour tissue, circulating tumour cells and micrometastases (harvested from lymph nodes) from individual patients.

In this Perspective, we describe the current treatment approaches for patients with conventionally staged CRC, discuss strategies used for widening the therapeutic ratio of these therapies, focusing both on minimizing toxicity and monitoring efficacy, and propose new tools for disease staging that could enable more personalized treatment decisions.

Adjuvant chemotherapy

Clinicians often present adjuvant chemotherapy to patients as 'taking out an insurance policy'; however, the 'cost' is substantial and the benefits cannot be guaranteed. For example, an estimated 50% of patients with stage III CRC will be cured with surgery alone, the disease will recur in 20–25% despite adjuvant chemotherapy and perhaps 25–30% will be cured by chemotherapy, offset by an associated toxicity-related mortality of 0.5–1% and grade 3–4 adverse events in 20–30% of patients[18]. This context is a clear indication that the therapeutic ratio of adjuvant chemotherapy needs to be improved by introducing better methods for selecting patients who will benefit the most and sparing from toxicities those who will not derive benefit.

What regimens?

One of the first trials of adjuvant chemotherapy for colon cancer demonstrated that patients receiving 5-fluorouracil and the antihelminthic drug levamisole had improved OS relative to those undergoing observation alone (3.5-year OS 71% versus 55%; $P = 0.006$)[19]. Although this trial received technical criticism over its small sample size

(~300 patients per arm), the absence of a placebo arm and the selection of levamisole, an agent with limited efficacy, it was a landmark study that prompted considerable interest in adjuvant chemotherapy. In the early 2000s, the QUASAR group built on the principles of medical statistics promulgated at that time to conduct a series of very large adjuvant trials. These principles included asking questions that can be answered with clearly measurable outcomes, performing data collection focused only on key clinical events and recruiting a sufficient number of patients. In one of these trials, 3,239 patients with CRC (of whom 91% had stage II disease) were randomly assigned to receive adjuvant chemotherapy with 5-fluorouracil and folinic acid or to observation (with chemotherapy offered upon disease relapse). This was probably the first and only randomized trial to demonstrate an OS improvement in patients with stage II CRC receiving adjuvant chemotherapy[20]. Assuming a 5-year mortality of 20% without chemotherapy, the relative risk (RR) of death demonstrated in this trial translated into an improvement in 5-year OS of 3.6% (95% CI 1.0–6.0%). In addition, this trial remains the largest (n = 948) in which patients with rectal cancer were randomly assigned to adjuvant chemotherapy or observation; subgroup analyses revealed a similar degree of benefit in these patients and those with colon cancer in terms of recurrence risk (RR 0.68, 95% CI 0.48–0.96, and RR 0.83, 95% CI 0.65–1.07, respectively) and OS (RR 0.77, 95% CI 0.55–1.08, and RR 0.84, 95% CI 0.66–1.07)[20]. The QUASAR group trial also demonstrated that patients do not derive any benefit from levamisole relative to placebo and that low-dose folinic acid (10 mg) is as effective as the conventionally accepted dose (100 mg)[20].

A series of subsequent trials demonstrated that oral capecitabine is at least equivalent to intravenous bolus 5-fluorouracil–folinic acid in term of OS (81.3% versus 77.6% at 3 years; P = 0.05)[21] and is better tolerated, and that a 12-month course of oral capecitabine does not confer an OS advantage over administration of this agent for 6 months[22].

The MOSAIC[23] and XELOXA[24] trials provided the next inflexional improvements, demonstrating that the addition of oxaliplatin to 5-fluorouracil–folinic acid (FOLFOX) or capecitabine (XELOX, also referred to as CAPOX) for 6 months significantly improves disease-free survival (DFS) but not OS at 5 years (77.6% versus 74.2% with 5-fluorouracil–folinic acid; HR 0.87, 95% CI 0.72–1.02; P = 0.15). Updated 10-year data from the MOSAIC trial confirmed an OS benefit with FOLFOX in patients with stage III disease (67.1% versus 59.0% with 5-fluorouracil–folinic acid; HR 0.80, 95% CI 0.66–0.96; P = 0.016)[23]. The addition of oxaliplatin, however, did not improve OS in those with stage II disease (79.5% versus 78.4%; HR 1.00, 95% CI 0.74–1.35; P = 0.98). Combination therapy comes with the costs of treatment-related deaths (0.5% and 0.6% in each arm in the MOSAIC and XELOXA trials, respectively) and a high rate of oxaliplatin-induced cumulative sensory neuropathy (92.1% and 65% of patients, respectively), which can affect the long-term quality of life of patients[25]. An analysis of pooled data from 11 studies conducted by the ACCENT and IDEA groups[26], in which patients with stage III colon cancer received FOLFOX or CAPOX for 6 months, suggested that early treatment discontinuation (defined as discontinuation before receiving a maximum of 75% of the number of planned cycles) was associated with worse DFS (3-year DFS 69% versus 78.8% without early discontinuation; HR 1.61, 95% CI 1.48–1.75; P < 0.001) and OS (74.7% versus 84.7% at 5 years; HR 1.73, 95% CI 1.57–1.91; P < 0.001); however these data have been somewhat overtaken by the wider findings from the IDEA group trials (discussed below).

The addition of the DNA topoisomerase I inhibitor irinotecan to 5-fluorouracil–folinic acid improves time to progression (median 6.7 months versus 4.4 months; P < 0.001) in patients with advanced CRC, and OS durations were longer (median 17.4 months versus 14.1 months: P = 0.03)[27]. Surprisingly, such an effect was not observed in the adjuvant setting (5-year OS 73.6% versus 71.3% without irinotecan; P = 0.09)[28]. Similarly, the anti-EGFR antibody cetuximab and anti-VEGFA antibody bevacizumab failed to confer any additional OS benefit in the adjuvant setting[29,30] when added to fluoropyrimidine–oxaliplatin. These observations all come from very large (involving several thousand patients) clinical trials, several of which had assembled formalin-fixed paraffin-embedded tissue biobanks with samples from the resected primary tumours. The existence of these biobanks enabled post hoc, retrospective molecular analyses to define patient subpopulations that might derive an OS benefit. Indeed, the clear mechanism of action of these targeted agents lead to hypothesis-based subgroup analyses, with the results indicating that patients with wild-type *KRAS* and wild-type *BRAF* tumours, or those with tumours characterized by microsatellite instability (MSI) and CD31 expression might derive a marginal benefit from cetuximab[31] and bevacizumab, respectively[30]; however, this evidence was not sufficiently compelling to warrant a change in clinical practice or further prospective studies focused on these selected patient populations.

Time of treatment initiation

In the QUASAR trial[20,32], patients could be enrolled up until 3 months after surgery; approximately 2,500 patients were treated within 6 weeks of surgery (chemotherapy versus observation: HR 0.73, 95% CI 0.55–0.96) and the remaining 2,500 between 6 and 12 weeks (chemotherapy versus observation: HR 0.83, 95% CI 0.62–1.12). Although this was a non-randomized comparison, no difference in OS was detected between patients with 'early' versus 'later' initiation of treatment.

Adjuvant treatment is typically initiated within 8 weeks of surgery. A subsequent meta-analysis of eight studies involving >13,000 patients suggested that starting treatment beyond this time frame is associated with worse OS (RR 1.2, 95% CI 1.15–1.26)[33], but data from the QUASAR trial provide a good argument for extending initiation of treatment up to 12 weeks after surgery.

