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Review

REVIEW

Challenges and solutions in patient treatment strategies for stage II colon cancer

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Abstract

Colorectal cancer remains one of the most common cancers worldwide and, despite improvements in treatment options for late-stage metastatic cancer, there are still questions surrounding how best to treat early-stage disease patients. Some recent advances have been made in the staging of cancer and improving the risk assessment of strategies for patient treatment. A number of high-risk features have been proposed that may help to stratify stage II cancer patients into groups that will truly benefit from adjuvant chemotherapy. Diagnostic tests are becoming available to measure these biomarkers, utilizing both currently available and novel technologies. This review will describe the challenges in treatment decisions for early-stage colon cancer and how personalized medicine can assist clinicians in making the best treatment choices for patients with stage II colon cancer in particular.

Key words: Colorectal cancer; treatment strategy; challenge

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, with a predicted 1.85 million new diagnoses worldwide in 2018, accounting for 10.5% of all cancer diagnoses—a number that is continuing to increase yearly. CRC accounts for 9.2% of all cancer-related deaths and, this year, CRC is projected to be responsible for 27.5% of all cancer-related deaths in Europe and 52.4% in Asia [1]. It is hoped that an increase in the adoption of nationwide screening programmes to facilitate earlier detection of CRC and improved treatment options for these patients would reduce the mortality rates associated with CRC over the coming years. The prevalence of CRC carries an economic burden to healthcare systems, resulting in approximately 10% of total cancer-related costs, with annual costs in the European Union of €13 billion. In the UK alone, 41,804 CRC new cases were diagnosed in 2015, accounting for 12% of the total cancer diagnoses. Of these CRC diagnoses, approximately 10% of patients are diagnosed with stage I disease, 35% are diagnosed with stage II disease, 35% with stage III disease and 20% with stage IV CRC [2–4].

Whilst treatment options for stage III and IV CRC patients are more straightforward, with clinical guideline information to assist clinicians in making treatment choices for their patients,

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the situation for stage II cancer patients is more unclear. This issue will be exacerbated by the fact that national screening programmes, with the aim of detecting patients with early-stage (stages I–II) CRC, will hopefully result in more patients being diagnosed at an earlier stage where there continues to be a debate over optimal management.

There is significant variation in choice of adjuvant therapy for stage II CRC patients internationally. In the UK, 50%–60% of stage II CRC patients receive adjuvant chemotherapy, a proportion of which is administered in combination with agents such as fluoropyrimidines and oxaliplatin, potentially over-treating the general population of patients so that a small minority might benefit. In the QUASAR (Quick and Simple And Reliable) study, it was demonstrated that adjuvant chemotherapy improves survival of stage II CRC patients, but that absolute improvement in overall survival (OS) was minimal (in the region of 3%–4%) [5]. A further implication of the over-treatment of patients is that the toxicity profile associated with adjuvant chemotherapy has a negative impact on the quality of life of patients [6].

The advancement of methods to better classify patients with early-stage colon cancer (e.g. a better delineation of stage IIA and stage IIB colon cancer patients) and the development of strategies for more personalized medicine in the form of *in vitro* diagnostic tests that can inform on the likely success of chemotherapy in these patients, may allow the populations of patients who will truly benefit from chemotherapy to be identified and treated appropriately, whilst those who have a high chance of cure by surgery alone can avoid toxic chemotherapy that is unlikely to give any survival benefits.

This review will focus on the recent advances in the staging of CRC and current and emerging treatment strategies for colon cancer patients, focusing on stage II colon cancer patients in particular. The currently available methods of risk assessment to determine chemotherapy offering that results in minimal risk to patients will be outlined, exploring the prognostic effects of available but currently underutilized tests such as microsatellite instability (MSI)/mismatch repair (MMR) and mutational testing. Newly available tools and those in development will also be discussed; the adoption of tests into clinical guidelines can stratify patients into groups of stage II colon cancer patients who may or may not benefit from standard adjuvant chemotherapy and those whom clinicians may recommend alternative approaches to.

For colon cancer grading and staging, is the current classification system fit for purpose, particularly in the case of stage II colon cancer patients?

In colon cancer, the staging of tumours is based on TNM classification, which has been the accepted standard for over 50 years, with the American Joint Committee on Cancer (AJCC) staging and the Union for International Cancer Control (UICC) TNM Classification of Malignant Tumours manuals now in their 8th editions [7, 8]. This system gives a clear indication of prognosis following tumour resection based on the primary and regional nodal extent of the tumour and the absence or presence of metastases. There have been some attempts to modify this classification to improve patients' outcomes, particularly in early-stage non-metastatic cancer, where more emphasis is placed on the importance of T category [9]. There is evidence from cancer registry analysis that enhanced weighting of T category in early-stage colon cancer patients may provide clinicians with an improved system of classifying colon tumours [10]. However, as the increasing need for personalized medicine and enhanced models of risk in cancer patients that can be adopted by the clinical community is recognized [11], further studies to validate this approach will be required.

Clinical guidelines have been developed to standardize the care of patients after diagnosis and to aid the physician in determining the appropriate treatment strategies for patients. The National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) guidelines are most commonly used by physicians to aid in treatment decisions [12, 13]. However, the information available to oncologists can often be complex, and therefore decisions must be made in the context of the wider multidisciplinary teams and additional information on tumour pathology and patient comorbidities, which may impact treatment decisions. For patients with non-metastatic cancer, risk of recurrence is closely linked to pathological stage. Generally, in stage I CRC patients, 5-year survival after tumour resection (but without adjuvant chemotherapy) is 85%–95%. Accordingly, stage I CRC patients are spared adjuvant chemotherapy. Stage III CRC patients have a 5-year survival after surgical resection of 30%–70%. In these patients, the proportional risk of death can be reduced by 20%–25% by using 5-fluorouracil (5-FU) and oxaliplatin combination therapies. It is therefore recommended that stage III CRC patients receive doublet combination chemotherapy.

