Since the MOSAIC study, oxaliplatin-based adjuvant chemotherapy has been the standard treatment of stage III colon cancer. Combination therapy with fluoropyrimidines and oxaliplatin has improved overall survival (OS) and reduced the risk of recurrence in patients with resected stage III colon cancer. However, only 20% of patients really benefit from adjuvant chemotherapy, exposing 80% of patients to unnecessary toxicity. Recent analyses of large multicenter adjuvant studies have focused on the prognostication of OS and disease-free survival in stage III colon cancer in order to reduce overtreatment and to find more accurate prognostic tools than those used for adjuvant treatment decision-making in stage II disease. Indeed, clinical and pathological prognostic factors, although important, are not sufficient to decide which stage III patients will benefit from adjuvant therapy, and biomarkers will help select patients that need adjuvant treatment. Molecular markers such as microsatellite status and BRAF and KRAS mutations have recently been explored, and molecular signatures have been identified as promising prognostic factor for OS. Furthermore, recent studies have highlighted the prognostic value of immune infiltration. This review focuses on pathologic, immunologic and molecular prognostic markers for stage III colon cancer that could help clinicians tailor adjuvant treatment in a comprehensive transversal approach.

**Key words:** stage III, colon cancer, prognosis, molecular classification, biomarkers
Pathological features and prognostic scores

Pathological staging remains the key to post-surgical prognostication. The most important factors in stage III disease are pT and pN status [12]. The 5-year cancer-specific survival rates for patients with pT4, pT3 and pT1–T2 tumors are ~30%, 63% and 88%, respectively [13], and 65% (95% CI 57%–72%) for pN1 tumours and 37% (95% CI 26%–48%) for pN2 tumours [13].

Beyond pT and pN staging, other pathological features are known to influence stage III colon cancer outcomes. Micrometastatic potential, evaluated in terms of vascular, lymphatic and perineural invasion, has been found to be even more important than pTNM status in some studies [14], but this is controversial. Perineural invasion has been correlated with worse disease-free survival (DFS) and overall survival (OS), specifically in stage III patients [15, 16]. Perineural and lymphovascular invasion seemed to be synergistic in some studies [16], but not in others [13]. Although these prognostic factors are widely used for adjuvant treatment decision-making in stage II disease, their individual roles in stage III disease need to be clarified.

The lymph node ratio (LNR), defined as the number of positive nodes divided by the total number of nodes removed, seems to have strong prognostic value. In a retrospective study, 24,477 patients were divided into 4 groups of LNR: LNR1 (<1/14), LNR2 (between 1/14 and 0.25), LNR3 (0.25–0.50) and LNR4 (0.50–1.0), with 5-year survival rates of, respectively, 64.8%, 56.2%, 45.1% and 29.6% (P < 0.0001) [17]. Other studies have confirmed these results [18, 19]. However, Berger et al. found that the LNR in N2 patients was only prognostic for cancer-specific survival and not OS [20]. This discrepancy may be explained by the use of different LNR cut-offs.

Three prognostic scores for OS and DFS have been created on the basis of clinical and pathological features [21–23]. The required information variously includes age, sex, pT and pN status, pre-operative carcinoembryonic antigen (CEA), histopathological grade, vascular invasion or lymphatic infiltration, total lymph node number, lymph node involvement, N+/N ratio, the use of adjuvant treatment and the adjuvant regimen (Table 1).

The MSKCC score predicts OS and DFS at 5 years for all stages of colon cancer, while the Numeracy score focuses on stage II and III disease. The ACCENT online tool is the only one dedicated to stage III colon cancer. It predicts 3- and 5-year OS with a concordance-index (c-index) of 0.66, which is suboptimal. For example, the TNM classification alone achieves a c-index of 0.60, and this rises to 0.68 after adding age, sex, grade and the numbers of total and positive lymph nodes [24]. No prognostic score is currently used in daily practice.

In the last decade, molecular assessment of colon cancer has become more routine, notably including mismatch repair (MMR) status, and BRAF and KRAS mutations. We and others have found that these molecular markers have prognostic value for DFS and OS.