Duration of adjuvant therapy

The IDEA international collaboration performed a pooled analysis[34] of six phase III randomized trials that were conducted across 12 countries. These trials involved ~13,000 patients with stage III CRC, compared 6 months versus 3 months of FOLFOX or CAPOX and had the primary end point of 3-year DFS. The non-inferiority margin for OS was set as a hazard ratio of 1.11. In all patients, 5-year OS was 82.4% versus 82.8% with 3 months and 6 months of therapy, respectively (HR 1.02, 95% CI 0.95–1.11; non-inferiority false discovery rate adjusted (FDRadj) P = 0.06). For those treated with CAPOX, 5-year OS was 82.1% versus 81.2% (HR 0.96, 95% CI 0.85–1.08; non-inferiority FDRadj P = 0.03), and for those receiving FOLFOX, it was 82.6% versus 83.8% (HR 1.07, 95% CI 0.97–1.18; non-inferiority FDRadj P = 0.34). Updated DFS results confirmed previous findings (HR 1.08, 95% CI 1.02–1.15; non-inferiority FDRadj P = 0.25). Importantly, this OS difference is considered by most clinicians to be too small to merit the inconvenience and toxicity of prolonged treatment.

Four of the six studies in the IDEA collaboration also involved patients with high-risk stage II CRC (n = 3,273)[35]. High-risk stage II CRC was defined by the presence of one or more of the following adverse features: T4 disease, poorly differentiated adenocarcinoma, invasion (vascular, perilymphatic or perineural), inadequate node harvest, or bowel obstruction or perforation. OS data are not yet mature in this group;

5-year DFS was 80.7% and 83.9% for 3 months and 6 months of treatment, respectively (HR 1.17, 80% CI 1.05–1.31; $P_{\text{non-inferiority}} = 0.39$). As the upper boundary of the confidence interval crossed the non-inferiority limit of 1.2, a 3-month course of treatment could not be deemed non-inferior to 6 months. A shorter duration of adjuvant therapy was associated with notable reductions in adverse events regardless of the chemotherapy regimen, especially for peripheral neuropathy (in 13% versus 36% of patients). The IDEA investigators concluded that the convenience, reduced toxicity and cost of a 3-month course of adjuvant CAPOX made this regimen a potential option in patients with high-risk stage II colon cancer in whom oxaliplatin-based chemotherapy is indicated. Interestingly, they also mentioned that the relative contribution of the factors used to define high-risk stage II disease needs to be better understood[27]. Consequently, the majority of treatment guidelines, such as those from ESMO[36], recommend adjuvant chemotherapy with CAPOX for 3 months. Some researchers, however, advocate for delivering more prolonged therapy (6 months) with CAPOX to patients with conventionally staged high-risk stage II colon cancer (T4 and/or N2 disease), despite the rather marginal clinical benefit.

Patient age

The average age of patients involved in the aforementioned clinical trials was 62 years, whereas the average age of patients presenting with CRC is 72 years. This discrepancy raises a question over the generalizability of the results of these trials. Given the typically long list of inclusion and exclusion criteria in clinical trial protocols, it follows that patients recruited to such studies do indeed represent a selected patient population. In the QUASAR trial[20,32], the OS benefits from 5-fluorouracil–folinic acid diminish by decile of age for older patients, with no benefit observed in patients aged ≥70 years. A pooled analysis of data from 4,819 patients with stage III CRC involved in the NSABP C-08, XELOXA, X-ACT and AVANT trials revealed that FOLFOX or CAPOX treatment is associated with an OS benefit over 5-fluorouracil–folinic acid in all age groups, with a HR of 0.77 ($P = 0.014$) in older patients[37], although the OS is more favourable in patients <70 years of age (HR 0.77; $P = 0.0004$). By contrast, a subsequent meta-analysis of pooled data from 1,985 patients involved in eight randomized trials showed that older patients with resected stage III CRC do not derive any OS benefit from the addition of oxaliplatin to chemotherapy (HR 1.02, 95% CI 0.82–1.27)[38], which is of course associated with excess symptomatic sensory peripheral neuropathy. Ongoing trials are recruiting older and frail patients to explore the utility of single-agent and doublet chemotherapy regimens versus control regimens (NCT02355379, NCT02316535 and NCT03828227). At present, clinicians must consider life expectancy and co-morbidities when discussing adjuvant therapy with older patients.

Neoadjuvant approaches

Neoadjuvant chemotherapy is predicated on the principle that delivering chemotherapy earlier in the growth cycle of a tumour should decrease the emergence of drug-resistant clones, downsize tumours to facilitate surgical removal, reduce micrometastatic burden prior to resection and avoid any post-surgical flare in the growth of residual malignant foci. In the phase III FOxTROT trial, with results published in 2023 (ref. 39), 1,053 patients with operable, non-obstructed colon cancer with CT-predicted stage T3–4, N0–2, M0 disease were randomly assigned (2:1) to receive three cycles of neoadjuvant FOLFOX versus upfront surgery (postoperative chemotherapy was available in both arms according to path stage). Patients who received neoadjuvant chemotherapy had a lower 2-year recurrence rate than those who only underwent surgery (16.9% versus 21.5%; RR 0.72, 95% CI 0.54–0.98; $P = 0.037$). However, the choice of 2-year recurrence rate as the primary end point was unusual and the differences in colon cancer-specific mortality and OS were not statistically significant. In this trial, patients with wild-type *RAS* tumours were also randomly assigned (1:1) to receive the anti-EGFR antibody panitumumab in addition to neoadjuvant FOLFOX, which did not provide any benefit in terms of recurrence rate. On the basis of baseline pretreatment CT scans, approximately 30% of patients enrolled in the trial had low-risk CRC and, theoretically, did not require chemotherapy, underlining the need to improve pretreatment disease assessment in further trials in this setting. Nevertheless, neoadjuvant FOLFOX was deemed well tolerated[40] and can be used as a downsizing procedure in patients for whom the multidisciplinary team feels that a complete resection might be difficult or not technically possible.

Immune-checkpoint inhibitors (ICIs) have revolutionized the treatment paradigm for mismatch repair-deficient (dMMR) and MSI-high (MSI-H) advanced-stage CRC[41], with emergent data in the neoadjuvant setting showing very promising results. In the NICHE trial[42], 32 patients with resectable dMMR/MSI-H colon cancer received neoadjuvant nivolumab plus ipilimumab. All 32 patients had a pathological response (defined as ≤50% viable residual tumour), with 31 (97%) having a major pathological response (<10% viable residual tumour) and 22 (69%) having a complete response[42]. Several other studies of neoadjuvant treatment have preliminary data available, showing broadly similar results[43].

Pharmacogenomic approaches to mitigate toxicity

Key regulatory bodies (such as the EMA and FDA) have released specific recommendations for the implementation of pharmacogenomics in clinical settings with the aim of mitigating treatment-associated toxicity[44,45]. Nevertheless, the efficacy of comprehensive genetic screening for alterations prior to commencing systemic therapy remains a contentious issue within the medical community.

