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Tumour Review

The evolving role of microsatellite instability in colorectal cancer: A review

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ABSTRACT

Microsatellite instability (MSI) is a molecular marker of a deficient mismatch repair (MMR) system and occurs in approximately 15% of colorectal cancers (CRCs), more frequently in early than late-stage of disease. While in sporadic cases (about two-thirds of MSI-H CRCs) MMR deficiency is caused by an epigenetic inactivation of MLH1 gene, the remainder are associated with Lynch syndrome, that is linked to a germ-line mutation of one of the MMR genes (MLH1, MSH2, MSH6, PMS2). MSI-H colorectal cancers have distinct clinical and pathological features such as proximal location, early-stage (predominantly stage II), poor differentiation, mucinous histology and association with BRAF mutations. In early-stage CRC, MSI can select a group of tumors with a better prognosis, while in metastatic disease it seems to confer a negative prognosis. Although with conflicting results, a large amount of preclinical and clinical evidence suggests a possible resistance to 5-FU in these tumors. The higher mutational load in MSI-H CRC can elicit an endogenous immune anti-tumor response, counterbalanced by the expression of immune inhibitory signals, such as PD-1 or PD-L1, that resist tumor elimination. Based on these considerations, MSI-H CRCs seem to be particularly responsive to immunotherapy, such as anti-PD-1, opening a new era in the treatment landscape for patients with metastatic CRC.

Introduction

Colorectal cancer is still a major public health problem in Western countries, representing the third most common cancer in both women and men. Actually the 5 year-overall survival rate approaches 65%, depending on stage of disease (90% in stage I, 15% in stage IV). Thanks to screening programs, in 2015 more than 70% of new cases underwent potentially curative resection [1].

Although traditional clinical-pathological staging remains useful in predicting the outcome, CRC shows a significant heterogeneity in both prognosis and response to therapy, even within the same pathological stage.

This clinical heterogeneity may be at least in part linked to genetic alterations that occur during the pathogenesis of CRC: in 85% of CRCs the process is driven by chromosomal alterations, either qualitative or quantitative (chromosomal-instability pathway), while in 15% is driven by a defective function of DNA MMR system (microsatellite-instability pathway) [2].

MSI represents a molecular hallmark of hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome (LS), usually linked to a germ-line mutation in one of MMR genes. Nevertheless, the majority of cases with MSI are sporadic, more often due to an epigenetic inactivation of hMLH1 [3,4].

The prevalence of CRCs with microsatellite instability (MSI) is different among disease stages: 15% in stage II–III (more common in stage II) [5,6], 4–5% in stage IV [7].

The current review summarizes the clinical-pathological features of MSI CRCs, the prognostic and predictive significance of MSI status in early-stage and metastatic disease and the implications on new drugs development.

Microsatellite instability: definition

MMR system is of pivotal importance for the rectification of DNA sequence mismatches during DNA replication. This repair system is mainly composed of four proteins (MLH1, MSH2, MSH6 and PMS2) interacting together to detect mismatches and cut out them, so that DNA polymerase and DNA ligase can resynthesize and rebind correct DNA strand [8].

Microsatellites are short DNA motifs of 1–6 bases repeated and distributed throughout the genome both in coding and non-coding

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regions. Owing to their repeated structure, microsatellites are particularly prone to replication errors that are normally repaired by the MMR system. Loss of function of one of the MMR proteins causes a deficient MMR system leading to the accumulation of mistakes in microsatellites, such as insertions or deletions, which result in a genetic instability. MSI may have an oncogenic potential when it occurs in coding regions of genes involved in several crucial cellular functions and pathways [9]. MSI is detected by PCR amplification of specific microsatellite markers or by immunohistochemical loss of expression of one of the above mentioned proteins. While in LS MSI is related to germ-line mutation in one of MMR genes (usually MLH1 or MSH2), in sporadic MSI CRCs there is usually an epigenetic inactivation of the hMLH1 gene via methylation of the gene promoter [10,11].