Mismatch repair

At the origins of carcinogenesis there are pathways responsible for three different tumour phenotypes: one associated with chromosomal instability, one with CpG methylator phenotype, and one with deficient DNA mismatch repair system [25–27]. DNA mismatch repair (MMR) deficiency is found in ~15% of colorectal cancers and induces the microsatellite instability (MSI)
phenotype, leading to accumulation of mutations in tumour DNA. Its prevalence decreases with tumour stage (I–II 20%, III 12%, IV 5%). About two-thirds of dMMR tumours are sporadic [28], while one-third are familial, being linked to the Lynch syndrome. Sporadic dMMR inactivation occurs in a context of a CpG island methylator phenotype [29]. Approximately half of sporadic dMMR cases are associated with BRAF mutations [28]. Sporadic dMMR colon cancer shares clinicopathological features with inherited dMMR colon cancer, but patients with sporadic dMMR are older at diagnosis than Lynch syndrome patients [28, 30].

Patients with stage II colon cancer and the dMMR phenotype have better clinical outcomes and lower recurrence rates without adjuvant treatment than patients with proficient MMR (pMMR or MSS) tumours [31–33]. However, the MMR phenotype appears to have less prognostic value in stage III colon cancer. A multicentre retrospective trial showed that DFS seems better in the case of dMMR tumours: the 3-year DFS rate was ∼27% higher compared with pMMR tumours [34]. Post hoc analysis of large prospective trials showed that patients with stage III dMMR tumours do not have better OS than those with stage III pMMR tumours [34–36]. In those trials MMR status was determined prospectively. However, in the analysis of N0147 trial by Sinicrope et al., there seemed to be a benefit in term of DFS only in patients with right-sided dMMR colon tumours [36]. The prognostic value of dMMR status was not confirmed in a recent pooled analysis of data from the PETACC-8 and N0147 trials [37]. In the pooled cohort of 4674 patients, the 3-year DFS was 75% for dMMR patients and 74% for pMMR patients (HR 0.87, 95% CI 0.71–1.07, P = 0.196).

A retrospective pooled analysis of several studies suggests that the dMMR phenotype is associated with resistance to adjuvant 5FU chemotherapy [38, 39]. In another retrospective pooled analysis of adjuvant trials, within the dMMR subset, patients with sporadic dMMR did not benefit from 5FU-based adjuvant treatments, contrary to patients with the Lynch syndrome [31]. However, in stage III colon cancer, the post hoc analysis of the NSABP C-07 and MOSAIC trials showed that patients treated with a standard fluoropyrimidine plus oxaliplatin combination, dMMR patients seemed to derive the same benefit as pMMR patients from 5FU plus oxaliplatin chemotherapy [40, 41].

Close overlap between MMR status and other characteristics such as tumour immune infiltration, CIMP phenotype and BRAF mutations may be responsible for the conflicting data reported over the past decade [42]. Finally, recent clinical trials have shown some efficacy of immune checkpoint blockers in patients with dMMR metastatic colorectal cancer [43], and these drugs will soon be evaluated in adjuvant trials for patients with dMMR stage III colon cancer (NCT02912559).

Cpg island methylator phenotype

CpG islands are genomic regions containing a large number of cytosine and guanine nucleotides, located in 5’ regulatory regions (promoter regions) [44–49].

The CpG island methylator phenotype (CIMP) is the most recently identified mechanism of colon cancer carcinogenesis. It is observed in ∼18% of colorectal cancers and is due to CpG island methylation in the promoter region of certain genes involved in malignant transformation. The CIMP phenotype is defined by hypermethylation of at least three out of the five pre-defined markers involved in carcinogenesis. The most common molecular abnormalities associated with CIMP tumours are BRAF, KRAS and TP53 mutations and the dMMR phenotype, although there is an imperfect overlap between CIMP and dMMR [27].