In stage II colon cancer patients, according to a number of studies comparing surgical resection alone vs surgical resection and adjuvant chemotherapy, the 5-year OS after surgical resection alone is approximately 80% [5]. Stage II colon cancer patients can be divided into stage IIA (pT3N0), stage IIB (pT4aN0) and stage IIC (pT4bN0) groups, where in all cases the tumour has yet to spread to the lymph nodes. Nevertheless, a proportion (around 20%) of stage II cancers carry the risk of micrometastatic disease and the main purpose of adjuvant therapy after surgery is to destroy these micrometastases before they develop further [14]. This suggests that patients with stage II CRC are most likely composed of a heterogeneous population of patients that consists of those curable by surgery alone (80%), those with micrometastatic disease that may not be susceptible to adjuvant chemotherapy (16%) and those with micrometastatic disease that would be eradicated by adjuvant chemotherapy (4%). Due to the wide range of survival within this group and the complexities of determining which category a given patient may fall into, the indication for adjuvant chemotherapy is less clear. Adjuvant chemotherapy is not routinely offered to stage II colon cancer patients unless they are deemed to be high-risk, where their tumours have other 'high-risk' pathological characteristics such as poor differentiation, tumour perforation, vascular, perineural or lymphatic invasion. They may also be considered high-risk if there was inadequate sampling of lymph nodes or presented as an emergency with bowel obstruction [12, 13]. Stage and grade of tumour should be considered using the above and any other important factors in order to reach a clinical decision on whether adjuvant chemotherapy is appropriate. However, these considerations alone, which are rather subjective and have low concordance rates when comparing individual pathologists, are not adequate to accurately designate stage II colon cancer patients into either low or high risk of recurrence. Whilst the 5-year OS rate for stage II colon cancer patients after surgery alone has typically been accepted as up to 80% due to the results of randomized studies

comparing surgical resection alone vs in combination with adjuvant therapy [15], there is increasing evidence that improved stratification of stage II colon cancer patients into groups that reflect the risk of micrometastases should improve patient outcomes. It follows that there is an unmet clinical need for better predictive markers to risk-stratify patients within this stage II subset so that there is a better understanding of patients who are at the greatest risk of recurrence and therefore who would most benefit from adjuvant chemotherapy. This would delineate clearer and more personal treatment pathways for patients within this group, minimizing over-treatment.

Chemotherapy strategies for early-stage colon cancer

Since the clinical introduction of 5-FU in the 1950s and subsequent introduction of the oral prodrug capecitabine [16], improvements in the treatment of colon cancer have been modest, with an emphasis on the administration of 5-FU in the adjuvant setting in combination regimens with leucovorin and oxaliplatin [17]. The decision on whether to administer adjuvant chemotherapy to patients and the specific types of regimens used is often based on patient comorbidities and associated toxicity profiles of the individual agents. Targeted therapies, such as monoclonal antibody therapy, which targets vascular endothelial growth factor such as bevacizumab and epidermal growth factor receptor (*EGFR*) such as cetuximab and panatimumab, have also been investigated as therapeutic options but have been shown to be of no benefit in the adjuvant setting in early-stage disease [18, 19].

Adjuvant chemotherapy in this setting would aim to eradicate the micrometastases remaining after surgical resection, thereby reducing the risk of metastatic-disease recurrence. A number of studies have been carried out to try and elucidate the true benefits of adjuvant therapy in stage II colon cancer patients (including those referenced here) but there are a number of caveats such as the use of retrospective analysis and meta-analysis combining patients treated with numerous different types of therapies and combinations where data interpretation has proved difficult, and where the true benefits may be difficult to determine. In addition, as stage II colon cancer patients only make up approximately one-third of CRC patients, with relatively low recurrence rates, a significant number of patients need to be enrolled and followed up for at least 5 years in order to identify any true benefits of adjuvant chemotherapy after surgery.

The current evidence for the benefits of adjuvant therapy in stage II colon cancer relies on data from the from the QUASAR and NSABP (National Surgical Adjuvant Breast and Bowel Project; C-01 to C-04) studies [5, 20]. The NSABP studies compared surgery alone to a range of adjuvant therapies in patients with stage II and stage III colon cancer. These individual studies suggested that adjuvant chemotherapy may provide a benefit over surgery alone and, in a combined analysis of all four trials comprising 1565 stage II colon cancer patients, the conclusion was that adjuvant chemotherapy decreased risk of death and improved cure for stage II colon cancer patients regardless of the presence or absence of high-risk features [20]. The aim of the QUASAR trial was to assess the potential benefits of adjuvant chemotherapy in patients with low-risk colon cancer and this trial enrolled 3239 stage II colon cancer patients who were randomized into two arms: surgery alone or surgery plus bolus 5-FU-based chemotherapy. Only a small proportion (628 patients) in this study had data available regarding high-risk features such as T4 status and vascular invasion. This study demonstrated a small absolute improvement in survival of 3.6%, with the benefits most obvious in the first 2 years after surgery [5]. More recently, the Phase III MOSAIC trial (Multicenter International Study of Oxaliplatin/5-FU/LV in the Adjuvant Treatment of Colon Cancer) [21] looked at the role of oxaliplatin used in combination with 5-FU (FOLFOX). However, while a small benefit was identified in stage III colon cancer patients, there was no improvement in OS in stage II colon cancer patients, even when these patients were divided into high- and low-risk groups [22].