Fluoropyrimidines

The inactivation of 5-fluorouracil and its oral prodrug capecitabine relies on the activity of dihydropyrimidine dehydrogenase, encoded by *DPYD*. *DPYD* deficiency or certain variants lead to protein truncation and a prolonged plasma half-life of 5-fluorouracil, exacerbating its toxicity[46]. Thus, individuals with certain genetic variants of *DPYD* are at a substantially increased risk of developing severe life-threatening toxicity (such as myelosuppression, mucositis or diarrhoea with risk of death) after receiving standard doses of 5-fluorouracil. More than 200 genetic variants of *DPYD* have been described[47,48] and the clinical implications of four of these variants, *DPYD**2A (IVS14+1G>A), c.2846A>T, c.1679T>G and c.1236G>A/HapB3, have been validated. Hence, the EMA recommends initial screening for these four variants to enhance patient safety through dose modulation[49]; this approach can improve overall patient outcomes and quality of life by minimizing toxicity. Although no large randomized, prospective trials have been done to explore whether patients harbouring these variants and receiving reduced doses have worse outcomes, in a study providing real-world evidence, fluoropyrimidine efficacy was similar in a population of patients who underwent *DPYD* genotyping and received appropriate dose reductions relative to a control population[50]; and the results of a small observational study suggest that differences in median OS were not statistically significant between 37 carriers of *DPYD**2A who received reduced doses and a matched cohort of non-carriers (27 months with regular dose versus 24 months with reduced dose; $P = 0.47$)[51]. Moreover, high tumoural

levels of dihydropyrimidine dehydrogenase have been correlated with induction of resistance to fluoropyrimidines[52]. Therefore, concluding that cancer cells carrying *DPYD* variants are potentially more sensitive to fluoropyrimidines seems logical. Researchers have also suggested that, given the fact that the effectiveness of a reduced dose of fluoropyrimidines has not been established, subsequent doses could be increased, perhaps by increments of 15%, in patients carrying relevant *DPYD* variants in the absence of serious adverse effects[53].

Most studies of the correlation between *DPYD* genotype and fluoropyrimidine toxicity have been performed in white individuals, among whom 9% have low levels of functional dihydropyrimidine dehydrogenase, and up to 0.5% have a complete loss of enzyme function[54]. Researchers investigated the incidence of *DPYD* deficiency in a cohort of 1,364 Asian patients with colon cancer enrolled in the JOIN and ACHIEVE trials of adjuvant chemotherapy, and reported an incidence of ~0.6% in these patients, with no clear association observed between *DPYD* deficiency and safety[55].

Researchers have demonstrated that an expansion of the panel of variants to include rarer variants that might affect specific toxicities or be more relevant in non-white populations might further improve patient safety outcomes[56,57]. ESMO[53], the Pharmacovigilance Risk Assessment Committee of the EMA[49] and other European clinical institutions recommend genotyping of *DPYD* and/or phenotypic testing of dihydropyrimidine dehydrogenase before treatment with fluoropyrimidines. Some evidence indicates that a high pretreatment serum concentration of uracil is predictive of severe, including fatal, fluoropyrimidine-associated toxicity and is a promising phenotypic biomarker to identify patients at risk of such adverse events[58]. Phenotypic screening, however, has not been adopted to the same extent as genotypic screening of *DPYD* across Europe. In France, mandatory *DPYD* deficiency screening prior to initiating treatment has been introduced on the basis of evidence-based pharmacogenetics and permits either genetic or enzyme assays[46]. In the USA, the National Comprehensive Cancer Network has not endorsed or implemented either approach, primarily owing to concerns that dose reduction in individuals with *DPYD* variants could compromise the effectiveness of treatment with fluoropyrimidines[59].

Topoisomerase I inhibitors

The most common severe adverse events from irinotecan are delayed diarrhoea and neutropenia[60]. Irinotecan is one of the substrates of UDP-glucuronosyltransferase (encoded by *UGT1A1*), which can inactivate this drug. The *UGT1A1**28 and *UGT1A1**6 variants correlate with decreased UDP-glucuronosyltransferase activity and increased risk of irinotecan-related severe toxicity[61]. Despite this correlation, *UGT1A1* genotyping is not standard practice in most hospitals, in part because the original pharmacogenetic and toxicity studies were performed in cohorts of patients receiving high-dose single-agent irinotecan[62], whereas nowadays this drug is usually administered at a lower dose and in combination with fluoropyrimidines.

Platinum-based therapy

The ERCC genes encode proteins that are crucial components of the DNA nucleotide excision repair system. Variations in these genes can lead to differences in toxicity among individuals receiving platinum-based chemotherapy[63]. The presence of the *ERCC1* single-nucleotide polymorphism rs11615, the so-called T allele mutation in *ERCC1*, has been linked to increased incidence of grade 1 neuropathy in patients receiving oxaliplatin, but no correlation with higher grade neuropathy has been described[64], which limits the clinical utility of genetic testing for this alteration.

Biomarkers to personalize treatment

ctDNA

The use of liquid biopsy-based assays, especially of circulating cell-free tumour DNA (ctDNA) assays, for the analysis various cancer types across different disease stages is rapidly increasing. In patients with advanced stage cancers, ctDNA analysis has clinical utility for treatment selection, efficacy monitoring and identification of the most appropriate treatment following drug resistance (reviewed in ref. 65). Furthermore, rapidly accumulating evidence supports the clinical validity of ctDNA analysis for the detection and monitoring of molecular residual disease (MRD) as a logical extension of these applications. The clinical utility of preoperative ctDNA evaluation in patients with CRC remains to be validated. By contrast, ctDNA is detectable in a limited number of these patients after surgery and is strongly associated with an increased risk of disease recurrence, regardless of initial ctDNA status prior to surgery[66]. Numerous studies have suggested that the presence of ctDNA is a surrogate for MRD and a strong predictor of clinical disease recurrence in patients with ctDNA-defined MRD-positive CRC, adding value to existing clinicopathological criteria as a prognostic factor[67,68] on multivariate analysis. In a study of 184 patients with stage II–III CRC, 27.5% had ctDNA detectable before surgery. The recurrence rate was 32.7% and 11.6% in patients with and without detectable ctDNA, respectively ($P = 0.001$)[67]. Similarly, in another study detection of ctDNA after surgery was a strong predictor of recurrence (HR 7.0, 95% CI 3.7–13.5; $P < 0.001$)[68].

In practice, ctDNA-based assessment of MRD status is typically performed ≥4 weeks after curative surgery and ≥2 weeks after completion of systemic therapy. For longitudinal monitoring, ctDNA is typically assessed every 8–12 weeks. At present, ctDNA-based MRD assessment assays can be categorized into two types: (1) tumour-informed assays, which detect ctDNA using mutational signatures inferred from genomic sequencing of the primary tumour to detect patient-specific genomic alterations; and (2) tumour-agnostic assays, which involve sequencing of a fixed gene panel and/or analysis of aberrant methylation in ctDNA, and do not require prior tumour tissue profiling[69]. Both approaches have been used in observational studies[68,70–82] (Table 1). In general, studies using tumour-informed assays have typically included more patients with stage II–III disease, whereas those using tumour-agnostic assays have tended to include more patients with stage III–IV disease. Nevertheless, the findings of these studies show that the presence of ctDNA after surgery in patients with CRC of various stages is closely connected to unfavourable recurrence rates and OS, regardless of the detection technique used (Table 1). Furthermore, in this setting, ctDNA assessment broadly results in two therapeutic decisions that are somewhat stage-specific. For patients with stage II CRC, ctDNA informs a positive selection approach, whereby only those at the highest risk of relapse are considered for adjuvant chemotherapy (that is, ctDNA-positive patients). Conversely, for those with stage III disease, the absence of ctDNA is used to identify those with a very good prognosis and a high chance of being cured by surgery, and thus unlikely to have sufficient absolute benefit from adjuvant chemotherapy (negative selection approach). Currently, ongoing trials are not focused on patients with stage I disease.