Lynch syndrome

LS, the most frequent form of hereditary CRC, is an autosomal dominant condition with incomplete but high penetrance, caused by an inactivating germ-line mutation of one of the four genes involved in the DNA MMR system (MLH1, MSH2, MSH6, PMS2). LS is characterized by early-onset colorectal and endometrial tumors and an increased risk of certain extra-colonic cancers, including tumors elsewhere in the gastrointestinal tract (e.g., stomach, small bowel, biliary tract), in the urinary collecting system (renal pelvis, ureter) and in the female reproductive system (ovaries). Recent population-based studies showed a lifetime CRC risk of about 52.2% in women and 68.7% in men and a median age at diagnosis of 61.2 years [10–12].

Amsterdam and Bethesda criteria were developed to identify potential LS patients candidates for genetic testing [13,14], as shown in Tables 1 and 2 [15,16]. Nowadays, many guidelines suggest two possible approaches to screen out LS: a universal one, that is to test every patient with CRC, and a selective one, that is to test every patient with CRC diagnosed prior than 70 plus patients diagnosed at older age who meet the Bethesda criteria, with the latest approach missing more than a quarter of patients with LS (Fig. 1) [17].

The results of immunohistochemistry and genetic testing show excellent concordance. Nevertheless, few cases of MSI cannot be detected by immunohistochemistry because missense mutations can lead to a dysfunctional protein, which is dismantled with loss of antigenicity [18,19].

The first proposed panel of MSI markers consisted of two mononucleotides (BAT-25, BAT-26) and three di-nucleotides (D2S123, D5S346, and D17S250) [20]. A new expert consensus recommend the use of a panel of 5 quasi-monomorphic mononucleotide repeats (BAT-25, BAT-26, NR21, NR24 and NR27), characterized by a constant number of nucleotide repeats and an identical size between individuals, unlike most microsatellites are polymorphic [15]. CRCs can be classified into microsatellite instability-high (MSI-H), and microsatellite instability-low (MSI-L), depending on the percentage of loci with MSI. In particular, a MSI-H phenotype is defined by the presence of at least two unstable markers among the 5 analyzed (or $\geqslant$30% of unstable markers if a larger panel is used). Conversely, most of the sporadic CRCs are designated as microstallite-stable (MSS), because they show chromosomal instability and a lack of MSI features [21].

With immunohistochemistry it should be considered that MMR proteins PMS2 and MSH6 cooperate with MLH1 and MSH2 respectively and their expression closely depends on the binding to the major partner (i.e. MLH1 and MSH2). Therefore, loss of expression of MSH2 is frequently associated with loss of expression of MSH6 and this pattern is highly suggestive of MSH2 germ-line mutation. Similarly, loss of expression of MLH1 is frequently associated with loss of expression of PMS2 and this pattern may results either from MLH1 germ-line mutation or from acquired somatic hypermethylation of the MLH1 gene promoter. Germ-line mutations of MSH6 and PMS2 are generally associated with isolated loss of expression of MSH6 and PMS2 protein respectively [22].

Clinical-pathological features

From a clinical point of view, MSI-H CRCs are diagnosed at a younger age, with a predominance in the right colon, frequently raised from sessile serrated adenoma and are diagnosed at an earlier stage as compared to MSS CRCs, most commonly in stage II [23,24]. Moreover, as opposed to LS cases, sporadic CRCs are characterized by older age at diagnosis and are more often associated with female sex and cigarette smoking [25–27].

Histologically, there are some peculiarities that may suggest the MSI status, beyond IHC or genetic testing. A great production of

Table 1
Bethesda revised guidelines for Lynch syndrome related CRC.

Tumors from individuals should be tested for MSI in the following situations:
1. CRC diagnosed in a patient who is less than 50 y.o
2. Presence of synchronous, metachronous CRC or other Lynch Syndrome related tumours, regardless of age (*endometrial, stomach, ovarian, pancreas ureter and renal pelvis, biliary tract and brain tumors, small bowel cancers, sebaceous gland adenomas and keratoacanthomas as seen in Muir-Torre syndrome*)
3. CRC with the MSI-H histology diagnosed in a patient who is less than 60 y.o (*presence of tumor-infiltrating lymphocytes, Chron's-like reaction, mucinous/signet-cell differentiation or medullary growth pattern*)
4. CRC diagnosed in a patient with one or more first-degree relatives with Lynch Syndrome-related cancers, with one of *the cancers* diagnosed before 50 y.o
5. CRC diagnosed in a patient with two or more 1st or 2nd degree relatives with Lynch Syndrome related cancers regardless of age

Adapted Ref. [15]

Table 2
Amsterdam II criteria.