The prognostic value of CIMP in stage III colorectal cancer has been evaluated in only three studies: 2 retrospective monocentric studies and 1 post hoc analysis of the CALGB 89803 prospective trial [50–52]. The retrospective study of Van Rijnsoever et al. found that patients with CIMP-positive tumours had worse survival after surgery alone than patients with CIMP-negative tumours. Among patients treated with chemotherapy, there was no difference in survival between the CIMP-positive and -negative subgroups [51]. A second retrospective study defined two CIMP statuses, correlated with BRAF (CIMP1) and KRAS (CIMP2) mutations [50]. Patients with CIMP1 tumours had worse DFS (HR 3.9, 95% CI 1.08–14.35, P = 0.015) and higher recurrence rates (53% for CIMP1, 18% for CIMP2 and 26% for CIMP-negative tumours) [50]. The post hoc analysis of tumoural samples collected prospectively during the CALGB 89803 trial, that compared fluorouracile with fluorouracile + irinotecan (IFL regimen), confirmed these data, showing that patients with CIMP+ tumours had worse OS than those with CIMP-negative tumours (HR 1.36, 95% CI 1.01–1.84) [52]. This last work highlighted an interaction between CIMP and MMR status: CIMP+/dMMR patients had better OS than CIMP+/pMMR patients (HR 0.42, 95% CI 0.23–0.77, P = 0.004), while MMR status had no impact in the CIMP-negative population. This study also suggested that CIMP+ patients arisen benefit in OS from irinotecan-based chemotherapy compared with fluorouracile alone [52]. This difference was not found with other chemotherapy regimens. However, these findings were not confirmed in any other cohort.

More data are needed on stage III colon cancer and CIMP. Indeed, there are no data from prospective trials with homogeneous population. Even if CIMP seems to confer a worse prognosis, its overlap with BRAF mutations and MMR status makes it difficult to determine the independent prognostic value of CIMP positivity.

**BRAF mutation**

BRAF is one of the three functional human RAF proteins [53, 54]. This kinase is involved in cell proliferation, differentiation and death. BRAF V600E represents ∼80% of BRAF mutations. It is present in ∼9%–12% of patients with early-stage disease and in 5%–10% of patients with metastases [55]. BRAF mutations are associated with more right-sided primary tumours and with an increased risk of peritoneal and distant lymph node metastasis [55]. Recent guidelines recommend specific and aggressive management of BRAF V600E disease, including triplet chemotherapy with anti-VEGF targeted therapy [56]. However, the prognostic value of BRAF mutation needs to be defined in early-stage colon cancer. Data concerning the role of BRAF mutation in stage III colon cancer are pretty consistent as they are from post hoc analysis of large prospective adjuvant trials or prospective cohorts.
In a recent post hoc analysis of the MOSAIC study, OS and recurrence-free survival (RFS) were assessed according to BRAF mutations, which were found in 94 (10.4%) of 902 stage II/III colon cancer patients. BRAF mutation did not confer a worse prognosis (HR 0.99, 95% CI 0.67–1.47, P = 0.966) [41].

In contrast, two studies suggest that BRAF is a prognostic factor in mixed stage II and III colon cancer [7, 57] and three studies have linked BRAF mutation to poor prognosis in stage III colon cancer (Table 2) [58–60]: BRAF-mutated patients had shorter times to relapse, survival after relapse and OS than BRAF wild-type/pMMR tumours. In a post hoc analysis of the PETACC-8 study, evaluating the prognostic value of BRAF and KRAS mutations, BRAF was prognostic only when combined with pMMR status (90% of the patients): BRAF-mutated/pMMR tumours had worse outcomes than BRAF wild-type/pMMR tumours (HR for OS 1.84, 95% CI 1.01–3.36, P = 0.046). The prognostic value of BRAF mutation was not found in the dMMR population [58]. In the other two studies, the prognostic value of BRAF mutation was not assessed with regard to MMR status [59, 60]. Finally, the interaction between BRAF mutation and MMR status was confirmed in a pooled analysis of two large adjuvant trials with more than 4000 patients (PETACC8 and N0147), in which BRAF mutation again seemed linked to a worse prognosis only in pMMR patients, being associated with time to recurrence, survival after relapse and OS [61].