Additional studies such as the NSABP C-07 trial [23] have also demonstrated the limited role for oxaliplatin in the stage II setting. There is some debate around whether the current standard for oxaliplatin containing adjuvant chemotherapy of 6 months of treatment is the most effective duration for patients with stage III and high-risk stage II colon cancer [24]. Some studies such as the large-scale international short-course oncology therapy (SCOT) study [25] have demonstrated that 3 months' duration of treatment has comparable efficacy to 6 months, whilst being associated with reduced toxicity and improved quality of life. It is important to consider both patient-survival outcomes and quality of life related to the side effects of chemotherapy, particularly where it is used in combination when considering what may be the best standard of care for these patients.

Risk assessment for colon cancer chemotherapy

The adverse events (AE) following treatment with chemotherapy are diverse and can vary greatly in terms of severity. 5-FU carries with it a range of toxicities, including vomiting, diarrhoea, neutropenia, thrombocytopenia, stomatitis/mucositis and palmar plantar erythrodysesthesia, also known as hand and foot syndrome. Approximately 20% of patients will experience grade 3/4 severe toxicities and 0.5%–1% of patients treated with 5-FU will suffer fatal toxicity, which is often associated with severe diarrhoea, neutropenia, thrombocytopenia or cardiac symptoms [26, 27]. The most common agent used in combination with 5-FU, oxaliplatin, is also associated with sensory peripheral neuropathy, which can be disabling and have an impact on the quality of life in patients where chemotherapy is successful and some efforts have been made to identify those patients at greatest risk of these side effects and how to manage their treatment [28].

The cost to patients, families and healthcare providers of severe fluoropyrimidine-induced or oxaliplatin-induced toxicity in combination therapies is considerable. The ability to anticipate a patient's likelihood of developing life-threatening toxicity would allow dose modification that could save lives without compromising the efficacy of treatment. There has been a concerted effort to identify and validate the relevant genetic pathways and potential biomarkers to allow fluoropyrimidine toxicity to be predicted [29, 30]. Metabolism of fluoropyrimidines involves a complicated enzymatic pathway involving the activity of a number of genes including dihydropyrimidine dehydrogenase (*DPYD*), which is responsible for catabolism of pyrimidines. After *DPYD* was first associated with 5-FU toxicity [31], a number of genetic variations in the *DPYD* gene were subsequently linked to fluoropyrimidine toxicity including large-scale studies and meta-analyses [6, 32, 33].

The association of 5-FU and capecitabine use with these dose-limiting toxicities is well documented and *DPYD* status is included as a contraindication in the summary of product characteristics for these drugs as approved by the European Medicines Agency (EMA) [34] and the Food and Drug Administration (FDA) [35]. These pieces of literature outline that *DPYD* deficiency is associated with rare, unexpected and severe toxicities, such as stomatitis, diarrhoea, mucosal inflammation, neutropenia and neurotoxicity in patients treated with 5-FU. Patients with low or absent *DPYD* activity are outlined as being at increased risk of severe, life-threatening or fatal adverse reactions and, although this status is difficult to define, it has been linked to certain homozygous or compound heterozygous mutations in the *DPYD* gene locus causing a complete or nearly complete loss of *DPYD* gene enzymatic activity. 5-FU administration in these cases would be contraindicated. Patients with partial *DPYD* deficiency (heterozygous mutations in *DPYD*) may be at greater risk of these adverse effects and it is important for the clinician to be able to assess the risk, taking into account the suitability of the treatment and, where prescribed, carry out dose adjustment as necessary and monitor patients closely.

Clinical guidelines have been developed to attempt to consolidate the wealth of data around fluoropyrimidine toxicity [36] where the most robustly investigated genetic variants associated with these severe AE have been identified. There are a number of genetic tests available and in development that include the genetic variants recommended in these guidelines and other parameters [37–39]. The link between the *DPYD* pathway and 5-FU toxicity is complex and does not take into account other severe toxicities such as cardiotoxicity, which may be found in as many as 1%–4% of patients and which can manifest in a variety of different ways, such as unstable angina, myocardial infarction, arrhythmia and sudden death. There are also important considerations to be made with regard to the ethnicity of patients; to date, most studies have focused on Caucasian populations in the USA and Europe, but recent studies, including a systematic analysis of population-scale genetic databases in large patient populations, have demonstrated allelic frequency differences in patients of South Asia [40]. In patients with East Asian ethnicity, including Chinese patients, there is increasing evidence that genetic variants of the *DPYD* gene associated with toxicity differ from those linked to toxicity in Caucasian patients [41, 42], although large-scale studies will be required to fully determine the genetic profile of Chinese patients most at risk, with these differences most likely due to the frequency of these genetic variants in any given population. Genetic tests developed to determine the risk of severe AE in large and ethnically diverse populations must take these differences into consideration when offering any genetic testing to patients.

