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What We Know About Stage II and III Colon Cancer: It's Still Not Enough

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Abstract

The introduction of oxaliplatin as adjuvant treatment for stage III colon cancer in 2004 has been the last practice changing progress in adjuvant treatment for patients with early colon cancer. Since then, many prognostic and predictive biomarkers have been studied, but only DNA mismatch repair status has been validated as having an important prognostic value. Accordingly, TNM and clinical-pathological patterns, such as pT4 lesions and lymph node sampling <12 nodes, are the main factors that guide physicians' choice regarding adjuvant treatment. More recently, many biomarkers showed promising results: POLE, ErbB2, CDX2, SMAD4, BRAF and KRAS. In addition to these, immune-contexture, molecular classification, and gene signatures could become new ways to better classify colon cancer patients with more discriminatory power than TNM. The aim of this review is to report the state-of-the-art of prognostic and predictive factors in the adjuvant setting and which of these could modify clinical practice and maybe replace TNM classification.

1 Introduction

Colorectal cancer (CRC) is a major cause of death globally, with an estimated 134,490 new cases and 49,190 deaths occurring in 2016 in the USA [1]. In 75% of cases the tumour is diagnosed when it is still in an early stage and surgery alone is potentially curative [2]. The role of adjuvant therapy has been well established for stage III colon cancer (CC) since 2004, when Thierry André and colleagues [3] demonstrated the benefit of fluoropyrimidine plus oxaliplatin in terms of disease-free survival (DFS) and overall survival (OS) [4, 5]. Much more controversial is the use of adjuvant therapy in stage II CC, in which the absolute benefit of single-agent 5-fluorouracil (5-FU) ranges from 2% to 5% [6]. Although the TNM stage system and the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) system are the main prognostic factors used in clinical practice, extensive intra-stage variability in outcome is well known, probably reflecting the

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heterogeneity of this disease. To develop more effective therapies, it is critical to identify prognostic and predictive markers of recurrence and identify novel targets of therapy for the patients who are potentially curable. Our review focuses on well-known prognostic factors and on those that could potentially modify clinical practice in the future.

2 Histopathological and Clinical Features

In stage III CC the standard of care is fluoropyrimide- and oxaliplatin-based chemotherapy, after surgical intervention. Adjuvant treatment should be administered as soon as the patient is medically able, but not later than 6–8 weeks after resection [7, 8]. The exact duration for this therapy is 6 months of FOLFOX (5FU, folinic acid and oxaliplatin) or XELOX (capecitabine and oxaliplatin), but results from completed trials aimed to demonstrate the non-inferiority of three months of chemotherapy vs the standard 6 months, are expected in a few months. A shorter duration of therapy, if equally efficacious, would be advantageous for patients and Health Care Systems. Therefore, the results from TOSCA trial [9], that is the first trial comparing 3 versus 6 months of adjuvant chemotherapy completing accrual within the international initiative of treatment duration evaluation (International Duration Evaluation of Adjuvant, IDEA) [10], are very crucial because they will profoundly impact clinical practice.

In this setting, the benefit of 5-FU in terms of reducing the risk of relapse is around 15% [11], plus the benefit of oxaliplatin that ranges in 4–6% [3–5]. Although almost 50% of patients are cured by surgery alone, we do not have any biomarkers available to select those patients who do not need chemotherapy and to prevent them from the exposure of chemotherapy toxicity.

In stage II, for low-risk patients the benefit derived from 5-FU adjuvant chemotherapy is very low. Only patients defined as at high-risk for relapse are considered for adjuvant treatment. The histopathological and clinical features to define a patient as high risk are: pT4 lesions, lymphovascular or perineural invasion, tumour presentation with perforation or obstruction, poorly differentiated histology, or lymph node sampling <12 nodes. In these patients, the use of oxaliplatin has no benefit and 5-FU or capecitabine are recommended [6].

3 Prognostic Biomarkers for Stage II and III CC

Researchers and clinicians need prognostic biomarker to decide when to treat and predictive biomarkers to decide which agents to use to treat patients. Unfortunately, there are no predictive biomarkers validated for neither stage II nor III CC, although much effort was invested and potential candidates have been identified.