The GALAXY study is a prospective observational arm of the ongoing CIRCULATE-Japan study. In GALAXY, researchers performed serial ctDNA testing in patients with clinical stage II–IV or recurrent CRC after complete surgical resection and reported on the primary DFS end

Table 1 | ctDNA studies assessing MRD in patients with CRC after surgery

Patient population (*n*)	Assay	Sampling time	Main findings	Ref.
Tumour informed				
Stage II colon cancer (230)	Safe-SeqS	4–10 weeks after surgery; serial follow-up for 24 months	ctDNA positivity after surgery associated with worse 3-year RFS (0% versus 90% in ctDNA-negative (HR 18, 95% CI 8–40; $P < 0.0001$)) in patients who did not receive adjuvant therapy; ctDNA positivity during surveillance predicted recurrence with a median lead time of 5.5 months	70
Stage III colon cancer (96)	Safe-SeqS	4–10 weeks after surgery; after completion of treatment	ctDNA positivity after surgery associated with worse RFS (HR 3.8, 95% CI 2.4–21.0; $P < 0.001$); ctDNA positivity after completion of treatment associated with estimated 3-year RFI in 30% versus 77% in ctDNA-negative (HR 6.8, 95% CI 11.0–157.0; $P < 0.001$)	71
Locally advanced rectal cancer (159)	Safe-SeqS	4–10 weeks after surgery	ctDNA positivity after surgery associated with considerably worse 3-year RFS (33% versus 87% in ctDNA-negative; HR 13.0, 95% CI 5.5–31.0; $P < 0.001$)	72
Stage I–III CRC (125)	Safe-SeqS	Day 30 after surgery	ctDNA positivity after surgery associated with a higher risk of recurrence (HR 7.2, 95% CI 2.7–19.0; $P < 0.001$); ctDNA positivity during surveillance predicted recurrence with a median lead time of 8.7 months	73
Stage IV CRC with upfront resectable liver metastases (54)	Safe-SeqS	4–10 weeks after surgery; after adjuvant therapy	ctDNA negativity after all treatment (surgery with or without adjuvant chemotherapy) associated with better RFS (0% versus 76% in ctDNA-positive (HR 14.9, 95% CI 4.94–44.7; $P < 0.001$))	75
Stage IV CRC treated with metastasectomy (58)	Ultra-deep targeted sequencing	3–4 weeks after surgery	ctDNA detection rate higher in patients with liver metastases ($P = 0.005$) or tumours ≥1 cm ($P = 0.018$), and was lower following a complete or partial response to chemotherapy	76
Stage II–III CRC (240)	GeneseeqPrime	Within 3–7 days of surgery; serial follow-up for 24 months	ctDNA positivity after surgery associated with a greater risk of recurrence (HR 10.98, 95% CI 5.31–22.72; $P < 0.001$); ctDNA positivity during monitoring predicted recurrence with an accuracy of 92% and a mean lead time of 5 months	77
Stage IV CRC treated with metastasectomy (112)	GeneseeqPrime	Median of 27 days after surgery	On multivariate analysis, ctDNA positivity was the most significant prognostic factor for DFS (HR 5.8, 95% CI 3.3–10.0; $P < 0.001$); ctDNA positivity after surgery predicted disease progression with a median lead time of 3.2 months and was associated with inferior OS (HR 16.0, 95% CI 3.9–68.0; $P < 0.001$)	78
Stage III CRC (160)	Signatera	Within 8 weeks of surgery (median 2 weeks); within 3 months after adjuvant treatment	Greater risk of recurrence in patients with ctDNA positivity after surgery (HR 7.0, 95% CI 3.7–13.5; $P < 0.001$) and following adjuvant chemotherapy (HR 51, 95% CI 154–167; $P < 0.001$); ctDNA positivity during surveillance predicted recurrence with a median lead time of 9.8 months	68
Stage I–IV CRC (1,039)	Signatera	4, 12, 24, 36, 48 and 72 weeks after surgery	ctDNA positivity associated with increased recurrence risk (HR 10.0, 95% CI 7.7–14.0; $P < 0.0001$) across all pathological stages; postoperative ctDNA positivity enabled identification of patients with stage II or III CRC who derived benefit from adjuvant chemotherapy (HR 6.6, 95% CI 3.5–12.3; $P < 0.0001$)	82
Tumour agnostic				
Stage IV CRC with liver metastases (63)	Guardant360	Median 13 months after surgery	ctDNA positivity associated with inferior 2-year OS from the date of liver resection (70% versus 100% in ctDNA-negative; $P = 0.005$)	74
Stage I–IV CRC (84)	Guardant Reveal	4 weeks after surgery; 4 weeks after adjuvant treatment	15 of 15 patients (100%) with ctDNA positivity and 12 of 49 patients (25%) with ctDNA negativity following final therapy (either surgery alone or end of adjuvant therapy) had disease recurrence	79
Stage III colon cancer (1,107)	Multiplex ddPCR	4–8 weeks After surgery	ctDNA positivity was an independent predictive factor for DFS (adjusted HR 1.55, 95% CI 1.13–2.12; $P = 0.006$); ctDNA positivity is also prognostic for OS (HR 1.65, 95% CI 1.12–2.43; $P = 0.01$); 3-year DFS was 66% versus 77% in ctDNA-positive and ctDNA-negative patients ($P = 0.015$)	80
Stage IV CRC with liver metastases (96)	Multiplex ddPCR	Within 3 months of surgery; every third month up to 36 months after surgery	RFS was significantly poorer in patients with ctDNA positivity compared than in those with ctDNA negativity after surgery (HR 4.5, 95% CI 2.1–−9.5; $P < 0.001$) or adjuvant chemotherapy (HR 8.4, 95% CI 3.1–23.1; $P < 0.0001$)	81

ctDNA, circulating cell-free tumour DNA; CRC, colorectal cancer; ddPCR, digital droplet PCR; DFS, disease-free survival; MRD, molecular residual disease; OS, overall survival; RFI, recurrence-free interval; RFS, recurrence-free survival.

point in 2022 (ref. 82). Patients with high-risk stage II–III disease and ctDNA-negative status 4 months after surgery had favourable 18-month DFS regardless of whether they received adjuvant chemotherapy or underwent observation (94.9% versus 91.5%, respectively; HR 1.71, 95% CI 0.8–3.7; $P = 0.16$). By contrast, patients with the same disease stage but a ctDNA-positive status derived significant benefit from adjuvant chemotherapy (18-month DFS of 61.6% versus 22.0% with observation; HR 6.59, 95% CI 3.5–12.3; $P < 0.0001$). The researchers also followed the

ctDNA clearance rate with serial sampling from baseline to 24 weeks after surgery. Although the number of events was small, the results suggest that the risk of recurrence was greater in patients without ctDNA clearance after surgery (HR 11, 95% CI 5.2–23.0; $P < 0.0001$). Therefore, postsurgical ctDNA status or lack of clearance (at 24 weeks) could guide a range of prospective clinical trials or treatment strategies, including observation only in patients with ctDNA-negative status, treatment intensification in those with ctDNA-positive status and/or addition of systemic agents from a different therapeutic class (such as irinotecan, mitomycin C or experimental drugs) in those without ctDNA clearance by week 24. The median follow-up duration of the GALAXY trial at the time of reporting was 16.7 months. Reporting of longer-term data is expected in late 2023 and might provide more definitive insights on the value of ctDNA testing for predicting OS.