Although trials in the metastatic setting are currently testing the efficacy of triple anti-MEK, anti-BRAF and anti-EGFR therapy, BRAF inhibition is not commonly used for BRAF-mutated colon cancer, owing to the modest efficacy of anti-BRAF therapies when given alone [62–64]. The efficacy of these combinations on metastatic disease will certainly lead to clinical trials dedicated to BRAF-mutated non-metastatic colon cancer.

Table 2. Studies evaluating BRAF and KRAS mutations as prognostic factors for stage III colon cancer

<table>
<thead>
<tr>
<th>Marker</th>
<th>Treatment</th>
<th>N</th>
<th>End points</th>
<th>HR for event occurrence</th>
<th>95% CI</th>
<th>Dataset</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>BRAF mut and pMMR</td>
<td>2559</td>
<td>OS</td>
<td>1.84</td>
<td>1.01–3.36</td>
<td>PETACC-8</td>
<td>Taieb et al. [58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DFS</td>
<td>1.74</td>
<td>1.14–2.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRAF mut and pMMR</td>
<td>2720</td>
<td>OS</td>
<td>1.43</td>
<td>1.11–1.85</td>
<td>NCCGT N0147</td>
<td>Sinicrope et al. [59]</td>
</tr>
<tr>
<td></td>
<td>BRAF mut</td>
<td>506</td>
<td>OS</td>
<td>1.66</td>
<td>1.05–2.63</td>
<td>CALGB 89803</td>
<td>Ogino et al. [60]</td>
</tr>
<tr>
<td></td>
<td>BRAF mut and pMMR</td>
<td>1253</td>
<td>OS</td>
<td>1.36</td>
<td>1.0–1.84</td>
<td>Epidemiological cohort</td>
<td>Lochhead et al. [57]</td>
</tr>
<tr>
<td></td>
<td>BRAF mut and dMMR</td>
<td>1253</td>
<td>CSM</td>
<td>1.60</td>
<td>1.12–2.28</td>
<td>Epidemiological cohort</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BRAF mut</td>
<td>902</td>
<td>OS</td>
<td>0.99</td>
<td>0.67–1.47</td>
<td>MOSAIC</td>
<td>André et al. [41]</td>
</tr>
<tr>
<td></td>
<td>BRAF mut and pMMR</td>
<td>1404</td>
<td>OS</td>
<td>1.56</td>
<td>1.02–2.39</td>
<td>PETACC-3</td>
<td>Roth et al. [35]</td>
</tr>
<tr>
<td>KRAS</td>
<td>KRAS mut</td>
<td>2559</td>
<td>OS</td>
<td>1.56</td>
<td>1.12–2.15</td>
<td>PETACC-8</td>
<td>Taieb et al. [58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DFS</td>
<td>1.55</td>
<td>1.23–1.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KRAS mut</td>
<td>2720</td>
<td>DFS</td>
<td>1.48</td>
<td>1.27–1.74</td>
<td>NCCGT N0147</td>
<td>Sinicrope et al. [59]</td>
</tr>
<tr>
<td></td>
<td>KRAS mut</td>
<td>378</td>
<td>CSM</td>
<td>1.49</td>
<td>1.02–2.15</td>
<td>Epidemiological cohort</td>
<td>Eklof et al., BJC (2013)</td>
</tr>
</tbody>
</table>

CSM, cancer-specific mortality; DFS, disease-free survival; OS, overall survival.

**KRAS mutation**

KRAS is an intracellular effector located downstream of epidermal growth factor receptor (EGFR). KRAS mutations represent 45% of metastatic colorectal tumours [65] and ~15%–37% of early-stage tumours [61, 66, 67]. KRAS mutation is more frequent in pMMR than in dMMR colorectal cancer [61]. In the metastatic setting, KRAS mutation confers resistance to anti-EGFR treatments [68, 69]. However, targeting the EGFR pathway with adjuvant treatment failed to improve DFS and OS in stage III colon cancer [10, 59].