3.1 CDX2

The role of Caudal-type homeobox transcription factor 2 (CDX2) as a prognostic biomarker and its association with advanced stage, CpG island methylator phenotype-high (CIMP-high), and high tumour grade has been widely demonstrated in the past [12–14]. More recently, its expression has been evaluated in 2115 tumour samples by Dalerba and

colleagues [15] (Table 1). The results revealed that without adjuvant chemotherapy, CDX2-negative tumours (only 5% of all patients) were associated with a lower rate of disease-free survival than CDX2-positive tumours. In addition, patients with stage II or stage III CDX2-negative CC might benefit from adjuvant chemotherapy and adjuvant chemotherapy might be a treatment option for patients with stage II CDX2-negative disease, who are commonly treated with surgery alone. These findings need to be further confirmed, ideally within the framework of prospective and randomized clinical trials.

3.2 MSI

Deficient DNA mismatch repair (dMMR) status is found in about 15% of CRCs. In fact, its prevalence is higher in early stage and decreased in advanced disease (20% in stage I-II, 12% in stage III and 4% in stage IV). The dMMR can be investigated by testing for loss of an MMR protein (immunohistochemistry) or for MSI using a PCR-based assay: MSI-high (MSI-H) tumours display loss of at least one MMR protein with immunohistochemistry (MLH1, MSH2, MSH6, and PMS2) or instability in two or more of the five microsatellite markers (BAT25, BAT26, D2S123, D5S346, and D17S250) with a PCR-based assay [30]. The dMMR status is one of the most studied and well-established positive prognostic factor, especially in early-stages of CC. In all the studies, microsatellite instability (MSI) has been associated with a better outcome in comparison of microsatellite stability (MSS), in terms of time to recurrence (TTR), relapse free survival (RFS) and OS [16–19, 31]. However, the prognostic role in stage III CC is less robust than in stage II. Moreover, in an important study by Sinicrope and colleagues [20] the authors noticed a possible connection between microsatellite status and the localization of primary tumour, with a positive prognostic factor only for right-sided tumours.

Probably because of the good prognosis for patients with stage II MSI-H CC, no evidence of benefit with adjuvant 5-FU has been found [32–34], and for patients with stage II MSI-H, adjuvant chemotherapy is not recommended.

In the future, potential treatment options for patients with MSI-H CC may include immune checkpoint inhibitors. The use of immune checkpoint inhibitors has revolutionized the treatment of some types of cancer, especially non-small cell lung cancer (NSCLC), melanoma, renal cancer, and head and neck and bladder. It has been recently demonstrated that MSI-H tumours have an increased mutational burden that could be responsible for the generation of neoepitopes, eventually recognizable by the immune system [35]. Indeed, numerous studies have demonstrated that MSI-H tumours are highly infiltrated with T cells, including cytotoxic T lymphocytes - CTLs [36–41], a well-established good prognostic factor. Furthermore, Le and colleagues have recently demonstrated the MSI status as a predictive marker for response to programmed death 1 (PD-1) blockade in patients with stage IV CRC [36], opening new therapeutic options for these patients even into first line treatment.

3.3 BRAF

In sporadic CRCs, BRAF mutation is seen in approximately 60% of MSI-H tumours and only in 5–10% of microsatellite stable (MSS) tumours [42]. BRAF V600E mutations are

associated with several clinicopathological parameters, and the ones most often reported are: proximal location, higher age, female gender, MSI-H, high grade, and mucinous histology.

The BRAF V600E mutation has been widely investigated, and its negative prognostic impact on stage II and III CC has been observed in numerous studies [17, 19–23, 31, 43–45]. BRAF mutation has the greatest impact in terms of OS, especially for left-sided and MSS tumours. The strong association between MSI-H and BRAF mutation makes it harder to distinguish the role of the distinct factors in prognosis. However, in a retrospective analysis of the PETACC-3 trial [21] there was no evidence for prognostic value in MSI or right-sided tumour groups. Nevertheless, in three different studies no prognostic role of BRAF mutations has been demonstrated [46–48]. To underline the strong correlations between these useful biomarkers, it has been recently demonstrated that in MSI-H tumours the concomitant evaluation of both BRAF and KRAS provides useful prognostic information beyond the evaluation of either variable separately. Most importantly, patients with double wild-type (dWT) cancers had a highly favourable survival with 5-year cancer-specific survival (CSS) of 93% (95% CI 84–100%) compared to patients with either BRAF or KRAS mutated cancers (5-year CSS 76%, 95% CI 67–85%), especially in stage II patients with dWT cancers in whom no cancer-specific deaths were observed [49].