Genetic testing to assist clinicians in determining the risk of severe AE and even death in these patients will be an important tool going forward, particularly in stage II colon cancer patients where treatment decisions are already complex. In addition to better understanding the benefits of chemotherapy for stage II colon cancer patients in terms of disease-free survival (DFS) and OS, it is also important to weigh up the risks associated with these therapies. The development of better tools and tests for stratification into responder groups where predictive biomarkers can allow improved therapeutic choices, together with tools that also help to determine which patients are at a high risk from AE, will be useful to clinicians in determining which patients will truly benefit from treatment after surgery.

Tools available for predicting chemotherapy benefits

The clinical guidelines published by the NCCN, American Society of Clinical Oncology and ESMO identify high-risk features that collectively include pathologic T4 status, poor differentiation, perforation, lymphovascular and perineural invasion and inadequate sampling of lymph nodes. These features are based on evidence from numerous studies associating these characteristics with high-risk stage II disease; consideration of adjuvant therapy is recommended in stage II colon cancer patients where high-risk features have been identified [12, 13]. Guideline recommendations can be rather subjective and previous studies have shown that concordance between different observers has been rather low for these high-risk features. The adoption of incorporating additional features of the tumour and biological markers, particularly where high-risk disease status is unclear, are being studied to determine whether they can enhance patient outcomes in stage II colon cancer patients.

The position of the tumour in the patient's colon (right or left side) can be used as a general prognostic tool for colon cancer where right-sided tumours are associated with poor prognosis, as they are typically poorly differentiated with a higher rate of mutations and more likely to be diagnosed at later stages of disease when compared with left-sided tumours. In terms of relevance in stage II disease, there are conflicting studies, with some indicating better prognosis in right-sided tumours and others showing no significant difference in the prognosis of tumours located on either side [43]. Further studies will be required to determine the true value of sidedness as a prognostic tool in stage II colon cancer.

Additional markers have been identified that may allow clinicians to stratify patients into low- vs high-risk categories and inform on treatment decisions. Approximately 15% of all tumours have a deficiency in DNA mismatch repair (MMR) genes and associated MSI. MSI status is classified as MSI-high (patients with deficient MMR status, MSI-H) or MSI-low (patients with proficient MMR status, MSI-L). MSI-H status is associated with improved OS and reduced lymph-node spread and metastasis in colon cancer [44]. MSI has also been shown to be a predictive marker for 5-FU therapy resistance [45]. The prognostic and predictive value of MSI status has important clinical implications, and usually supports the decision not to offer adjuvant chemotherapy to this subgroup of stage II colon cancer patients. MSI status may also inform on the suitability of immune checkpoint monoclonal antibody therapy in some patients [46].

Mutations in the *BRAF* gene, which encodes the B-Raf proto-oncogene serine/threonine kinase, particularly those with the V600E mutation (occurring within the activation segment of the kinase domain), seem to be significantly associated with not only reduced OS, but also MMR status, with the highest OS rate seen in patients with *BRAF*-wild type and MMR-deficient tumours (5-year OS, 89.7%) and the lowest OS rate in patients with *BRAF*-mutant and MMR-proficient tumours (5-year OS, 69.1%) [47]. Further studies should help to determine the significance of this finding in stage II colon cancer patient populations. *KRAS* (Kristen rat sarcoma) mutations are found in approximately 40% of colon cancers and lead to activation of the RAS/MAPK signalling pathway. Whilst *KRAS* status may play a role in predicting the efficacy of monoclonal antibody therapy in advanced metastatic disease such as cetuximab [48], there seems to be limited prognostic value for this mutation, as demonstrated in the PETACC-3 trial (Pan European Trial Adjuvant

Colon Cancer) in which no significant benefit in relapse-free survival or OS was demonstrated [49].

Other potentially important markers include loss of 18q heterozygosity as a surrogate marker for chromosomal instability during mitosis. However, despite some clinical trials in this area, including two Phase III co-operative trials [50], no sufficient association between 18q loss of heterozygosity and 5-year DFS or OS was identified. Dysregulation of microRNA (miRNA) expression, where these non-coding RNAs prevent the gene expression of target messenger RNA and disrupt transcription, has also been associated with prediction of MSI status and disease recurrence in MSI stable stage II colon cancer and may prove to be a helpful tool in successfully identifying patients with poor prognosis [51].

More recently, there have been advances in gene-expression profiling being used as both a prognostic and a predictive tool in the management of cancer. The emerging significance of circulating tumour DNA as a marker of residual micrometastatic disease may prove useful [52], particularly where there is a lack of tools available, such as in the case of stage II colon cancer. However, this is likely to require the utilization of further developments in technology, particularly with regard to sample enrichment [53], as current approaches have only demonstrated clinical utility in later-stage disease [54].

Caudle type homeobox 2 (*CDX2*) expression analysis by immunohistochemistry (IHC) has been shown to be a prognostic factor in stage II and III CRC according to Tomasello *et al.* [55]. Low *CDX2* expression was been shown to be a negative prognostic factor distinguishing between high and low mortality/disease progression with a hazard ratio (HR) of 0.5 [95% confidence interval (CI) = 0.38–0.66, $P < 0.001$] in a large 14-study meta-analysis and has appeal based on its accessibility and low cost. However, a more recent retrospective study assessing *CDX2* levels by expression and IHC was only able to demonstrate prognostic value with respect to 5-year OS in stage IV CRC patients with a HR of 2.38 (95% CI = 1.26–4.48, $P = 0.0074$) [56], demonstrating potential utility in guiding adjuvant chemotherapy in stage III CRC patients.