Recently, in patients with BRAF wild-type tumours, the impact of KRAS mutation on prognosis was suspected through epidemiological cohorts studies [70]. This detrimental role of KRAS mutation on stage III colon cancer outcome was confirmed by post hoc analysis of prospectively collected data from adjuvant studies such as PETACC-8 and N0147 (Table 2) [58, 59, 70, 71]. Though it has been suggested that codon 12 and 13 did not bear the same prognostic value [72] more recent data in larger cohorts with better clinical follow-up, showed that both exon 2 mutations are poor prognostic markers [71, 73, 74]. Contradictory results were obtained in a post hoc analysis of the PETACC-3 trial in 894 patients with stage III colon cancer, where KRAS mutations were associated with neither relapse nor survival [7]. Finally, a pooled analysis of the PETACC-8 and N0147 trials confirmed a 1.5 higher risk of relapse and death in KRAS mutant, than in the KRAS wild-type population, for TTR, DFS and OS [61]. In this cohort of 4411 stage III colon cancer patients, as with BRAF, this prognostic value was only found in pMMR patients [61]. Screening for KRAS and NRAS mutations (by expanded RAS analysis) is now mandatory in metastatic colon cancer before targeting the EGFR pathway [56]. Unfortunately, expanded RAS analysis was not used in the majority of the studies described...
above. It could provide complementary information needed to understand the lack of efficacy of anti-EGFR therapies in early-stage colon cancer.

HER2 and other molecular factors

HER2 (Erbb2) is a transmembrane receptor of the EGFR family [75]. Its activation plays a role in cell proliferation and differentiation, and in apoptosis inhibition [75]. The reported prevalence of HER2 overexpression/amplification in colon cancer varies widely, from ~3%–47% in the different cohorts, but it is generally below 10% [76–78].

The prognostic value of HER2 overexpression in this setting is controversial [77, 79–81]. Most studies have shown that HER2 expression is not associated with outcome but rather with the presence of metastases [79–81]. However, Park et al. found that tumours with HER2 overexpression had a higher recurrence rate and were associated with poorer 3- and 5-year survival rates [77]. These results were confirmed by a retrospective analysis of stage III colon cancer samples from 2559 patients included in PETACC-8 randomized trial [78]. In this cohort, HER2 mutation was observed in 3.8% of cases and was associated with higher rate of recurrence (HR 1.55 [95% CI 1.02–2.36], P = 0.04) and shorter OS (HR1.57 [95% CI 0.99–2.5], P = 0.05) in multivariate analysis [78]. Differences between studies could be explained by the different techniques used to assess HER2 expression. The PETACC8 study used the next-generation sequencing colon cancer panel V2 (for mutations in exons 19–21, and for amplification) plus immunohistochemistry [78]. The results of the phase II trial of trastuzumab plus lapatinib in HER2-amplified metastatic colon cancer are encouraging for this kind of dual blockade in early-stage colon cancer [82]. However, the only evidence of HER-2 blockade efficacy we have in oncology concern breast cancer and, recently, gastric cancer [83–85]. In colon cancer, we will have to wait for further phase III studies results assessing the dual blockade of the HER-2 pathway by both trastuzumab and lapatinib to confirm HER-2 as a valuable target for metastatic and non-metastatic colon cancer.

Other molecular features have been described as prognostic factors. Caudal-type homeobox transcription factor 2 (CDX2) is an important regulator of intestinal development and is involved in colon oncogenesis [86, 87]. Colon cancers without CDX2 overexpression are often associated with aggressive features such as advanced stage, poor differentiation, vascular invasion, BRAF mutation, and the CpG island methylator phenotype [88–91]. Recently, Dalbera et al. showed that a lack of CDX2 expression was associated with worse outcome in stage II and III colon cancer (HR for disease recurrence 3.44, 95% CI 1.60–7.38, P = 0.002). In stage III colon cancer, both CDX2 positive and negative tumours benefit from adjuvant treatment [92, 93]. Moreover, though very promising these findings on CDX2 prognostic and predictive value have now to be confirmed in external series based on patients enrolled in clinical trials in order to increase the quality of clinical data and the robustness of this marker before becoming a new daily practice usable tool.