At least three relatives must have cancer associated with Lynch Syndrome (colorectal, endometrium, small bowel, ureter or renal-pelvis); all of the following criteria should be present:
– One must be a first-degree relative of the other two
– At least two successive generations must be affected
– At least one relative with cancer associated with Lynch Syndrome should be diagnosed before age 50
– FAP should be excluded in the CRC cases
– Tumors should be verified by pathological examination

Adapted Ref. [16].

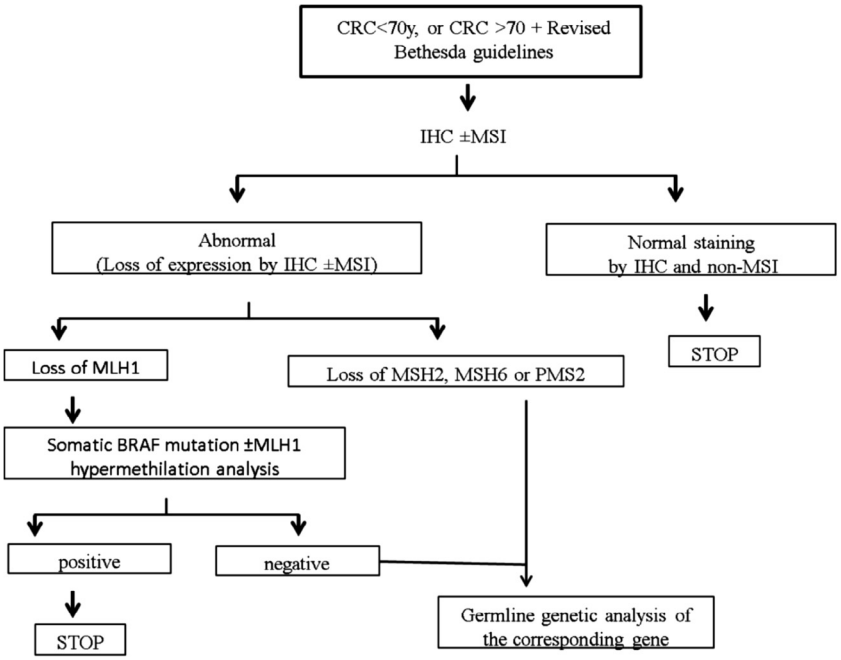

Fig. 1. The diagnostic alghorithm for Lynch syndrome: the selective approach [13].

mucin with extracellular accumulation often correlates with MSI, within different percentage of tumour area. Also signet-ring cell differentiation, with intracytoplasmic filling with mucin, causing lateral dislocation of the nucleus, is frequently observed, coexisting or not with extracellular mucin. Oftentimes MSI-H CRCs show poor differentiation, with a peculiar incidence of medullary carcinoma, a rare histotype, characterised by sheets of cells with vesicular nuclei and cytoplasmatic eosinophilia [28–30].

Important lymphocytes infiltration, even with a Crohn's like reaction, is prominent in MSI CRCs. This is due to the lack of MMR system with the consequent accumulation of frame-shift mutations that causes the transcription and translation of peptides with altered amino acid sequences (neo-antigens), that are presented by HLA class I and are recognized by cytotoxic T cells (CTL) [31,32]. Tumor-infiltrating lymphocytes could be divided into stroma-infiltrating lymphocytes (SIL) and intra-tumor cell infiltrating-lymphocytes (ITCIL), that lie beyond the basal membrane of the tumour cells. MSI-H CRCs show a severe infiltration of ITCIL, that are antigen-restricted cytotoxic T-lymphocyte (CD8+), with no difference between LS and sporadic MSI-H CRCs [33]. In the study by Tougeron et al. the number of frameshift mutations and their presence in specific target genes (ASTE1/HT001, HNF1A and TCF7L2) correlates to the number of infiltrating CTL. Notably, neo-antigens are presented by antigen presenting cells (APC) also to CD4+ T lymphocyte, that are responsible for the secretion of cytokines that enhance and sustain the cytotoxic activity of CTL, mainly depending on the Th1 polarisation [28,34,35].