Oncotype Dx Colon® (Genomic Health) can be used in patients with a positive colon biopsy to provide a colon recurrence score to determine the aggressiveness of the tumour and therefore the risk of recurrence after surgery. This assay consists of 12 cancer-related genes (representing androgen signalling, stromal response, cellular organization and proliferation) and provides a discrimination between patients with good and poor survival profiles with a HR of 1.47 (95% CI = 1.01–2.14, $P = 0.046$) [57]. These tests are expensive, costing several thousand dollars per test; whilst Oncotype Dx Breast® has been recommended for use in the USA, the clinical performance of Oncotype Dx Colon has precluded its use as a prognostic test in the clinical setting. Other gene-expression-based tests include (i) ColoPrint (Agendia) consisting of an 18-gene-expression panel with potential clinical utility in stage II CRC, which assesses the risk of recurrence (high vs low) and thus the benefit of adjuvant therapy (HR = 2.16, 95% CI = 1.28–3.65, $P = 0.004$) [58], and (ii) ColDX (Almac), a 634-probe signature gene-expression profile, grouping patients into high vs low risk of recurrence/cancer-related death and thus informing on the likely benefit of adjuvant chemotherapy (HR = 2.13, 95% CI = 1.3–3.5, $P < 0.01$) [59]. The performance of gene-expression-profiling-based prognostic tests has the caveat of being reliant on the extraction of good-quality RNA from tumour-tissue samples and the use of gene-expression technology on samples, which can drive up the cost and reduce the cost-effectiveness of such tests to healthcare systems. An approach that can use digital pathology to provide a measure of the risk of recurrence that can be adopted into current treatment pathways would be advantageous.

A number of studies provide strong evidence supporting the prognostic use of ploidy status as a marker of chromosomal instability measured by DNA cytometry and increasingly by digital microscopy in a number of cancer and pre-cancerous lesions, including prediction of disease progression of ulcerative colitis towards CRC and prognosis in patients with stage II carcinomas of both the colon and rectum, reviewed in Danielsen *et al.* (2016) [60]. More recently, a novel methodology for the analysis of chromatin structure (nucleotyping) within tumour cells was used to assess prognosis in patients with early-stage CRC including patient samples from the QUASAR 2 study, which demonstrated that nucleotyping could stratify patients more precisely than MSI where patients with heterogeneous chromatin had worse prognosis than those with homogeneous chromatin [61]. It will be important to develop the technology for studying both DNA and chromatin status as prognostic tools for stage II colon cancer patients given the evidence of their role in tumour progression.

There is also some evidence that the tumour microenvironment plays a role in tumour progression. One method by which the tumour microenvironment can be measured is via the proportion of epithelial tumour tissue vs stroma in tumour-tissue sections. The tumour-stroma percentage has been confirmed as a prognostic factor in CRC studies in stage II and III CRC patients from the VICTOR trial, with OS and DFS being significantly lower in patients with a high percentage of tumour stroma (>50%) with 5-year OS and DFS for stroma-high vs stroma-low patients of 69.0% vs. 83.4% and 58.6% vs. 77.3%, respectively [62]. The use of such parameters in combination with current pathological assessments could provide a low-cost addition to the TNM status and, for example, MSI status. These two parameters were combined in a study by Danielsen *et al.* [63] in which the prognostic value of the combination biomarker was assessed in 2624 patients with early-stage CRC, including over 1000 patients with stage II disease. In this study stage (II vs. III and pT4 vs. pT3), ploidy, stroma-tumour fraction, tumour grade and age were all found to be significantly prognostic alone, with ploidy and tumour-stroma fraction significantly prognostic in multivariate modelling. The combination of DNA ploidy and tumour-stroma fraction allowed stratification of stage II CRC patients into three clinically useful groups with 5-year cancer-specific survival (CSS) rates of 90% vs. 83% vs. 73% (HR = 1.77, 95% CI = 1.13–2.77 and HR = 2.95, 95% CI = 1.73–5.03, $P < 0.001$), respectively (Figure 1) [63].

Given the increasing evidence of the immune system in cancer progression, studies have been conducted to determine the impact of infiltrating T-cells on risk of recurrence. The Immunscore® Colon test from HalioDx assesses the risk of recurrence and thus the benefit of adjuvant therapy by measuring the presence of $CD3^+$ and $CD8^+$ T-cells in tumour-tissue samples and assigns a score based on the level of immune-cell infiltration. This score is linked to a patient risk category based on total time to relapse. The assay identifies a subgroup of high-risk (immunoscore-low) patients who are likely to benefit from adjuvant chemotherapy and has clinical utility in stage II and stage III colon cancer patients (stage II high vs. low immunoscore, HR = 3.03, 95% CI = 1.92–4.76, $P < 0.001$) [64]. It is becoming increasingly apparent that the context of the tumour microenvironment is very important in