Somatic mutations of PIK3CA are present in 10%–20% of colorectal cancer, basically confined to exon 9 and exon 20. Mutations of PIK3CA seem to predict the lack of response for anti-EGFR therapy, especially mutations in exon 20, for patients with RAS wild-type tumours [94, 95]. In addition, there is moderate evidence of the predictive value of PIK3CA mutations for adjuvant aspirin, coming from two epidemiological cohorts from the US [96]. Ongoing randomized controlled trials will allow to definitely answer this question in the forthcoming years.

Preliminary data indicate ctDNA can be detected in a high proportion of patients with advanced CRC, suggesting promise as a, prognostic and predictive test that should have broad community acceptance. Preliminary results showed that ctDNA is detectable at diagnosis in the majority of patients with non-metastatic CRC. In this 68 patients population ctDNA, based on 15 frequent mutations, was detected in 41 (60%) of patients before surgical removal of the primary. With a 15-month follow-up all 5 patients that recurred had positive ctDNA at diagnosis, whereas CEA was elevated in only 1 of these cases. [97]. The presence of ctDNA after surgery seems thus a major prognostic biomarker with an HR of 18 (95% CI: 7.9–40) for the risk of recurrence [98]. The potential for ctDNA as a prognostic or predictive marker for patients with early stage cancer, should be further explored.

Molecular classification

Improvements in DNA sequencing techniques have allowed researchers to explore more than one mutation at a time, providing insights into colon cancer biology. The Cancer Genome Atlas Network analysed 224 colorectal tumours by whole-exome sequencing and integrative analysis of genomic data [99]. This work showed that there are two types of colorectal cancer: hypermutated and non-hypermutated tumours. Similarly, Guinney et al. defined four consensus molecular subtypes (CMS) of colon cancer, based on transcriptomics, using surgical samples of all stages (I–IV; 41% stage III) [100]. Each CMS has genetic specificities (Table 3). CMS4 tumours were associated with worse OS and relapse-free survival after adjustment for clinicopathological features, dMMR status, and BRAF and KRAS mutations: the 5-year OS rates were 62% in the CMS4 group and 74%, 77% and 75% in the CMS1, 2 and 3 groups, respectively. Patients with CMS1 tumours had worse survival after relapse, with a median of 9 months, versus 35 months for CMS2 tumours, 20 months for CMS3 tumours and 24 months for CMS4 tumours (Table 3). Song et al. retrospectively analysed the NSABP-C07 data and found that only patients with stage III CMS2 tumours seemed to benefit from fluorouracil + oxaliplatin adjuvant chemotherapy as compared with fluorouracil alone [101]. However, this was not found in the validation cohort of this study and it needs to be confirmed in external series [101].

The differences in the prognosis of CMS groups confirm that the molecular mechanisms of each subtype are clinically relevant. It is conceivable that activation of genes involved in the metastatic process confers a worse prognosis.

This classification could help to select patients for adjuvant treatment by providing prognostic and predictive information, and could also help to tailor individual treatment. Yet, the only data available on the predictive value of the CMS signature on the benefit of adjuvant chemotherapy come from a retrospective study with chemotherapeutic regimens that are not standards anymore. The assessment of the predictive value of CMS has now...
to be assessed on patients treated with a fluoropyrimidine- and oxaliplatin-based adjuvant regimen. Moreover, the feasibility of CMS in daily practice, even for prognostication, is currently difficult due to technical and cost constraints, but may be interesting to categorize patients as a stratification parameter in future adjuvant clinical trials.

Recently, interesting results have been reported on the epithelial to mesenchymal transition. Activation of genes involved in this transition seems to undermine the benefit of adjuvant chemotherapy and could also predict the efficacy of some targeted therapies [102–104].