3.4 KRAS

Different important studies examined the prognostic impact of specific KRAS mutations in CC, considering BRAF mutational status as a confounding variable. The presence of KRAS mutations (in the BRAF wild type (WT) population) confers a worse prognosis, with reduced DFS and OS [24–26, 50] in resected tumours. In fact, the importance and the impact of KRAS mutation on prognosis is still debated and not clear, since the large PETACC-3 translational study [31] and data from the National Surgical Adjuvant Breast and Bowel Project (NSABP) clinical trials C-07 (n = 1836) and C-08 (n = 463) [19] reported no prognostic value in terms of OS, RFS, and TTR [51]. Mutation of KRAS occurs in 40% of sporadic CRCs, and it is an established predictor of absence of response to epidermal growth factor receptor (EGFR)–targeted agents in the metastatic setting [52], but in the adjuvant setting targeting this pathway failed to improve DFS and OS in both PETACC-8 and NO147 trials [53, 54]. Furthermore, no definitive results exist concerning the predictive role of KRAS with FU/FA chemotherapy [17]. More recently, J. Taieb and colleagues presented results from the PETACC-8 trial (cetuximab + FOLFOX vs FOLFOX) in full WT patients (RAS & BRAF). Although cetuximab did not significantly improve TTR, DFS, or OS in patients with RAS WT or RAS & BRAF dWT tumours (HR ranging from 0.77 to 1.05, all p > 0.05), the curves clearly separated after 2–3 years and stayed so at 5-year follow-up, encouraging investigation of these important results in a prospective analysis [55].

3.5 ErbB2

Following the MOSAIC trial, no advances have been made in the adjuvant treatment setting. ErbB2 amplification has been recently shown as a potential targetable alteration in metastatic CRC in the HERACLES trial [56]. In this proof-of-concept, multicentre, open-label, phase 2 trial, 27 patients with HER2-positive metastatic CRC (mCRC), refractory to standard therapy were enrolled. The patients received dual-targeted therapy with

trastuzumab and lapatinib, showing a disease control rate of 78%, with 34% partial responses (PR) or complete responses (CR). This discovery reinforces the interest in studying the occurrence and the prognostic role of ErbB2 alterations in stage III CC where we need to improve adjuvant strategies. Recently, P. Laurent-Puig and colleagues showed the poor prognostic impact of ErbB2 alterations (mutations in 1% or amplification in 3%) in about 1800 tumour samples from the PETACC8 trial [27]. Altogether, ErbB2 alterations were present in 64 patients (3.8%). In a univariate analysis, ERBB2 alterations were associated with shorter time to recurrence (HR: 1.55 [95%CI: 1.02; 2.36] $p = 0.04$) and shorter overall survival (HR: 1.57 [0.99; 2.5] $p = 0.05$). This prognostic value was maintained after adjustment for treatment, RAS mutation, histological grade, tumour location, pT and pN status, bowel obstruction or perforation, and venous or lymphatic embolism. In conclusion, its poor prognostic value supports the testing of anti-ErbB2 therapies in the adjuvant setting in investigational trials.

3.6 POL-E

DNA mismatch repair and DNA polymerase (POLE and POLD1) proofreading are responsible for genomic stability. Recent data by Domingo and colleagues [57] demonstrated that pathogenic POLE proofreading domain mutations occur in 1% of CRC, in which they are responsible of an ultramutated status. POLE mutations are correlated with younger age, male sex, and right-sided tumours, and with a strong tendency to mutual exclusivity with dMMR. In this study, POLE-mutant CRCs displayed significantly increased CD8+ cell infiltrates compared to pMMR tumours and higher expression of cytotoxic markers and immune checkpoints. More importantly, POLE-mutant status has been shown to be a very strong positive prognostic factor in terms of tumour recurrence and DFS, especially in stage II patients (HR = 0.22, $p = 0.014$ in CRC recurrence). This strong signal needs to be validated in prospective clinical trials.