Results from the DYNAMIC trial, the first randomized controlled trial that assessed the utility of ctDNA-guided treatment decisions in CRC, were reported in 2022 (ref. 83). In this trial, patients with stage II colon cancer were randomly assigned to ctDNA-guided management, involving adjuvant chemotherapy in patients with a ctDNA-positive status at 4–7 weeks after surgery and no adjuvant therapy for ctDNA-negative patients, versus standard management guided by clinicopathological factors. At a median follow-up of 37 months, fewer patients in the ctDNA-guided group received adjuvant chemotherapy (15.3% versus 27.9% with standard management). Moreover, ctDNA-guided treatment was deemed non-inferior relative to standard management on the basis of 2-year recurrence-free survival (RFS) of 93.5% and 92.4%, respectively, and 3-year RFS of 91.7% versus 92.4%. The results of DYNAMIC provide evidence supporting the notion that postoperative ctDNA positivity is a promising biomarker to guide adjuvant treatment decisions in patients with stage II CRC.

Several trials designed to evaluate whether ctDNA can be used as a dynamic biomarker to optimize adjuvant chemotherapy through escalation or de-escalation are currently underway (Table 2). The results of these studies will help to understand the effects of different adjuvant chemotherapy regimens in patients according to ctDNA-defined MRD status (such as fluoropyrimidines with or without oxaliplatin; 5-fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX); or 5-fluorouracil, folinic acid, oxaliplatin and irinotecan (FOLFOXIRI)).

Studies of novel treatment approaches for resected CRC are also harnessing ctDNA-based MRD detection technologies. ELI-002 is a vaccine targeting G12D or G12R-mutated *KRAS* that is being tested in patients with ctDNA-defined MRD-positive solid tumours in the AMPLIFY-201 trial (NCT04853017). Autogene cemuveran is an mRNA-based vaccine being tested in patients with stage II–III ctDNA-positive CRC after resection (NCT04486378). The CLAUDE trial is testing the microbiome-derived peptide vaccine (mimicking the tumour-associated antigens FOXM1 and BIRC5) EO2040 in combination with nivolumab in patients with ctDNA-defined MRD following resection of stage II–IV CRC (NCT05350501). Other adjuvant trials are testing atezolizumab plus bevacizumab in patients with ctDNA-defined MRD-positive gastrointestinal cancers, including CRC (MRD-GI; NCT05482516), and temozolomide plus irinotecan

Table 2 | Ongoing randomized trials investigating ctDNA-guided adjuvant chemotherapy strategies in patients with CRC

Trial	Location	Phase	Disease characteristics	Interventions	Primary end points
COBRA (NCT04068103)	USA	II/III	Stage IIA colon cancer	Arm 1: surveillance; arm 2: FOLFOX or CAPOX (6 months) in ctDNA-positive patients versus surveillance in ctDNA-negative patients	ctDNA clearance rate and RFS in ctDNA-positive patients
CIRCULATE (NCT04089631)	Germany	III	Stage II colon cancer	ctDNA-positive patients randomly allocated to surveillance versus capecitabine	DFS in ctDNA-positive patients
PRODIGE 70 -CIRCULATE (NCT04120701)	France	III	Stage II colon cancer	ctDNA-positive patients randomly allocated to surveillance versus mFOLFOX6 (6 months)	DFS in ctDNA-positive patients
IMPROVE-IT (NCT03748680)	Denmark	II	Stage I–II CRC	ctDNA-positive patients randomly allocated to surveillance versus FOLFOX or CAPOX (6 months)	DFS in MRD-positive patients
CIRCULATE-US (NCT05174169)	USA	II/III	Stage III colon cancer	ctDNA-negative patients randomly allocated to surveillance versus mFOLFOX (3–6 months) or CAPOX (3 months); ctDNA-positive patients randomly allocated to mFOLFOX or CAPOX (6 months) versus FOLFIRINOX (6 months)	ctDNA positivity and DFS in patient subgroups defined by ctDNA positivity
CLAUDIA (NCT05534087)	South Korea	III	Stage II–III colon cancer	ctDNA-positive patients randomly allocated to mFOLFIRINOX (3 months) versus FOLFOX or CAPOX (3 months)	DFS in patient subgroups defined by disease stage
AFFORD (NCT05427669)	China	III	Stage II–III CRC	ctDNA-positive patients randomly allocated to mFOLFOXIRI (6 months) versus mFOLFOX (6 months)	DFS in patient subgroups defined by disease stage
ALTAIR (NCT04457297)	Japan	III	Stage III CRC	ctDNA-positive patients randomly allocated to TAS-102 versus placebo (both for 6 months)	DFS in patient subgroups defined by disease stage
VEGA (jRCT1031200006)	Japan	III	Stage II–III colon cancer	ctDNA-negative patients randomly allocated to CAPOX versus observation (both for 3 months)	DFS in patient subgroups defined by disease stage

CAPOX, capecitabine and oxaliplatin; CRC, colorectal cancer; ctDNA, circulating cell-free tumour DNA; DFS, disease-free survival; FOLFIRINOX, 5-fluorouracil, folinic acid, irinotecan and oxaliplatin; FOLFOX, 5-fluorouracil, folinic acid and oxaliplatin; mFOLFOX6, modified FOLFOX; mFOLFOXIRI, modified FOLFOX and irinotecan; MRD, molecular residual disease; RFS, recurrence-free survival; TAS-102, trifluridine and tipiracil fixed-dose combination.