Table 3. CMS subgroups and their associated molecular characteristics, according to Guinney et al. [100]

<table>
<thead>
<tr>
<th>Subtype</th>
<th>CMS1 (MSI/immune)</th>
<th>CMS2 (canonical)</th>
<th>CMS3 (metabolic)</th>
<th>CMS4 (mesenchymal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>14%</td>
<td>37%</td>
<td>13%</td>
<td>23%</td>
</tr>
</tbody>
</table>

Clinical features
- Female
- Right sided lesions
- Higher histopathological grade
- Left sided

More advanced stage (III or IV)

Associated mutations
- dMMR, CIMP high, hypermutation
- BRAF mutations
- Immune infiltration and activation
- SCNA high
- WNT and MYC activation
- Mixed MMR status, CIMP low, SCNA low
- KRAS mutations
- Metabolic deregulation
- SCNA high
- Stromal infiltration
- TGFβ activation
- Angiogenesis
- HER2 high level amplification

Outcomes
- Worse survival after relapse
- Superior survival after relapse
- Worse relapse free and overall survival

5-year overall survival rate (95% CI)
- 74% (69–79)
- 77% (74–80)
- 75% (70–80)

62% (58–66)

5-year relapse-free survival rate (95% CI)
- 75% (70–80)
- 73% (70–77)
- 73% (68–80)

60% (55–65)

Median survival after relapse
- 9 months
- 35 months
- 20 months
- 24 months

5-year overall survival rate (95% CI)
- 74% (69–79)
- 77% (74–80)
- 75% (70–80)

5-year relapse-free survival rate (95% CI)
- 75% (70–80)
- 73% (70–77)
- 73% (68–80)

CIMP, CpG island methylation phenotype; SCNA, somatic copy number alteration.

Table 4. Comparison of the existing molecular signatures for treatment decision in localized colon cancer

<table>
<thead>
<tr>
<th>Signature</th>
<th>Disease setting</th>
<th>N</th>
<th>End point</th>
<th>Results for stage III</th>
<th>Dataset</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncotype Dx</td>
<td>II–III treated</td>
<td>892</td>
<td>5-year recurrence rate</td>
<td>Recurrence rate:</td>
<td>Clinical trial (NSABP-C-07)</td>
<td>Yothers et al. [105]</td>
</tr>
<tr>
<td>colon cancer</td>
<td>colon cancer</td>
<td></td>
<td></td>
<td>Low: 21% (95 CI 16–26),</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intermediate: 29% (95 CI 24–34),</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High: 38% (95 CI 30–46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncotype Dx</td>
<td>II–III treated</td>
<td>688</td>
<td>3-year RFS and OS</td>
<td>–</td>
<td>Clinical trial (PETACC-3)</td>
<td>Di Narzo et al., [108]</td>
</tr>
<tr>
<td>colon cancer</td>
<td>colon cancer</td>
<td></td>
<td></td>
<td>5-year RFS: Low: 78.2% (95 CI 49.9–90.7),</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High: 47.2% (95 CI 25.8–68.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coloprint®</td>
<td>I–II–III treated and untreated</td>
<td>206</td>
<td>5-year RFS</td>
<td>5-year RFS:</td>
<td>Prospective cohort</td>
<td>Salazar et al., J Clin Oncol (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low: 82% (95 CI 50–90.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veridex®</td>
<td>II</td>
<td>688</td>
<td>3-year RFS, SAR and OS</td>
<td>–</td>
<td>Clinical trial (PETACC-3)</td>
<td>Di Narzo et al., [108]</td>
</tr>
</tbody>
</table>

Molecular signatures

Supervised prognostic genomic signatures have been developed to summarize the prognostic information provided by molecular factors. The main genomic signatures have been widely analyzed in both retrospective and prospective studies, including Oncotype Dx Colon Cancer®, ColoPrint®, Veridex® and GeneFx Colon® [105–107]. Most validation studies focused on stage II disease, but several included stage III disease, especially using the Oncotype Dx Colon Cancer® signature [105]. These genomic signatures can classify the risk of recurrence as low or high (Table 4).
In theory, the information provided by these signatures is very important. However, the accuracy of these scores is controversial, especially in stage III disease. Indeed, these signatures divide patients into three groups, but the benefit of adjuvant treatment of the large intermediate-risk group is unclear. Moreover, prediction of recurrence is not very good for stage III disease. For example, Oncotype Dx Colon Cancer predicts 5-year recurrence rates for low, intermediate and high-risk stage IIIA/B colon cancer of, respectively, 21% (95% CI 16%–26%), 29% (95% CI 24%–34%), and 38% (95% CI 30%–46%) [105].