From a molecular point of view the MSI-H status could be suspected also by different mutation status in two oncogenes. BRAF and KRAS are two mutually exclusive mutated oncogenes that encode for serine/threonine kinase leading to stimulation of the mitogen-activated protein kinase pathway (RAS-RAF-MEK-ERK) [36]. BRAF V600E mutations can be useful to rule out LS, because they frequently occur in sporadic MSI CRCs, caused by MLH1 promoter methylation, especially in C-region, while rarely can be detected in LS-related CRCs, except for cases associated with PMS2 germ-line mutation. By contrast, KRAS mutations (in codons 12 or 13) are inversely correlated with MSI-H status [37–39].

Interestingly, in a recent work Stadler et al. demonstrated that a multigene NGS tumor profiling panel can reliably discriminate between deficient-MMR (dMMR) and proficient-MMR (pMMR) CRCs on the basis of a mutational load cut-off, providing simultaneously information about mutational status of RAS, BRAF and other genes [40].

Prognostic role of MSI in early and metastatic disease

MSI-H CRCs have peculiar clinical features and a better prognosis compared to MSS tumors. In some reports this survival advantage seems to be independent of tumour stage [41,42], whereas in others seems to be confined to stage II [43] or stage III [44], but these data are likely confounded by the effect of adjuvant chemotherapy (Table 3) adapted Ref. [8].

A meta-analysis evaluated 1277 MSI-H stage I–IV CRC patients from a total of 32 eligible studies. The authors found that the effect of MSI on prognosis was independent from patient selection and was maintained in both the early and advanced setting, with a 35% reduction in the risk of death (HR 0.65, 95% CI 0.59–0.71). This

Table 3
Summary of results of studies with 5FU-based chemotherapy in MSI-H CRCs.

References	Stage	Chemotherapy regimen	Results
[55]	III	5FU-LEV	Benefit
[54]	III	5FU-based	Benefit
[57]	IV	5FU-FO	Benefit
[3]	II-III	5FU-FO	Detriment
[59]	II-III	5FU-based/5FU-LEV	None
[36]	All	5FU-based	None
[52]	All	5FU-based	None
[58]	All	5FU-based	None
[53]	II-III	5FU-based	None
[74]	II-III	5FU-based	None
[62]	II-III	5FU-FO	Detriment
[40]	All	5FU-based	None
[41]	II-III	5FU-based	Benefit in LS
[42]	II-III	5FU-FO	Benefit
[65]	II-III	5FU-FO	Benefit in stage III

Abbreviations: FO, folinic acid; 5-FU, 5-fluorouracil; LEV, Levamisol. Adapted Ref. [8].

analysis included untreated patients as well as patients treated with 5-FU-based chemotherapy and the benefit was maintained when restricting the analysis to clinical trial participants [45].

Afterwards, data from a meta-analysis by Guastadisegni et al. confirmed the association between MSI and favourable prognosis in 31 eligible studies providing overall survival (OS) data from 1972 stage I–IV MSI-H patients [46]. The better outcome was found in terms of OS, disease-specific survival (DSS) and disease-free survival (DFS). Subsequent clinical trials confirmed that dMMR tumors have a significantly reduced recurrence rate as compared to pMMR ones [47,48].

In a retrospective analysis of the QUASAR study, that included 1913 CRC patients who were randomly assigned to receive or not adjuvant treatment with 5-FU/LV, the prognostic value of MMR was similar in the presence and absence of chemotherapy. Supporting these data, in the 5-FU control arm of the PETACC3 trial in more than 600 stage II–III patients, the good prognostic effect of MSI remained significant ($p = 0.0077$) [48,49].