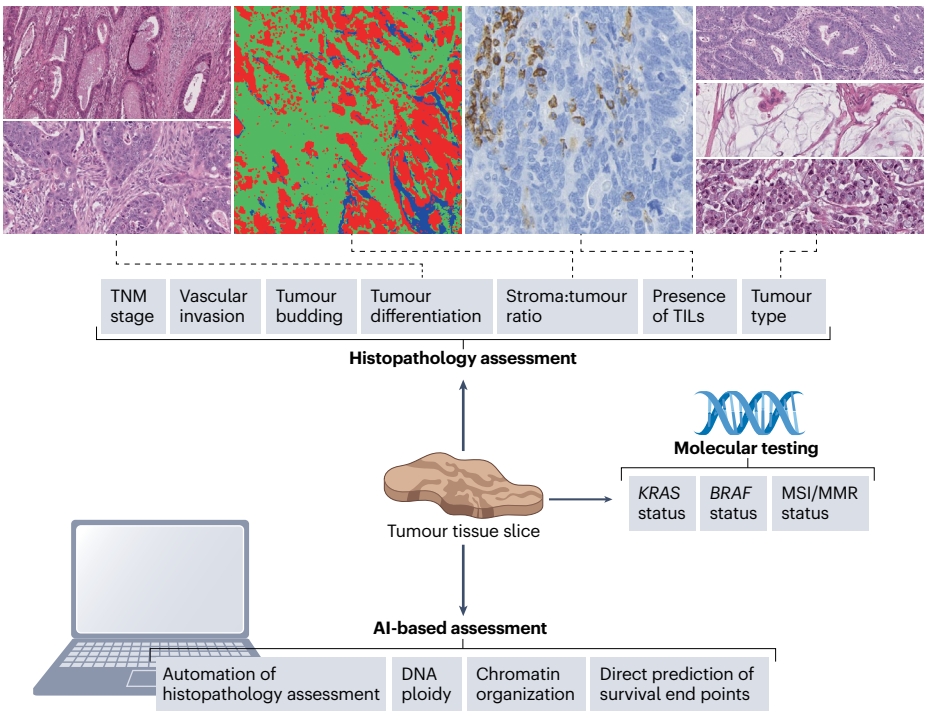

Fig. 1 | Factors that potentially affect prognosis of colorectal cancer. The figure shows examples of important tissue-based biomarkers in colorectal cancer (CRC). Besides the traditional pathological assessments of Tumour, Node, Metastasis (TNM) staging of tumour differentiation, multiple biomarkers have emerged as important predictors of patient outcome, such as vascular invasion, tumour budding, stroma to tumour ratio and presence of tumour infiltrating T cells (TILs). Artificial intelligence (AI) can be used to automate such assessments and also to characterize histopathological aspects that are currently not considered by pathologists as part of their routine assessments. AI can be trained to 'learn' how to identify and combine histopathological features in order to predict the outcome in the patient directly from images of histopathology sections. AI can also be combined with different tissue preparations; for example, isolation and DNA-specific staining of individual nuclei followed by automatic measurement of DNA content (ploidy) or chromatin organization in cancer cell nuclei. Finally, tumour tissue can also be used for sequencing or testing of specific molecular features. Some of these features, such as *KRAS* and *BRAF* mutational status, and microsatellite instability (MSI) and mismatch repair (MMR) status, have been shown to have prognostic or predictive value in some patients with CRC. Overall, tumour tissue harbours a wealth of information that can be captured by biomarkers and used to personalize treatment.

(ERASE-TMZ; NCT05031975) and the trifluridine and tipiracil fixed-dose combination TAS-102 plus irinotecan (NCT04920032) in those with ctDNA-defined MRD-positive CRC.

Some issues need to be considered before ctDNA can inform adjuvant chemotherapy decisions in routine clinical practice. The main challenge when using ctDNA-based MRD assays is the occurrence of relapse in patients deemed to have MRD-negative status, which might result from inadequate analytical sensitivity. Standardizing pre-analytical variables and refining ctDNA assays to improve sensitivity and reproducibility are therefore needed. Molecular profiling using multiomic analyses beyond genomics, including methylomic, fragmentomic and proteomic profiling, is another potential approach to improving the sensitivity of ctDNA-based MRD assays[84]. Some of the ongoing randomized trials previously mentioned additionally use serial ctDNA testing to address the problem of MRD-negative relapses and start adjuvant therapy at the time of ctDNA detection. Another challenge is the way in which the analyses are conducted. The choice of a ctDNA assay should consider three aspects: sensitivity and specificity, genome coverage and turnaround time. Tumour-informed tests have the advantage of a high analytical sensitivity, detecting allele variants with frequencies as low as 0.01%, and a low likelihood of false-positive results from clonal haematopoiesis of indeterminate potential. These tests, however, might not enable the detection of all mutations relevant to MRD or the identification of new mutations emerging following treatment-related selection pressure, both of which could be important in terms of selecting treatment for patients with disease relapse. The advantages of tumour-agnostic assays include logistical simplicity, quick turnaround times, the capacity to conduct the test even in the absence of primary tumour tissue and the capability to identify MRD even after clonal development of micrometastases[85]. One final consideration is that, although clinical studies generally use survival-related end points, benefits beyond survival (including quality of life, geographic availability and economic cost of testing) need to be considered before fully integrating ctDNA into the clinic.

Tissue-based biomarkers

Researchers have identified and characterized to some degree a wealth of tissue-based biomarkers (Fig. 1). The most widely clinically applicable biomarkers are those derived from staging systems, which indicate the extent of tumour growth, spread to lymph nodes and distant parts of the body (TNM stage), and the presence of residual tumour after surgery (R stage). Although tumour differentiation is another established biomarker in a range of cancer types, its prognostic importance in CRC is rather limited[86]. Tumour budding, or the presence and level of single

cancer cells or small clusters of cancer cells at the invasion front of the tumour, is associated with adverse patient outcomes[87]. Vascular invasion, particularly extramural vascular involvement but also vascular invasion within the bowel wall, also correlates with recurrence and mortality in patients with CRC[88].

The complex interactions between cancer cells and their local microenvironment can affect cancer progression and response to therapy[89,90]. A high stroma to tumour ratio (that is, a higher proportion of stromal component relative to the epithelial component in haematoxylin and eosin-stained sections) indicates an unfavourable prognosis[91]. In this context, a concept that has received special attention is the association between immune infiltrates and patient outcomes[92]. Initial observations suggesting that this biomarker has strong prognostic value culminated in the formation of an international consortium that defined the consensus Immunoscore assay (based on $CD3^+$ and $CD8^+$ T cell counts at the invasive margin and at the core of a tumour, with higher scores indicating higher infiltration) and investigated its prognostic value in thousands of patients with stage I–III colon cancer[93]. In a validation dataset consisting of 978 patients, they observed recurrence within 3 years in 14%, 24% and 36% of patients with a high, intermediate and low Immunoscore, respectively. The association with recurrence remained statistically significant after adjusting for established prognostic biomarkers. DFS and OS were also significantly associated with Immunoscore. Despite these data, the company responsible for marketing and distributing Immunoscore has discontinued this assay owing to a lack of commercial traction, which might be related to its price and inconclusive clinical utility.

Molecularly targeted therapies matched to specific tumour genotypes, or protein or RNA expression profiles, have been tested in patients with CRC, often in those with advanced stage disease[94–96]. Patients with advanced stage CRC without *KRAS* mutations can be selected for treatment with anti-EGFR antibodies[97]. *KRAS* status also has prognostic value in early stage CRC as demonstrated by analysis of patients with stage II CRC in the QUASAR trial in which risk of recurrence was significantly higher in those harbouring *KRAS*-mutant tumours than in those with wild-type *KRAS* tumours (28% versus 21%; RR 1.40, 95% CI 1.12–1.74; $P = 0.002$) but did not differ significantly between those with mutant *BRAF* tumours and those with wild-type *BRAF* tumours ($P = 0.36$). Neither biomarker predicted benefit from chemotherapy[98].

Researchers have suggested that molecular testing in early stage CRC should be based on DNA MMR/MSI status and assessment of DNA content (ploidy) in cancer cells[99] rather than specific driver mutations[100]. Patients with MMR deficiency or MSI, accounting for 10% to 15% of stage II CRCs, have a reduced risk of recurrence and adjuvant chemotherapy is not indicated in these patients[101,102] as demonstrated in the QUASAR study in which MMR deficiency was an independent prognostic variable for improved OS (HR 0.31, 95% CI 0.15–0.63; $P < 0.001$)[20,32]; the recurrence rate in patients with dMMR tumours was half that in patients with MMR-proficient tumours (11% versus 26%; RR 0.53, 95% CI 0.40–0.70; $P < 0.001$). A debate exists about whether dMMR tumours are resistant to 5-fluorouracil, but no evidence of this mechanism was found in the QUASAR study in which the survival benefits of chemotherapy relative to the control observation group were the same regardless of MMR status. As previously discussed, MMR status has been used to identify patients for neoadjuvant treatment with ICIs[42].