3.7 SMAD4

The SMAD4 tumour-suppressor gene (TSG) codes for a common intracellular mediator of the TGFb superfamily signalling pathway: it is involved in the regulation of cell proliferation, differentiation, apoptosis, and cell migration, and it is one of the most commonly altered pathways in human cancers. Analysis of the PETACC-3 trial [16] indicated loss of SMAD4 expression, found in 21% of patients, as a biomarker of poor prognosis in terms of RFS (HR = 1.47, 95% CI = 1.19 to 1.81, $P < 0.001$) and OS (HR = 1.58, 95% CI = 1.23 to 2.01, $P < 0.001$) both in stage II and stage III CC. Moreover, SMAD4 loss was statistically significantly more frequently in stage III than in stage II (23% vs 18%, $p = 0.03$). Nevertheless, the impact of SMAD4 loss (73/293 cases) was not statistically significant in terms of RFS or in OS for patients in stage II CC in a more recent study [28].

3.8 CIMP

The transcriptional inactivation of tumour suppressor genes by promoter hypermethylation is an epigenetic phenomenon involved in carcinogenesis of CC. The CpG island methylator phenotype is one of the most recently recognized mechanisms of colorectal carcinogenesis [58]. The CIMP phenotype is due to CpG island methylation in the promoter regions of

certain tumour suppressor genes involved in malignant transformation. The contradictory results published regarding the role of CIMP as a prognostic biomarker could be due to an overlap between the CIMP+ phenotype and the MSI phenotype, associated in 50% of cases with BRAF mutation. The most common molecular and clinical features of CIMP+ colon cancer are female sex, older age, proximal tumour location, BRAF mutation, wild-type KRAS and TP53 genes, and MSI [59].

In two different studies in stage III CRC, CIMP+ has been associated with shorter OS for those patients treated with surgery alone as compared to CIMP− patients [60] and the CIMP + subgroup with BRAF mutation and proximal tumour location had a significantly worse DFS [61]. Five other studies have investigated the prognostic value of CIMP+ in mixed stage II and III CRC: three studies showed a decrease in DFS in the CIMP+ group [62–64], although no significant difference in DFS between CIMP+ and CIMP− was noticed in the other two [65, 66]. Finally, Donada et al. [67] have studied the prognostic impact of CIMP in stage II CRC and found a benefit of adjuvant 5-FU in terms of OS in CIMP+ patients.

In conclusion, the prognostic impact of the CIMP+ phenotype remains very controversial, since many factors, especially BRAF mutation and MSI status, could influence the results. Moreover, the lack of standardization and consensus for the definition of CIMP status introduces another important bias. Although it appears that CIMP+ phenotype is associated with decreased OS and DFS in MSS patients, it warrants further investigations.

4 Immune Contexture

The complex interplay between the immune system and cancer has been a matter of research for decades, but only recently a deeper knowledge in this field has led to the development of effective immunotherapy as a novel treatment option for different types of cancer [68]. The recognition of the dual role that the immune system has in regards to cancer development led to the three “Es” of the cancer immunoediting theory — Elimination, Equilibrium, and Escape [69].

The immune contexture consists of type of immune cells, their location, density, and functional orientation and can influence tumour invasion, recurrence, and metastasis [70].

In CC, mounting evidence indicates immune infiltration as a crucial prognostic factor. Beginning in 2006, Galon and colleagues demonstrated the immune-contexture as a positive and independent prognostic factor [71–78] for early stage CC, with a stronger discriminatory power of standard TNM. For this reason, the authors developed the Immunoscore® (IM): a prognostic tool based on numbers of lymphocyte populations (CD3/CD45RO) in the tumour core and invasive margins. Recently, a Society for Immunotherapy of Cancer-led international consortium of 23 pathology expert centres from 17 countries validated the Immunoscore® in 1336 patients with stage I/II/III CC [29]. In the training set TTR was shorter among 332 patients (48.1%) with Low-IM (0 or 1 IM) CC vs. 358 patients with High-IM CC (HR = 0.35; 0.23–0.52; $P < 0.0001$). In the internal validation set with 630 patients, TTR was also shorter among 303 patients with Low-IM CC vs. 327 patients with High-IM CC (HR = 0.54; 0.34–0.84; $P = 0.006$). In both groups, results were independent of

age, sex, tumour stage, and sidedness. Among patients with stage II CC, the difference in TTR between Low and High-IM was significant both in the training set (HR = 0.27; $P < 0.0001$) and in the internal validation set (HR = 0.46; $P = 0.014$). We may conclude that Low-IM identified a subgroup of patients with high-risk stage II CC, but such a potentially important prognostic factor must be validated in a prospective cohort of patients in order to establish its impact in daily clinical practice. Moreover, since it may predict the efficacy of immunotherapies as adjuvant treatment, its use could become extremely important in the near future.