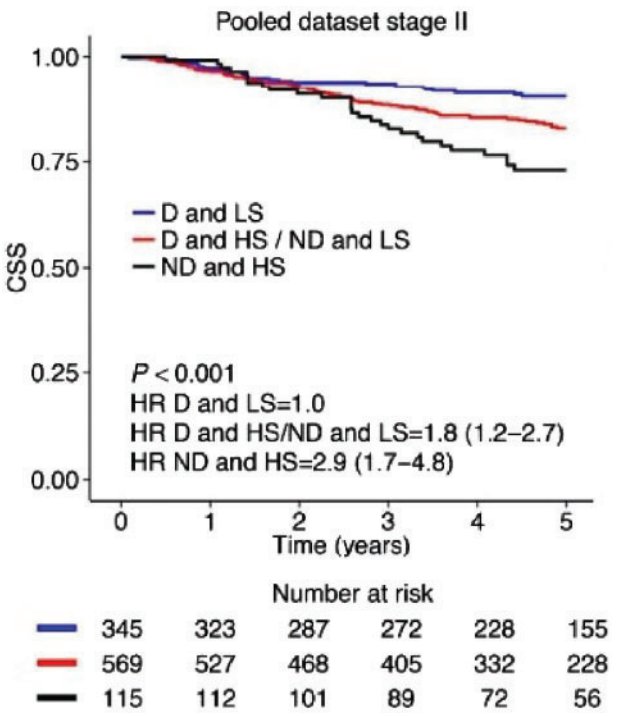

Figure 1. Kaplan–Meier plot illustrating cancer-specific survival (CSS) for stage II patients with tumours that were diploid and low stroma (D and LS), diploid and high stroma or non-diploid and low stroma (D and HS/ND and LS) and non-diploid and high stroma (ND and HS). Taken from [63]. Reproduced from an original article published in *Annals of Oncology* © ESMO with the authors' permission.

tumour progression in stage II colon cancer and the development of future tools to assess this in a cost-effective manner will provide clinicians with enhanced tools to allow them to fully assess the risk/benefit ratio of adjuvant chemotherapy. Table 1 outlines the performance characteristics and further details on prognostic tests currently available for stage II CRC.

Future tools for the stratification of early-stage colon cancer patients

The diagnostic value of clinical genomics is fundamentally limited by the short read lengths achievable with established non-NGS (next-generation sequencing) technology. Multiple potential sources of inaccuracy are encountered between the production of a pool of sequenced short reads, through assembly and alignment, before finally calling and reporting the sequence data [65]. Highly polymorphic regions pose a challenge to assembly, particularly in terms of computational burden. In addition, regions of repeated sequence, short-term repeats, segmental duplication, pseudogenes and transposon-derived repeats all contribute to confounding attempts at identifying the correct genomic position of a given read and thus give rise to an unacceptable loss of accuracy for clinical purposes.

These technical issues are at the heart of the current rush to establish long-read NGS technology in a clinical setting. Emerging NGS technologies such as those under development by Oxford Nanopore promise to deliver reliable, low-cost sequencing platforms with read lengths in the hundreds of kilobases. Access to such technology will vastly improve the ability of clinicians and researchers to accurately identify clinically relevant variants such as single-nucleotide polymorphisms and indels [66], but also to identify clinically relevant structural motifs that are currently beyond our capabilities.

Despite its limitations, non-NGS technology has already proved to be an effective tool for stratifying patient populations. Screening for specific mutations in *EGFR* and anaplastic lymphoma kinase (*ALK*) are established techniques used for targeting specific kinase-inhibitor therapies. Combining genomic data with checkpoint inhibition therapies is currently exhibiting a great deal of promise, particularly with respect to immune-oncology therapies such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death-ligand 1 (PD-L1) [67].

Applications such as pharmacogenetics have also shown promise, particularly in targeting therapies, not only in terms of predicted efficacy, but also for the purpose of avoiding serious AE. With respect to CRC, one of the most prescient applications is the genotyping of patients ahead of receiving fluoropyrimidine-based chemotherapy in order to identify those at risk of severe toxic AE [39]. Liquid biopsy refers to the collection and analysis of circulating tumour cells, cell-free nucleic acid and tumour-derived exosomal vesicles that have been shed by the tumour or metastatic site into a patient's blood or other fluids. An extensive array of high-quality studies have recently been published demonstrating the ability to extract clinically relevant information using such approaches [68]. Furthermore, diagnostic techniques using liquid biopsy to direct anti-EGFR treatment in no-small-cell lung cancer (NSCLC) have been approved for use in Europe and the USA. As such, it is clear that the potential for liquid biopsy in detection, guiding treatment and monitoring is significant.

With respect to CRC, a number of potential liquid biopsy biomarkers have been investigated. Investigations focusing on the extraction and sequencing of cell-free DNA (cfDNA) have shown the potential utility of liquid biopsy in assessing the *KRAS*, *BRAF* and *p53* status of a specific tumour [69]. Studies using quantitative reverse transcription PCR (RT-qPCR)-based technologies have shown the significant potential of individual microRNAs (miR-221, miR-21, miR-92a, miR-141 and miR-155) in terms of early detection and as prognostic parameters. Furthermore, a few studies have demonstrated the potential utility of multiplexed panels of microRNA with the ability to detect early-stage disease [70].

Digital pathology promises huge improvements in many aspects of primary and secondary diagnoses, from increased access to specialist pathology services to speedier determination of surgical margins. Further to this, the digitization of high-quality whole-slide images, driven by access to appropriate hardware, opens up a huge range of possibilities around the use of static image-analysis algorithms and is increasingly harnessing techniques such as machine learning-based image analysis to assist the pathologist in screening and diagnosing specimens [71]. The utility of digital-image analysis has already been demonstrated clinically over a range of indications at both the gross and histological levels and is commonly referred to as computer-aided diagnosis. Additionally, two of the CRC-specific diagnostic assays mentioned previously use image analysis of tissue as the basis for the underpinning technology [63, 64]. Combining the ease and speed of access provided by digital pathology architectures with the advances in traditional and Artificial Intelligence (AI)-based image-analysis algorithms for histology and cytology would seem to provide an ideal environment for the development of further precision medicine technologies. The current brisk pace of big data/bioinformatics developments is of particular note. Advances in a number of areas are driving this: access to cheap, scalable processing