The use of machine learning approaches has enabled the identification of a number of biomarkers in CRC. Some of these approaches automate pathological evaluations, such as that of tumour differentiation[103] and identification of tumour buds and poorly differentiated cell clusters[104], whereas others enable tissue analyses that pathologists could but usually do not perform owing to time constraints and limited accuracy, such as detection of certain cell types for subsequent classification[105] or prediction of MMR/MSI status from routine histopathology sections[106]. A machine learning-based biomarker that reflects the chromatin organization in cancer cell nuclei has been shown to be prognostic in a range of cancer types[107] and its prognostic value in early stage CRC has been confirmed by independent research teams using new external datasets[108,109]. Several research groups have attempted to predict OS directly from routine histopathology sections using deep learning approaches (a type of machine learning), enabling the integration of a wide range of features into a single biomarker according to their estimated prognostic value[110–112]. DoMore-v1-CRC, one such deep learning-based biomarker, was developed using data from 2,473 patients with stage I–III CRC[113]. DoMore-v1-CRC was validated in a geographically diverse patient cohort according to a predefined protocol specifying the primary analysis, which is essential to obtaining reliable performance estimation (in particular, for deep learning-based biomarkers)[114]. In the external validation dataset, comprising 1,110 patients with stage III or high-risk stage II CRC from the QUASAR 2 trial[30], DoMore-v1-CRC classified 24% and 63% of patients as having poor and good prognosis, respectively, and was a good predictor of cancer-specific survival in these groups (HR 3.84, 95% CI 2.73–5.43 ($P < 0.0001$); and HR 3.04, 95% CI 2.07–4.47 ($P < 0.0001$), after adjusting for established prognostic biomarkers)[113].

Despite a tendency in the field towards considering biomarkers individually rather than as an integrated whole, some groups are building and evaluating tools that integrate multiple biomarkers. Our group developed a method for automatic estimation of the stroma to tumour ratio and integrated it with the assessment of DNA ploidy to obtain a stronger prognostic biomarker, in particular, for stage II CRC[115]. Another group developed a tool that integrates $CD8^+$ T cell and stroma to tumour fractions for the same purpose[116]. RNA signatures have been defined based on the expression levels of multiple cancer-related genes. In CRC, common patterns of gene expression have been used to define four consensus molecular subtypes; however, the prognostic value of these assays seems rather limited[117,118].

We integrated DoMore-v1-CRC with pathological staging biomarkers to form a clinical decision support (CDS) system for optimizing adjuvant chemotherapy in patients with stage II–III CRC without residual disease[119] (Fig. 2). In an external validation dataset, this CDS system had strong prognostic value for cancer-specific survival (HR 3.06, 95% CI 1.73–5.42 ($P = 0.0001$), for intermediate versus low risk; and HR 10.7, 95% CI 6.39–17.9 ($P < 0.0001$), for high versus low risk). In the development dataset, the CDS-defined low-risk group had a better prognosis than the conventionally defined low-risk group (3-year cancer-specific survival of 96.2% versus 94.1%) and so did the CDS-defined intermediate-risk group (3-year cancer-specific survival of 85.1% versus 82.4%). By contrast, the CDS-defined high-risk group had a worse prognosis than the conventionally defined group (3-year cancer-specific survival of 50.5% versus 61.0%). Of note, the improved prognostic ability is also indicated by the fact that the cancer-specific survival hazard ratios for the comparison of the conventionally defined intermediate-risk versus low-risk groups (HR 1.13, 95% CI 0.55–2.32) and high-risk versus low-risk groups (HR 6.12, 95% CI 3.09–12.10) are lower than those for the comparisons of CDS-defined groups (previously discussed) in the external validation dataset. Perhaps an even more

important advantage with the CDS system compared with conventional stratification is the marked increase in the proportion of patients classified as low risk (41.4% versus 13.2% in the external validation dataset). Given the well-described fact that these patients have excellent survival, this advantage translates into a substantial increase in the number of patients for whom the disadvantages of adjuvant chemotherapy could outweigh the benefits. Omitting adjuvant chemotherapy in these patients should be safe, in particular, if combined with serial monitoring of ctDNA during follow-up to initiate treatment in a timely way in the very few patients who might eventually have disease recurrence. We evaluated the ability of biomarkers such as tumour differentiation, lymphatic invasion, venous vascular invasion, stroma to tumour ratio, Immunoscore, and *KRAS*, *BRAF* and MMR/MSI status to supplement the CDS system, but none was confirmed to improve the prognostic value when adjusting for the multiple comparisons.

Decisions based on tissue-based biomarkers versus ctDNA

Basing adjuvant treatment decisions on tissue-based biomarkers has the clear advantage that relevant data can be available within a few days of surgery, providing adequate time for discussing the findings and planning any adjuvant treatment before starting the treatment is suitable. By contrast, if clinicians only use ctDNA to guide treatment, relevant data will not be available until several weeks after surgery. Indeed, assessment of ctDNA status is recommended 4–8 weeks after surgical resection, because cell-free DNA from surgical trauma remains elevated for up to 4 weeks. A plausible alternative would involve complementing results from tissue-based biomarkers with ctDNA to identify more patients in need of adjuvant treatment. In comparison to analysing ctDNA in all patients, offering adjuvant treatment to those deemed to have high-risk disease based on tissue-based biomarkers and assessing ctDNA only in those with low-risk disease would enable initiation of treatment in the adjuvant setting in more patients, and a reduction of economic costs and personnel requirements. This precision oncology approach also has clear advantages over using only tissue-based biomarkers, because it reduces the possibility of some patients being classified as low risk using tissue-based biomarkers even though they could later have disease recurrence and die (Fig. 3).

Conclusions

Experts in the field now acknowledge that combining conventional histopathological methods (including assessment of MMR/MSI status), artificial intelligence-generated digital pathology tools and ctDNA