5 Sidedness

An increasingly large amount of evidence is accumulating showing that colon tumours proximal and distal to splenic flexure are distinct clinical and biological entities. A recent meta-analysis included 66 studies with about 1.5 million patients with a median follow-up of 65 months [79]. Left-sided primary tumour location was associated with a significantly reduced risk of death (HR = 0.82; 0.79–0.84; $P < 0.001$) in all stages. Studies that included only patients with stage IV disease ($n = 20$) compared with those that included patients with stages I to III only ($n = 25$) showed a significantly greater effect on mortality for patients with left CC (HR = 0.73, 0.69–0.78 vs HR = 0.84, 0.79–0.89; $P < .001$ for subgroups difference). Although this meta-analysis has some limitations, such as a notable heterogeneity of included studies, it confirms the important role of sidedness in CC, both in metastatic and adjuvant settings. The biological, embryological, and genetic differences between right and left CC have to be taken into account as a stratification factor for future studies to establish the efficacy of drugs and patients' prognosis [80].

6 Consensus Molecular Classification

Cancer genomes can now be systemically studied in their entirety within a single day, opening new incredible opportunities to analyse and find patient-specific cancer mutations, eventually responsible for tumour progression. Therefore, individual cancer sequencing may provide the basis of personalized cancer management. Some studies tried to classify different types of CC based on gene expression [81–86] to identify subgroups of patients with distinct prognosis and to improve the current disease stratification based on clinico-pathological variables. Because of the extreme variability of all these classifications, Guinney and colleagues [87] formed an international consortium and identified four Consensus Molecular Subtypes (CMS) with distinctive characteristics: CMS1 (14%) are hypermutated, MSI, and exhibit increased expression of genes associated with a diffuse immune infiltrate, mainly composed of TH1 and cytotoxic T cells, along with strong activation of immune evasion pathways, frequent occurrence of BRAF mutations, females with right-sided lesions and worse survival after relapse, and CIMP-H status; CMS2 (37%) are epithelial, and show marked WNT and MYC signalling activation; CMS3 (13%) are associated with mixed MMR status, CIMP and SCNA low, KRAS mutations, and metabolic deregulation; CMS4 (23%) showed clear upregulation of both genes implicated in epithelial-to-mesenchymal transition (EMT) and of signatures associated with the activation of transforming growth factor (TGF)-β signalling, angiogenesis, matrix remodelling pathways, and the complement-mediated inflammatory system, and they had worse OS and RFS,

irrespective of patient cohort; 13% of samples showed mixed features. The 5-year OS rates were 62% for CMS4, 74% for CMS1, 75% for CMS3 and 77% for CMS2: these differences in prognosis with unsupervised gene expression signatures confirm the clinical relevance of the intrinsic biological processes implicated in each CMS.

Interesting for this review, the authors separately analysed prognosis in the subset of patients enrolled in PETACC-3 trial (stage II and III CC) with similar results in terms of OS, RFS, and survival after relapse.

On the one hand, the impact of this classification in daily practice is still low, because it has to be validated before it can be a useful tool for oncologists. On the other hand, it could be used in clinical trials to select patients for tailored therapy in the adjuvant setting.

7 Gene Signatures

Many different gene signatures have been tested in several retrospective and prospective studies, but none of them is used in clinical practice as a decision-making tool. Moreover, only few gene signatures have been validated in external independent data sets: OncotypeDX®, GeneFx® Colon, ColoPrint®, OncoDefender-CRC®, and ColonPRS® are currently available [88]. Nevertheless, only OncotypeDx® and GeneFx® Colon have been investigated in co-variable analysis with MSI status and pT4 on FFPE samples, collected from prospective, randomized clinical datasets.