Table 1. Comparison of commercial tests currently available for stage II CRC, including an assay description, performance figures and validation cohort sizes

Test	Company	Description	Performance	Clinical evidence	References
CDX2	Various	CDX2 expression, FFPE tumour tissue analysed by IHC assay and expression to determine prognosis	Hazard ratio low risk vs. high risk HR = 0.5 (95% CI = 0.38–0.66) $P < 0.001$ Hazard ratio high risk vs. low risk HR = 1.03 (95% CI = 0.63–1.68) $P = 0.91$	Meta-analysis, 6291 patients with colorectal cancer Retrospective study, 422 stage II colorectal cancer patients	[55, 56]
Oncotype DX Colon ®	Genomic Health	Twelve gene signatures, FFPE tumour tissue analysed in a CLIA laboratory to determine whether disease is likely to relapse post surgery	Hazard ratio high risk vs. low risk HR = 1.47 (95% CI = 1.01–2.14) $P = 0.046$	QUASAR, 1436 patients with stage II colon cancer CALGB 9581, 690 patients with stage II colon cancer NSABP C-07, 892 patients with stage II and III colon cancer SUNRISE, 587 patients with stage II and III colon cancer	[57]
ColoPrint	Agendia	Eighteen gene signatures analysed in a CLIA laboratory using fresh frozen tissue to determine whether disease is likely to relapse post surgery	Hazard ratio high risk vs. low risk HR = 2.16 (95% CI = 1.28–3.65) $P = 0.004$	A total of 416 patients with stage II colon cancer	[58]
ColDx	Almac	A total of 634 probe signature gene-expression profiles from FFPE, to determine whether disease is likely to relapse post surgery. Application in stage II CRC	Hazard ratio high risk vs. low risk HR = 2.13 (95% CI = 1.3–3.5) $P < 0.01$	A total of 144 patients with stage II colon cancer A total of 393 patients with stage II colon cancer	[59]
Immunoscore Colon	HalioDx	Digital pathology-based quantification of density of IHC-stained $CD3^+$ and $CD8^+$ T-lymphocytes in the tumour. Application in stages II and III CRC	Hazard ratio high risk vs. low risk HR = 3.03 (95% CI = 1.92–4.76) $P < 0.0001$	A total of 1981 patients with stage II colon cancer	[64]
ColoProg	Oxford Cancer Biomarkers	Digital pathology-based quantification of DNA copy number (ploidy) and tumour microenvironment (stroma) from FFPE, to determine whether disease is likely to relapse post surgery. Application in stage II CRC	Hazard ratio high risk vs. low risk HR = 2.95 (95% CI = 1.73–5.03) $P < 0.001$	QUASAR 2, 394 patients with stage II colon cancer Gloucester, 358 patients with stage II colon cancer OUH-Aker, 277 patients with stage II colon cancer	[63]

FFPE, formalin fixed paraffin embedded; IHC, immunohistochemistry; CLIA, clinical laboratory improvement amendments; OUH, Oxford University Hospitals; QUASAR, Quick And Simple And Reliable; CALGB, Cancer and Leukemia Group B; NSABP, National Surgical Adjuvant Breast and Bowel Project.

power, cheap data storage, open-source-code architectures and access to distributed, connected data-collection systems make the environment ripe for development.

Advances in bioinformatics underpin current and future advances in precision medicine as a necessity, given the increasing complexity of our understanding of disease. A more comprehensive understanding of the multi-parametric nature of disease necessitates the capture, analysis and interpretation of clinically relevant parameters at genetic, epigenetic, protein and pathway levels [72]. The advent of big data and its various enabling technologies allows the capture, storage and sorting of such data, making it a valuable tool in the initial steps towards precision technologies [73]. Given the ever growing list of technologies that allow big data capture, it is likely that significant future developments will centre on better ways to curate and annotate these data, as well as frameworks driving ease of access. The analysis and interpretation of these data are driven by developments in bioinformatics techniques to actionable clinical endpoints. As such, improvements in mathematical techniques driving superior modelling, analysis and interpretation will remain of primary importance in order to realize the true potential of precision healthcare [74].

The clinical utility of precision diagnostic technology is inextricably linked to the ability to extract and interpret clinically actionable information. In ideal cases, novel precision diagnostic technologies will augment current treatment practice in a non-disruptive manner. However, it is clear that, with the accelerating pace of development, there will be a range of technologies that, by their very nature, will be highly disruptive to existing treatment pathways. The latter scenario presents a significant challenge to adoption and thus patient access. In order to counter this, it is imperative that assay manufacturers engage at an early stage in the development process with clinical key opinion leaders as well as healthcare providers and regulators in order to mitigate the challenges in adopting disruptive technologies.

Furthermore, the advent of precision medicine technology presents additional challenges in terms of patient/clinician interaction. Clinicians themselves must be prepared to ensure adequate patient awareness and input into the decision-making process [75]. This is a non-trivial endeavour when clinicians are faced with explaining and interpreting complex diagnostic data and their relevance to a particular case. One approach to dealing with this is to promote engagement between patient advocacy groups, clinicians and manufacturers to provide publicly accessible tools and materials to help give depth and relevance to the diagnostic information from the viewpoint of someone undergoing treatment.

Healthcare providers and insurers will also be impacted by emerging precision medicine technologies. Aside from the changes to existing treatment pathways, providers will have to establish frameworks for supporting more heterogeneous treatment options along with concomitant reimbursement and health economic strategies [76].