Moreover, in a multicentre retrospective analysis of 521 stage I–III MSI-H CRCs, bowel obstruction, stage T4 and vascular emboli were found to be independent predictors of poor DFS in multivariate analysis, thus highlighting the importance of the screening for vascular emboli to guide the decision whether or not to offer adjuvant chemotherapy in these patients [50].

Another interesting point is the role of BRAF mutations, whose relationship with MMR status in early-stage disease is still a matter of debate.

In a post-hoc analysis of the PETACC3 trial the impact of BRAF mutational status on OS was restricted to MSI-L and MSS tumors, while no prognostic significance was shown in the 190 MSI-H tumors [49]. This hypothesis was further corroborated by Samowitz et al. in a large US database: in 900 patients with stage I–IV disease, the BRAF V600E mutation did not affect the excellent prognosis of MSI tumors [51].

Focusing on this topic, Lochhead et al. evaluated a large US database and suggested that patients with MSI-H/BRAF^{WT} have the best prognosis, while BRAF mutant tumors are associated with the highest mortality [52]. This hypothesis was confirmed in the CALGB 89803 adjuvant trial: in 34 patients with MSI-H/BRAF^{MT} tumors the prognostic role of MSI was balanced by BRAF mutation, resulting in the same survival as patients with MSS/BRAF^{WT} tumors; in other words, survival was longer in patients with MSI-H/BRAF^{WT} disease and intermediate in patients with MSS/BRAF^{WT} or MSI-H/BRAF^{MT} disease, whereas patients with MSS/BRAF^{MT} had the worst prognosis [53].

Therefore, collectively these data demonstrate that in early-stage disease BRAF mutation has a negative prognostic effect in MSS and MSI-L CRC, whereas in MSI-H tumors seem to partially mitigate the good prognostic effect of MSI.

In metastatic setting few data about the role of MMR status are available, mainly because of the low prevalence of MSI in stage IV, thus supporting the hypothesis that MSI CRCs have a lower metastatic potential [54,55].

In a series of recurrent CRCs, lower OS from diagnosis to death (OS1) and from recurrence to death (OS2) were observed in patients with MSI-H tumors. Of note, they also had a lower recurrence rate, more local and peritoneal recurrences, were less likely to undergo curative resection and did not benefit from conversion therapy, reflecting the intrinsic chemoresistance of these subsets of metastatic CRCs [56].

These data were confirmed in a pooled analysis of 4 phase III studies in first-line treatment of metastatic disease (CAIRO, CAIRO2, COIN, FOCUS): in 153 MSI-H patients median PFS and OS were significantly worse as compared to pMMR patients (PFS 6.2 vs 7.6 months, HR 1.33; 95% CI 1.12–1.57, $p = 0.001$ and OS 13.6 vs 16.8 months, HR 1.35; 95% CI 1.13–1.61, $p = 0.001$) [57].

In an another retrospective analysis, 55 patients with dMMR metastatic CRC were evaluated and did not appear to have improved outcomes (OS 15.4 months, 95% CI 10.61–17.74) even following R0/R1 metastasectomy (OS 33,8 months, 95%CI 14,5–76,5) as compared to historical pMMR controls. In this setting, patients with MSI-H/BRAF^{WT} tumors had an improved overall survival when compared with MSI-H/BRAF^{MT} tumors (17.3 vs 10.1 months; $p = 0.029$) [58].

Therefore, based on the above mentioned clinical data in the metastatic setting, it seems likely that MSI can have a negative prognostic role and that BRAF mutation can at least in part drive the poorer outcome observed.

Predictive role of MSI in adjuvant and metastatic setting

Regarding the role of MSI in predicting chemotherapy efficacy in early-stage CRC, a growing body of literature seems to suggest a lack of benefit of 5-FU chemotherapy in patients with dMMR, although the data are conflicting [5,42,59–66], as shown in Table 3 adapted Ref. [8].