OncotypeDx® was first validated in the Quasar trial, in which it could separate patients with high risk of recurrence (RR) and low RR (High Recurrence Score, 3- year RR of 22% and low RS, 3-year RR of 12%) [89], and it remained significant also in a multivariate analysis with MSI status and pT4. These results have been further confirmed in two other clinical trials: CALGB 9581 [90] and NSABP C-07 [91]. In the former, the prognostic value of RS was most evident in the subgroup of T3 MSS patients: 5-year RR in the prespecified low and high RS groups were 13% and 21%, respectively. In the latter, average 5-year recurrence risk rates (low, intermediate, and high) were: stage II, 9%, 13% and 18%; stage III 21%, 29%, and 38%. No predictive role has been demonstrated in all these studies. Finally, in the Sunrise study [92], patients with stage II disease in the high-risk group had a 5-year risk of recurrence comparable to patients with stage IIIA to IIIB disease in the low-risk group (19% vs 20%), whereas patients with stage IIIA to IIIB disease in the high-risk group had a recurrence risk similar to that of patients with stage IIIC disease in the low-risk group (approximately 38%). Therefore, OncotypeDx® is the most largely and independently validated gene signature, but its discriminatory power is too weak to be used in daily clinical practice, and it might have to be integrated with other determinants.

GeneFx® is a DNA microarray-based gene signature developed using FFPE tumour samples of 215 stage II CC patients [93]. The signature could discriminate patients with higher relapse rate and cancer-related death with a HR of 2.53 ($p < 0.001$). More recently, it has been significantly associated with RFI after adjustment for other prognostic factors (HR = 2.13; 95% CI, 1.3 to 3.5; $P < 0.01$) in multivariable analysis [94].

8 Circulating Tumour DNA

Genomic profiles of circulating cell-free tumour DNA (ctDNA) were shown to match those of the corresponding tumours, with important implications for both molecular pathology and clinical oncology. Analyses of liquid biopsies can be used to monitor response to treatment, assess the emergence of drug resistance, and quantify minimal residual disease (MRD), both in adjuvant and metastatic settings [95]. Indeed, ctDNA levels can be used to monitor MRD after surgery or other curative treatments and liquid biopsies can be applied to the monitoring of response and/or resistance to systemic therapy.

The ctDNA is a promising biomarker for the noninvasive assessment of cancer burden. Recently, a study published in Science Translational Medicine [96] evaluated the ability of ctDNA to detect MRD in 1046 plasma samples from a prospective cohort of 230 patients with resected stage II CC. It showed that ctDNA detection has 48% sensitivity and 100% specificity in the prediction of radiologic recurrence at 36 months in postoperative patients. Three-year RFS estimates were 0% for the ctDNA-positive group and 90% for the ctDNA-negative group. However, an additional 16 of 164 patients (9.8%) experienced disease recurrence but were ctDNA-negative. Furthermore, the authors also noted a higher specificity for ctDNA testing over CT scans. Patients treated with chemotherapy who had detection of ctDNA after treatment were associated with a higher risk of recurrence (HR, 11; 95% CI, 1.8–68; $P = 0.001$). In patients not treated with adjuvant chemotherapy, 11 out of 14 (79%) patients with ctDNA detection postoperatively, had recurred at a median follow-up of 27 months; recurrence occurred in only 16 (9.8%) of 164 patients with negative ctDNA (HR = 18; $P < 0.001$). From these results, authors noted that ctDNA could be a real-time marker of response to adjuvant chemotherapy, faster and less invasive than a CT scan, but this must be validated in a larger cohort of patients. Indeed, detection of ctDNA after resection of stage II CC may identify patients at the highest risk of recurrence and help inform adjuvant treatment decisions.

More recently, a systematic review strongly suggested that patients with ctDNA-positive CRC have an unfavourable prognosis, both in terms of DFS and OS [97], even if there are major limitations in this analysis, such as clinical and methodological heterogeneity of studies included.

The development of novel technologies such as ctDNA is already implemented and used in clinical practice for patients with NSCLC and is likely to become an additional tool to monitor patients with gastrointestinal (GI) cancer in real time on a molecular level in the future.

9 Conclusion

In the last decades, although mounting evidence has been shedding light on multiple factors that can influence the prognosis of patients with early CC, no changes in adjuvant treatment have been made based on these biomarkers. Furthermore, the number of effective treatment options for patients in the adjuvant setting has not increased, despite many drug-targetable factors having been discovered. Unfortunately, none of these drugs showed efficacy in early

CC patients so far. The only standardized and efficacious treatment is 5-FU + oxaliplatin-based chemotherapy (oxaliplatin only in stage III). The exact duration of chemotherapy (3 vs 6 months) will be addressed by the results coming from the TOSCA trial expected in a few months, which will have strong impact on our clinical practice. More importantly, we still cannot recognize which patient really needs chemotherapy and which patient is already cured by surgery alone: this exposes the patient to chemotherapy adverse events without any benefit.