Future directions and discussion

Improved screening strategies for the detection of colon and rectal cancer, at a population level, have clear implications in terms of improved patient outcomes connected to early-stage detection. More effective screening strategies would likely shift the proportion of patients presenting such that a significantly larger proportion would be detected at stages I and II and proportionally fewer at later stages III and IV. The implications, in terms of improved patient outcomes, are well documented; CRC treatment outcomes vary greatly, depending on the stage at diagnosis [77]. Additionally, CRC is the third most common cancer worldwide. These factors alone provide a compelling reason to promote such screening programmes with the overall goal of optimizing early-stage diagnosis. In the context of this discussion, the introduction of a precision diagnostic element to such a programme would theoretically allow the targeted deployment of available resources in order to best serve patient subpopulations, not least due to the large number of treatment options available. As such, it is important to consider the economic and practical implications of such programmes within socialized and private healthcare systems. The first of these is to examine the economic and social outcomes of improved DFS rates. Screening programmes of the types described in this review will not affect the overall incidence of disease; however, due to the larger proportion of early-stage detections, it is true to say that the number of individuals requiring multi-intervention late-stage care will decrease. Given that late-stage treatments account for the majority of the costs associated with treatment at a population level [78], and that the cost of treatment increases with stage [79], there is a clear economic argument in favour of such programmes.

In terms of the socioeconomic impact of such programmes, early detection provides for far more flexible treatment options [78]. Patients are also more likely to have a temporary stoma than a permanent stoma if their colon cancer was diagnosed at an early stage [80]. Earlier diagnosis would therefore allow more patients to maintain/retain a normal lifestyle for longer. This is particularly pertinent in both young and old populations. In the case of younger patients, to avoid the stigma and embarrassment associated with colostomy bags, etc. and, in the case of the elderly, it would allow more individuals to retain higher levels of independence and reduce the implied care burden on social services and familial-care structures.

In this respect, it would seem apparent that improved early detection through screening would yield multiple economic benefits outside simply reducing the cost of treatment. It would allow patients, through improved DFS rates, to re-enter the labour force and thus increase the number of economically productive life years. In addition, in lowering the care burden on familial-care structures, it would allow those who would otherwise be charged with either full- or part-time care to remain in the workforce and thus contribute to economic productivity, and would also reduce the required resource allocation within social-care systems and, as such, allow the repurposing of limited resources to other areas.

Another possible implication of increased survival rate driven by earlier detection and intervention is the implied increase in resources required to effectively monitor patients post treatment. It is a requirement of screening methods that they do not create an unmanageable workload. One fundamental advantage of a successful early-detection screening programme would be that larger numbers of people would be living longer post treatment. While it is possible to argue that effective screening tools could be applied to the post-treatment patient pool as well as the population, it is unlikely that biomarker-based screening tools would supplant traditional endoscopy/imaging-based monitoring tools in the post-treatment population. As such, it would seem prudent, particularly in a socialized healthcare system, to consider the increased resources required to carry out more frequent monitoring (endoscopies) on an expanded post-treatment population, and on the increased number of high-risk individuals screened in the first place (who may be false positives, unknown until colonoscopy). This would not only require the setting-up of larger numbers of examination facilities, but would necessitate the recruitment and training of large numbers of endoscopists to carry out the procedures.

It could be argued that, behind breast, cervical and prostate cancer, CRC is the indication most likely to benefit from improved screening strategies and patient stratification. This is principally driven by the large number of treatment options and a high curative rate of cases diagnosed at an early stage. It is true to say that diagnostic tools for effective patient stratification are of limited use without a concomitant number of effective treatment options. A basic argument would be what the point of stratifying a patient population is if there is only one treatment option available. As such, one of the key factors in assessing the potential clinical utility of a given diagnostic/screening strategy and patient stratification is whether or not a relevant range of treatment options exists, which would allow clinicians to tailor treatments in response to personalized diagnostic information. Viewed in such light, CRC and colon cancer in particular, behind only breast and prostate cancers, appears ripe to benefit from a personalized medicine-screening approach.

Conclusions

In the absence of any major therapeutic advances in the management of early-stage colon cancer, it is clear that a personalized approach that delivers existing drugs to those patients at highest risk of recurrence and that could avoid the over-treatment of patients who have a high chance of being cured by surgery alone will gain increasing support from the clinical community. It is possible to envisage a large, prospective randomized trial based on biomarker-driven patient selection.

An example of such a trial could be where the hypothesis would be to establish the effectiveness of the ploidy/stroma biomarkers [63] (Figure 1) to stratify early-stage (stages II and IIIa) colon cancer patients into three prognostic groups with individual management protocols. The primary objective would be to determine DFS in the following three settings: (i) biomarker-determined low risk of recurrence: observation only; (ii) biomarker-determined intermediate risk of recurrence: randomize patients to single-agent capecitabine 2500 mg/m^2 for 6 vs 3 months; (iii) biomarker-determined high risk of recurrence: randomize patients to capecitabine for 6 months vs capecitabine plus oxaliplatin (CAPOX) for 3 months. Such studies will be vital to truly deliver on the promise of personalized medicine for stage II colon cancer patients.

Authors' contributions

S.F., G.A.M. and E.M.A.M. wrote the manuscript and D.J.K. contributed to the manuscript and reviewed the final versions. All authors read and confirmed the final version of the manuscript.

Conflict of interest

D.J.K. is a company director of Oxford Cancer Biomarkers Limited.

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