In the prospective validation of Oncotype Dx Colon Cancer, only high- and intermediate-risk patients seemed to benefit from the adjunction of oxaliplatin to 5FU [105], but there were few events in the different groups and these results thus need to be confirmed.

None of the current signatures has been evaluated in a real-life setting. This would need testing to be randomized after removal of the primary tumour, in order to determine the influence on treatment decisions and patients’ outcome.

Recent prognostic signatures based on apoptotic genes have been tested in stage III colon cancer [109]. The APOPTO-CELL-PC3 is the more accurate of them, and divides the patients into three groups depending on their apoptosis competency and their Procaspase-3 level of expression. With this signature, high risk patients had increased risk of relapse (HR 3.90, 95% CI 1.59–9.37, \( P = 0.008 \)) and death (HR 9.40, 95% CI 2.06–41.98, \( P = 0.002 \)). Despite the fact that those signatures have been validated externally, they cannot be used in daily practise because of the too small number of patients included in the studies.

### Immunologic features

In 1986, lymphocyte infiltration (LI) of rectal carcinoma was linked to a better prognosis [110]. More than 300 publications on colorectal carcinoma treated with various therapies have confirmed this seminal publication [111–113]. In 2005, Pagès et al. showed that abundant CD3 + CD8+ and CD3 + CD4+ T lymphocyte infiltration was associated with better survival. In addition, a high density of T cells was associated with less nodal invasion and metastatic recurrence [14]. A prognostic LI immunoscore has been developed [113, 114], based on numbers of lymphocyte populations (CD3/CD45RO, CD3/CD8 or CD8/CD45RO) in the tumour core and invasive margins. This score identifies three groups of clinical outcome. It was very recently shown to be reproducible and robust in a large multicentre assessment conducted in 17 countries and involving more than 3800 samples, including a subset of stage III disease [115].

Another immunoscore is based on automated LI evaluation (linear quantification of lymphoid infiltration, LQLI). This method divides tumours into 3 patterns of CD3+ lymphocyte distribution between the tumour front of invasion, the tumour core, and surrounding tissues. Low-level LI (LQLI pattern 3) was associated with worse DFS in this study [HR 6.02, (95% CI 2.74–13.18), \( P < 0.001 \) in multivariate analysis] [116]. This score has been recently validated prospectively, using a predefined statistical hypothesis, in a large phase III trial population of stage III patients who received FOLFOX-based adjuvant treatment [117].

Though some of these immunoscores are now considered as robust and reproducible and others have been validated with predefined statistical hypotheses, none of them are currently usable in daily practice. To do so, we are still waiting for a score that is validated with multivariate analyses integrating all potentially confounding clinical and molecular prognostic factors such as a dMMR phenotype, BRAF and RAS mutational status, in prospectively randomized patients from clinical trials, using predefined statistical hypotheses and in an integrated approach to therapeutic decision-making.
In future, such scores will be implemented in clinical decision-making for post-surgical treatment of stage III colon cancer, both to refine prognostication and to predict the efficacy of immunotherapies.

Conclusion

Although the treatment of stage III colon cancer has barely changed since 2004, our knowledge of prognostic factors and carcinogenesis has advanced considerably in the last decade (Figure 1). However, the prediction of adjuvant chemotherapy and targeted therapies benefit in molecular subclasses are mainly based on in vitro and retrospective datasets, and need to be confirmed prospectively to allow personalized treatment in the future.

Alongside long-standing clinical and histological factors, new immunological and molecular factors are assisting with prognostication. For example, MMR, BRAF, KRAS and HER2 alterations, and immune infiltration, should probably be taken into account in the near future.

Existing scores only use one set of prognostic tools: molecular, clinical or immunological. Now, large-scale analyses of pooled data from recent clinical trials will help to integrate these factors in a global approach needed to extend advances made in the metastatic setting to patients with localized disease.

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