Concerning prognostic factors, the most validated and important ones for our practice are pT4 and MSI status, since all other biomarkers need to be further investigated and validated before they can be used in clinical practice. Accordingly, universal MMR or MSI testing should be performed for all patients with early CC: stage II MSI-H tumours may have a good prognosis and do not benefit from 5-FU adjuvant therapy. In addition to this, MSI-H patients could be involved in future clinical trials with immunotherapy as adjuvant treatment.

Nevertheless, very promising results have been shown for POLE, SMAD4, CDX2, ErbB2, KRAS, and BRAF as prognostic biomarkers, and especially POLE (immunotherapy), ErbB2 (targeted therapy), and CDX2 (chemotherapy for CDX2-neg patient) hold potential as predictive factors.

The immunoscore® may be a new way to classify patient both as a prognostic and a predictive factor, since it may predict the benefit from immunotherapy, but it must be validated in a prospective cohort of patients.

Although the development of next generation sequencing (NGS) could make molecular classification more and more available in every cancer center, the CMS is still too complicated to become a real tool useful in daily clinical practice.

Between all gene signatures tested, the most validated is OncotypeDx®, which can be useful as an integration for other determinants, but data available so far are not sufficient to recommend the use of these tools to determine adjuvant therapy.

A new and very promising technology is ctDNA that may identify patients with stage II CC at high risk of recurrence of both post resection and post adjuvant chemotherapy, but larger studies are needed to confirm this.

In the future, the challenge is to integrate all these promising biomarkers on how to guide us on which patient benefits from chemotherapy and which patient needs other types of treatment (immunotherapy or targeted therapy).

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Key Points

No significant development in treatment strategies for colon cancer patients in the adjuvant setting has been made in the last decade.

Many predictive and prognostic biomarkers showed promising results to better determine patients' prognosis and treatment response.

Further studies are needed since these biomarkers can change our clinical practice.

Table 1

Summary of prognostic and predictive biomarkers in stage II and III CC

Variable	Study	Sample size	Disease stage and characteristics	1° Endpoint	HR	CI 95%	*p* value	Type of analysis	Ref
MSI vs MSS	PETACC-3	1404	II and III	OS	0.49	0.34–0.69	0.001	univariate	[16]
				RFS	0.47	0.31–0.72	<0.001	univariate	
	QUASAR	1913	II and III	RFS	0.53	0.40–0.70	<0.001	univariate	[17]
	ACCENT	2723	III treated	OS	0.79	0.65–0.97	0.023	univariate	[18]
				TTR	0.80	0.66–0.97	0.025	univariate	
		307	II untreated	TTR	0.27	0.10–0.75	0.012	univariate	
				OS	0.27	0.10–0.74	0.011	univariate	
	NSABP C-07/C-08	1796	II and III	OS	0.64	0.46–0.89	0.008	multivariate	[19]
				TTR	0.48	0.33–0.70	0.0001	multivariate	
	NO147	2580	III	DFS	0.82	0.64–1.07	0.1403	multivariate	[20]
			III left	DFS	1.71	0.99–2.95	0.0564	multivariate	
			III right	DFS	0.71	0.53–0.94	0.0184	multivariate	
	CALGB89803	891	III left	DFS	1.58	0.72–3.46	0.2817	multivariate	
			III right	DFS	0.59	0.41–0.86	0.0039	multivariate	
BRAF MUT vs WT	PETACC-3	1423	II and III	OS	1.78	1.26–2.53	0.0009	univariate	[21]
				RFS	1.30	0.94–1.81	0.1174	univariate	
				SAR	2.48	1.74–3.53	<0.0001	univariate	
		697	II and III, MSS/left	OS	6.41	3.57–11.52	<0.0001	univariate	
				RFS	3.57	2.02–6.31	0.0005	univariate	
		1054	II and III, MSS	OS	2.82	1.58–4.30	<0.0001	univariate	
	QUASAR	1584	II and III	RR	0.84	0.57–1.23	0.4	univariate	[17]
	NSABP C-07/C-08	2226	II and III	OS	1.46	1.20–1.79	0.0002	univariate	[19]
				TTR	1.02	0.82–1.28	0.86	univariate	
				SAR	2.31	1.83–2.95	<0.0001	univariate	
		201	II and III, MSI	OS	1.76	0.88–3.49	0.11	multivariate	
		1534	II and III, MSS	OS	1.58	1.23–2.04	0.0004	multivariate	