Preclinical data showed that the presence of a dMMR status is associated with resistance to 5-FU [67,68]. A pooled analysis of 5 clinical trials by Ribic and colleagues [5], in which patients with stage II and III colon cancer were randomized to 5-FU plus levamisole or leucovorin versus surgery alone, demonstrated a better survival of patients with dMMR CRC treated with surgery alone and a lack of benefit of 5-FU-based chemotherapy. These results were confirmed in a subsequent analysis where the cases above mentioned were combined with those of a previous study of the same group. In particular, in patients with stage II dMMR tumors treatment was associated with reduced overall survival (HR, 2.95; 95% CI 1.02–8.54, $p = 0.04$) [69].

In the QUASAR trial, chemotherapy with 5-FU plus folinic acid compared to surgery alone improved survival in patients with stage II CRC [70]; interestingly, recurrence rate for dMMR tumors was half that of pMMR tumors (11% vs 26%; risk ratio [RR], 0.53; 95% CI 0.40–0.70, $p < .001$), but the chemotherapy efficacy did not differ significantly by MMR status [47].

In a pooled data analysis from the ACCENT database, time-to-relapse (TTR) and OS differ between dMMR and pMMR stage II CRC patients treated with surgery alone (TTR, HR 0.27; 95% CI 0.10–0.75; $p = 0.01$; OS HR 0.27; 95% CI 0.10–0.74, $p = 0.01$) but this advantage was attenuated in patients treated with 5-FU adjuvant chemotherapy (TTR HR 0.81; 95% CI 0.55–1.19, $p = 0.29$; OS, HR 0.87; 95% CI 0.61–1.26, $p = 0.47$). The prognostic role of MSI was confirmed also in stage III CRC patients treated with surgery alone (TTR HR 0.59; 95% CI 0.28–1.23, $p = 0.16$; OS HR 0.69; 95% CI 0.35–1.36, $p = 0.28$) and a significant survival benefit for 5-FU monotherapy vs surgery alone was seen both in patients with pMMR tumors (5-year survival rate = 71% vs 54%) and in patients with dMMR tumors (5-year survival rate = 77% vs 59%) [71].

In another analysis by Sinicrope and coworkers, 2141 patients with stage II and III CRC treated within randomized trials of 5-FU-based chemotherapy were evaluated: patients with dMMR CRC have reduced recurrence rates, delayed time to relapse, and improved survival rates, as compared to patients with pMMR CRC; a subset analysis suggested that any treatment benefit was restricted to suspected germ-line versus sporadic tumors. In addition, patients with stage III dMMR CRC treated with adjuvant 5-FU had a lower 5-year recurrence rate compared to patients receiving surgery alone (22% vs 37%; $p = 0.04$) [47].

Therefore, while adjuvant 5-FU chemotherapy does not seem to have an impact on the already good prognosis of patients with stage II MSI-H CRC, it should be considered as an option in patients with stage III MSI-H CRC.

Three phase 3 randomized trials have established the role of oxaliplatin added to a fluoropyrimidine (5-FU, Capecitabine) as the current standard of care for adjuvant treatment of stage III CRC patients [72–74].

In contrast to 5-FU, preclinical studies demonstrated that MSI tumor cells are sensitive to oxaliplatin [75], but to date few data with conflicting results have been published regarding the relationship between MMR status and oxaliplatin efficacy [76–78].

An analysis of patients with stage II and III CRC enrolled in NSABP C07 trial (5-FU plus folinic acid ± oxaliplatin) and C08 (FOLFOX ± bevacizumab) showed that dMMR was associated with an improved prognosis based on recurrence (HR 0.48, 95% CI 0.33–0.70, $p < 0.0001$), but was not predictive for oxaliplatin benefit [79].

Conversely, in a recent update of the MOSAIC phase III trial, comparing bolus/infusional fluorouracil plus leucovorin (LV5FU2) with LV5FU2 plus oxaliplatin (FOLFOX4), dMMR was an independent prognostic factor (HR 2.02; 95% CI 1.15–3.55, $p = 0.014$) and HRs for DFS and OS benefit in the FOLFOX4 arm were 0.48 (95% CI, 0.20–1.12) and 0.41 (95% CI 0.16–1.07), respectively, in patients with stage II and III dMMR CRC [6].