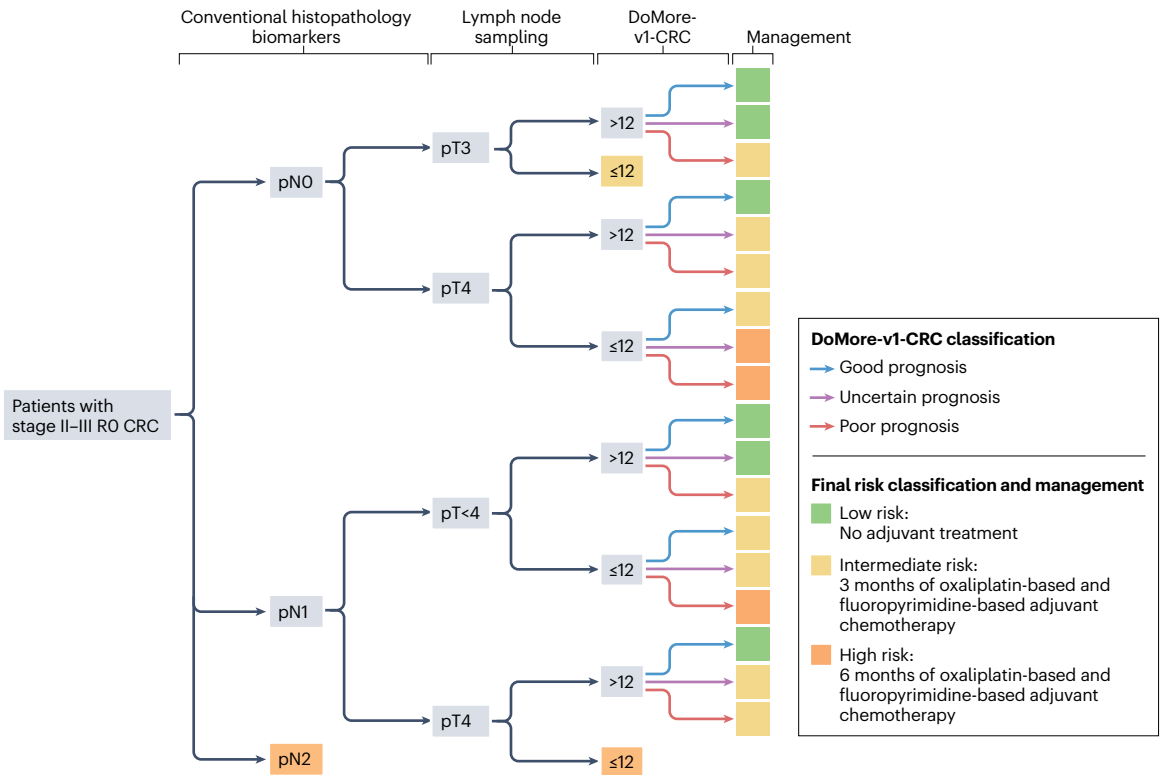

Fig. 2 | Combination of digital biomarker and conventional histopathological prognostic indices. Depiction of how DoMore-v1-CRC[113], a specific digital biomarker, integrates conventional histopathology biomarkers (pN stage, pT stage and number of sampled lymph nodes) to risk-stratify patients more accurately and personalize adjuvant treatment. DoMore-v1-CRC classifies a patient as having good, uncertain or poor prognosis based on an automatic analysis of an image of a histopathology section. The biomarkers are combined in a decision tree that was developed with the aim of providing a risk stratification system that could easily be used to support clinical decisions on adjuvant chemotherapy. The resulting recommendations are no adjuvant treatment in patients with low risk, and 3 months or 6 months of adjuvant chemotherapy in those with intermediate or high risk, respectively. The lack of treatment in low-risk patients could be combined with serial monitoring of circulating cell-free tumour DNA during follow-up. An alternative in intermediate-risk patients might be 6 months of capecitabine only. Finally, high-risk patients could be considered for participation in trials investigating the efficacy of more intense adjuvant treatments because the benefit of such regimens might be optimal in these patients. The proposed clinical decision support system exemplifies how the integration of different tissue-based biomarkers can guide the selection of adjuvant treatment and further personalize cancer care. CRC, colorectal cancer.

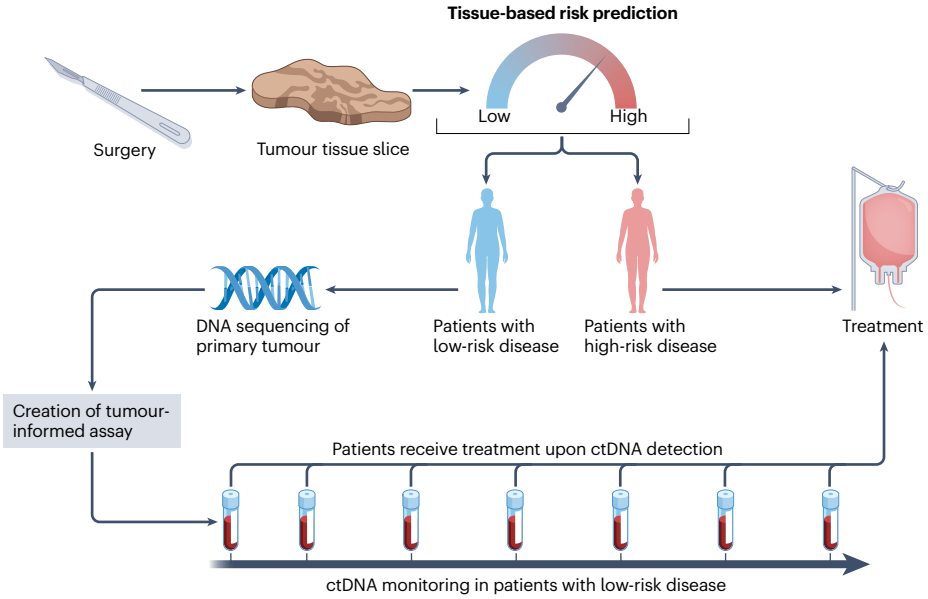

Fig. 3 | Combination of tissue-based biomarkers and ctDNA to improve patient management. After surgery, tissue derived from colorectal cancers can be characterized using different pathological, artificial intelligence and molecular biomarkers. These characteristics can be integrated to form a tissue-based risk prediction available only a few days after surgery. Patients classified as having high-risk disease can be offered adjuvant treatment based on their individual risk profile. Those with low-risk disease can undergo serial monitoring for circulating cell-free tumour DNA (ctDNA) during follow-up. To use a tumour-informed ctDNA assay, DNA sequencing of the primary tumour is performed to detect patient-specific genomic alterations. A tumour-informed assay is then created to identify mutational signatures detected in the primary tumour in blood ctDNA (using a liquid biopsy approach). Serial monitoring of ctDNA can be performed in low-risk patients no earlier than 4 weeks after curative surgery and every 8–12 weeks afterwards. Patients with molecular residual disease detected in ctDNA assay should immediately receive adjuvant treatment, typically oxaliplatin-based and fluoropyrimidine-based chemotherapy. By enabling initiation of treatment in the adjuvant setting for more patients, reduction of economic costs and personnel requirements, and identification of more patients with high-risk disease, this precision oncology approach improves the management of patients compared with approaches using only tissue-based biomarkers or ctDNA monitoring.

analysis will enable increasingly refined prognostic stratification of patients with CRC after surgery, might help to select certain patients for neoadjuvant treatment and will influence discussions about the relative benefits of adjuvant chemotherapy between clinicians and patients. The use of adjuvant chemotherapy is being made safer by using pharmacogenetic analysis of *DPYD* variants (which is being refined through further genome-wide association studies) or phenotypic assessment of enzyme activity, and other pharmacogenomic biomarkers are being explored.

Evidence suggests that the proportional reduction in recurrence risk or improvement in cancer-specific survival (20–25%) with adjuvant chemotherapy is relatively constant across the different prognostic risk groups, and therefore supports rational decision-making[20,32]. For example, in patients with a predicted 5-year OS of 90–95%, the harms from adjuvant chemotherapy outweigh the benefits; those with a predicted 5-year OS of 80%, 70–80% and 30–70% would receive single-agent capecitabine, 3 months and 6 months of treatment with FOLFOX or CAPOX, respectively; and those with a predicted 5-year OS of <30% would be considered for trials of novel treatments or dose-intense regimes, such as FOLFOXIRI. This concept could be further tested in a prospective randomized clinical trial; for example, a non-inferiority design and a cohort size of ~2,000 patients would enable comparing the use of conventionally available pathological data with the use of novel tools (such as the CDS system) to determine which patients do and do not have low-risk disease and thus could be spared from or receive adjuvant chemotherapy, respectively. These trials could also incorporate ctDNA analysis in treatment decisions. The results of these interventions will enable a new paradigm in which patients with CRC will receive truly personalized adjuvant chemotherapy.

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All the authors researched data for the article, contributed substantially to discussion of the content and wrote the manuscript. L.Y., A.K., H.E.D. and D.J.K. reviewed and/or edited the manuscript before submission.

Competing interests
H.E.D. reports filing a patent application entitled "Histological image analysis" with International Patent Application Number PCT/EP2018/080828. A.K. and H.E.D. report filing a patent application entitled "Histological image analysis" with International Patent Application Number PCT/EP2020/076090. A.K., H.E.D. and D.J.K. are founders of DoMore Diagnostics. D.J.K. is a founder of Oxford Cancer Biomarkers. The other authors declare no competing interests.

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