Variable	Study	Sample size	Disease stage and characteristics	1° Endpoint	HR	CI 95%	*p* value	Type of analysis	Ref
	CALGB89803	506	III	OS	1.66	1.05–2.63	0.015	univariate	[22]
		428	III, MSS	OS	1.61	0.96–2.69	NA	multivariate	
	NO147	2515	III	DFS	1.34	1.11–1.63	0.0028	univariate	[20]
		2176	III, MSS	DFS	1.32	1.01–1.73	0.0437	multivariate	
		304	III, MSI	DFS	1.58	0.88–2.82	0.1220	multivariate	
	Meta-analysis of 7 RCT	8721	II and III	OS	1.42	1.25–1.60	<0.0001	univariate	[23]
				DFS	1.26	1.07–1.48	0.006	univariate	
KRAS MUT vs WT	PETACC-3	1423	II and III	OS	1.09	0.86–1.37	0.4826	univariate	[21]
				RFS	1.04	0.85–1.27	0.7245	univariate	
				SAR	1.04	0.82–1.32	0.7222	univariate	
	NSABP	2081	II and III	OS	1.09	0.92–1.29	0.33	univariate	[19]
	C-07/C-08								
				TTR	1.12	0.94–1.32	0.21	univariate	
				SAR	1.11	0.92–1.34	0.30	univariate	
	QUASAR	1583	II and III	RR	1.40	1.12–1.74	0.002	univariate	[17]
	Prospective cohort	1075	I-IV BRAF WT, KRAS codon 12 mut vs wt	CCSM	1.30	1.02–1.67	0.037	multivariate	[24]
				OS	1.24	1.02–1.51	0.029	multivariate	
			I-IV BRAF WT, KRAS codon 13 mut vs wt	CCSM	0.86	0.58–1.27	NS	multivariate	
				OS	0.96	0.71–1.30	NS	multivariate	
	NO147	2478	III BRAF WT, KRAS codon 12 mut vs wt	DFS	1.52	1.28–1.80	<0.0001	multivariate	[25]
			III BRAF WT, KRAS codon 13 mut vs wt	DFS	1.36	1.0–1.77	0.0248	multivariate	
	PETACC-8	1629	III	TTR	1.56	1.2–1.92	<0.001	multivariate	[26]
		1043	III BRAF WT, left, KRAS codon 12 mut vs wt	TTR	1.96	1.5–2.56	<0.0001	multivariate	
			III BRAF WT, left, KRAS codon 13 mut v wt	TTR	1.59	1.0–2.56	0.051	multivariate	
ERBB2 alterations vs WT	PETACC-8	1800	III	TTR	1.55	1.02–2.36	0.04	univariate	[27]
				OS	1.57	0.99–2.5	0.05	univariate	
CDX2 neg vs pos	Independent and retrospective cohorts	466	II and III	DFS	2.73	1.58–4.72	<0.001	multivariate	[15]
		314	II and III	DFS	2.42	1.36–4.29	0.003	multivariate	
SMAD4 loss vs no loss	PETACC-3	1381	II and III	OS	1.58	1.23–2.01	<0.001	multivariate	[28]
				RFS	1.47	1.19–1.81	<0.001	multivariate	

Variable	Study	Sample size	Disease stage and characteristics	1° Endpoint	HR	CI 95%	*p* value	Type of analysis	Ref
Low IM vs High IM	Prospective cohort	690	I, II and III	TTR	0.35	0.23–0.52	<0.0001	univariate	[29]
		630	I, II and III	TTR	0.54	0.34–0.84	0.006	univariate	

MSI: Microsatellite Instability, *MSS*: Microsatellite Stable, *OS*: Overall Survival, *RFS*: Relapse Free Survival, *TTR*: Time To Relapse, *HR*: Hazard Ratio, *CI*: Confidence Interval, *mut*: mutation, *WT*: Wild Type, *SAR*: Survival After Recurrence, *DFS*: Disease Free Survival, *RR*: Response Rate, *CCSM*: Colorectal Cancer-Specific Mortality, and *IM*: Immunoscore