To complicate this issue further, in an analysis of the NO147 trial (a phase III trial comparing FOLFOX versus FOLFOX plus Cetuximab in stage III KRAS wild-type CRC), favorable DFS was observed for dMMR proximal tumors and any survival benefit was lost in distal and N2 tumors, suggesting that the predictive role of MMR status may depend on tumor location and nodal status [80].

Furthermore, in the AGEO study a large dataset of 433 dMMR stage II and III CRCs treated with surgery alone or surgery plus adjuvant chemotherapy consisting of fluoropyrimidine plus or minus oxaliplatin was analyzed [81]. As compared to surgery alone, adjuvant oxaliplatin-based chemotherapy improved DFS, contrary to fluoropyrimidine alone, with a statistically significant benefit in multivariate analysis limited to stage III tumors and to sporadic rather than LS cases.

Taken together these results, although controversial and deriving from retrospective analysis, seem to suggest the ability of oxaliplatin to overcome the detrimental effect of fluoropyrimidine monotherapy in dMMR CRCs, with the greatest benefit likely limited to certain subgroups (stage III, proximal location, N1 disease, sporadic cases).

In addition, a recent work by Dalerba and colleagues demonstrated that loss of CDX-2 identified a distinct phenotype of stage II CRC with worse prognosis and which seems to benefit from adjuvant chemotherapy [82]. To add complexity to this intricate scenario, MSI-H tumors appear to be characterized by an enrichment in CDX-2 negative tumors as compared to MSS ones; the relationship between these two factors deserve further evaluations, but it seems counterintuitive the correlation between a factor associated with good prognosis and probably resistance to 5-FU such as MSI with the CDX-2 negative status, which, on the contrary, confers a bad prognosis and a benefit from adjuvant chemotherapy.

Another crucial point concerns the predictive role of MSI in locally advanced rectal cancer (LARC) treated with neo-adjuvant fluoropyrimide-based chemo-radiation. Recently, de Rosa and colleagues retrospectively analyzed a series of 62 patients with germline dMMR rectal cancer, demonstrating a striking 27.6% of pathological complete response rate and outstanding stage-adjusted survival rates. Identification of a dMMR rectal cancers should not only trigger confirmatory germ-line testing, but could also influence the extent of surgical resection that should take into account several factors such as the presence of a synchronous colonic lesion, the tumor height, the clinical stage and the need for pelvic irradiation [83].

In contrast to early-stage disease, very few studies have analyzed the predictive role of MMR status in the metastatic setting, since only 4% of stage IV CRCs are MSI-H.

In a meta-analysis published by Des Guetz and colleagues, among 964 patients, 91 of whom had MSI-H tumors, no benefit of chemotherapy in terms of response rate for MSI-H compared with MSS tumors was found [84].

These results could therefore have implications especially in the management of liver metastases potentially eligible to surgery, where objective response could convert to surgery patients with initially inoperable disease. Since neo-adjuvant chemotherapy represents the standard of care, knowledge of MMR status, along with RAS and BRAF mutational status, could inform and guide clinical decision making also in this setting.

Immunotherapy in MSI colorectal cancers

As already stated, MSI-H CRCs manifest an important inflammatory response against which cancer cells line up several immune-escape strategies. For instance, beyond CD4+ and CD8+ T-cells the tumour microenvironment promotes the accumulation of Tregs that aim to turn the immune response towards cancer cells with the secretion of TGFβ and IL10 [32,85,86]. Moreover, there is an upregulation of inhibitory molecules such as checkpoint inhibitors (PD1, PDL1, CTLA4, lymphocyte-activation gene 3 and IDO) that, as well as Tregs, counterbalance the activation of the immune system with the classic "exhaustion" phenotype of TILs. The upregulation of immune checkpoints inhibitors is a consequence of the response to cytokines, like INFγ, that are part of the intratumoral immune activation. The consequence of this negative control is the inability of the immune system to eradicate cancer cells [87,88].

To overcome this obstacle, on the basis of a phase I trial evaluating anti PD-1 antibody therapy in patients with treatment-refractory solid tumours, in which only one case between CRCs, that turned out to be MSI, showed a complete durable response, Le et al. published a phase II study evaluating Pembrolizumab in patients with dMMR and pMMR CRC and a cohort of patient with neoplasms other than colorectal characterised by MMR deficiency [89,90]. The study reached the primary end point, showing a striking immune-related and RECIST objective response rate of 40% (4 of 10 patients), a disease control rate of 90% and an immune-related PFS rate at 20 weeks of 78% (7 of 9 patients) in the cohort of dMMR CRCs. Conversely, in the cohort of pMMR CRCs no immune-related and RECIST objective response rate were observed and the immune-related PFS rate at 20 weeks was 11%. Of note, all 6 patients with sporadic dMMR CRCs had and objective response, whereas only 3 of 11 (27%) patients with LS CRCs had a response. One explanation could be that germ-line dMMR CRCs had a lower number of frameshift mutations compared to the sporadic coun-

Table 4
Summary of ongoing clinical trials with anti-PD1 (±anti CTLA-4) in metastatic MSI-H CRC.

Trial identifier	Drug/s	Phase	Setting	References
KEYNOTE-164 (NCT02460198)	Pembrolizumab	II	Pre-treated	[91]
KEYNOTE-177 (NCT02563002)	Pembrolizumab	III	1-st line	[92]
CHEKMATE-142 (NCT02060188)	Nivolumab, Ipilimumab	I/II	Pre-treated	[93]

terpart, but this difference could also be due to the different pathogenesis of these 2 types of CRC [34].

To better clarify the potency of this new approach, a phase II (KEYNOTE-164) and a phase III (KEYNOTE-177) clinical trials evaluating Pembrolizumab in CRCs with MSI or MMR deficiency are ongoing [91,92]. Another trial (CHECKMATE-142) testing Nivolumab (anti PD-1) monotherapy and the association between Nivolumab and Ipilimumab (anti-CTLA-4 antibody) in MSI CRCs is ongoing [93] (Table 4).

Conclusions and future perspectives

Although LS is the most frequent cause of heritable CRC, it is still frequently underrecognised and underdiagnosed. In this context, it is of paramount importance to have widely available and easy-to-perform tests, in order to select patients who deserve further evaluation with a confirmatory germ-line testing and to offer the appropriate management to the patients and their relatives.

As regard early-stage CRC, MSI is a clear prognostic factor, especially in stage II, and a predictive marker of resistance to 5-FU, although literature data on this topic are conflicting. Despite this, some grey areas persist, in which there is room for improvement and for a shared decision making with the patients, for example in 'high risk' MSI-H stage II disease.

Differently from stage II, in stage III CRC it is unlikely that the MSI status would influence the choice of treatment (FOLFOX or XELOX). Nevertheless, a relevant and practical question is: should we treat with 5-FU or Capecitabine alone a patient with stage III MSI-H CRC not candidate to receive oxaliplatin?

In metastatic disease, the active immune microenvironment of MSI-H tumors makes these patients good candidate for enrollment in clinical trials with immune targeted therapies such as Nivolumab or Pembrolizumab, though only few patients have MSI-H metastatic CRC.

In conclusion, MSI CRC is an heterogeneous group of sporadic and heritable diseases which may behave differently, according to other characteristics (such as BRAF mutational status). MSI studies are difficult because of the low incidence of this characteristic, in different stages of the disease, with different standard treatments over time. In the future, it would be desirable to develop worldwide collaboration to obtain a sufficient number of patients to shed light on many unanswered questions. Furthermore, given the unprecedented outcome achieved with immunotherapy that would probably represent a 'game changer' in the management of metastatic MSI-H CRCs, clinical trials in other settings (neoadjuvant or adjuvant) are eagerly awaited.

Conflict of interest

The authors declare that they have not conflicts of interest.

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Author contribution

FG and MB conceived and designed the manuscript. All authors analysed and interpreted the data, and drafted the article. All the authors revised it critically for important intellectual content, and final approved the version to be submitted.

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None.

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