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Review

Microsatellite instability and chemosensitivity in solid tumours

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Sara Cherri, Ester Oneda, Silvia Noventa, Laura Melocchi and Alberto Zaniboni

***Abstract*:** The use of biomarkers that influence a targeted choice in cancer treatments is the future of medical oncology. Within this scenario, in recent years, an important role has been played by knowledge of microsatellite instability (MSI), a molecular fingerprint that identifies defects in the mismatch repair system. This knowledge has changed clinical practice in the adjuvant setting of colon cancer, and its role in the neoadjuvant setting in gastric tumours is becoming increasingly interesting, as well as in endometrial cancers in both early and advanced diseases. Furthermore, it has undoubtedly conditioned the first lines of treatment in the metastatic setting in different types of cancers. The incidence of MSI is different in different cancer types, as well as in early cancers *versus* metastatic disease. Knowing the incidence of MSI in the various histologies can provide insight into the potential use of this biomarker considering its prognostic value, especially in the early stages, and its predictive role with respect to treatment response. In particular, MSI can guide the choice of chemotherapy treatments in the adjuvant setting of colon and perioperative setting in gastric tumours, which could lead to immunotherapy treatments in these patients in both the early stages of the disease and the metastatic setting where the response to immunotherapy drugs in diseases with MSI is now well established. In this review, we focus on colon, gastric and endometrial cancers, and we briefly discuss other cancer types where MSI could have a potential role in oncological treatment decisions.

Keywords: cancer, chemotherapy, colon cancer, endometrial cancer, gastric cancer, microsatellite instability

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Introduction

Oncological clinical practice is increasingly aimed at identifying personalized and targeted drugs for the treatment of tumours. This has led to the search for targetable markers that identify patients who are more susceptible to a given treatment. This research primarily concerns biological drugs targeting certain molecular characteristics of the disease, unlike the use of chemotherapeutic drugs for which clinical research has not yet led to the identification of predictive response markers that would allow personalized treatments. However, in recent years, an aid to determining patient eligibility for standard chemotherapy treatments and identifying a proportion of unresponsive patients has been identified by increasing knowledge regarding the significance of microsatellite instability (MSI).[1] MSI is a molecular fingerprint that identifies defects in the mismatch repair system (dMMR). This mechanism involves a series of mismatch DNA repair enzymes, which are MutL homologue 1 (MLH1), MutL homologue 3 (MLH3), MutS homologue 2 (MSH2), MutS homologue 3 (MSH3), MutS homologue 6 (MSH6), postmeiotic segregation increased by 1 (PMS1) and postmeiotic segregation increased by 2 (PMS2). During normal DNA replication, the MSH2/MSH6 and MSH2/MSH3 heterodimeric complexes are responsible for detecting and binding DNA mismatch errors, while MLH1/PMS2 heterodimers are responsible for excision and resynthesis of the correct DNA bases in mismatch sites.

Correspondence to:
Sara Cherri
Department of Clinical Oncology, Fondazione Poliambulanza, Via bissolati 57, 25124, Brescia, Italy.
sara.cherri@poliambulanza

Ester Oneda
Silvia Noventa
Alberto Zaniboni
Department of Clinical Oncology, Fondazione Poliambulanza, Brescia, Italy

Laura Melocchi
Department of Anatomical Pathology, Fondazione Poliambulanza, Brescia, Italy

Therefore, loss of transcription or functional defects in one or more proteins of this fundamental control complex results in a systemic defect and consequent unsuccessful DNA repair.[2] This favours the accumulation of mutations in brief repetitive DNA sequences called microsatellites, which are distributed throughout the coding and noncoding genome and are generally unstable and identical in all cells from an individual. They are particularly sensitive to DNA mismatch errors because the constant repeat of the same nucleotides causes slippage of the DNA polymerase and errors in the absence of an efficient correction system. MSI is the result of variation in the number of mononucleotide and dinucleotide repetitions in tumour cells compared with normal cells, and their difference in length can be used as a surrogate to indirectly identify a mismatch repair system deficiency[3] (see Figure 1).

Standard diagnostic methods for identifying MSI in cancer include testing MSI status using molecular biology techniques[4] or, in daily clinical practice, evaluating expression of the four MMR proteins by immunohistochemistry (IHC) on histological tissue sections as a valid surrogate to identify tumours with a higher probability of instability,[5] though there is a slight and well-documented discordance between these two methods.[6] IHC is a relatively simple and widely available technique in all pathology laboratories that enables identification of the specific defective MMR protein by comparing expression between tumour cells and adjacent normal tissue.[7,8] On the contrary, IHC can be subject to preanalytical issues and fixation artefacts (false negatives), as well as aberrant or heterogeneous staining and false-positives because 6–7% of MSI tumours retain MMR IHC expression due to catalytically inactive mutated MMR proteins or other more complex molecular and epigenetic mechanisms underlying MSI, such as polymerase epsilon (POLE) mutations or MLH1 promoter methylation.[9,10] In these cases, and generally when IHC results are indeterminate or open to interpretation, MSI molecular testing should be performed according to Bethesda[11] and European Society for Medical Oncology (ESMO) guidelines.[12] MSI testing is based on polymerase chain reaction (PCR) amplification of microsatellite markers using different panels available comprising a combination of mononucleotide and dinucleotide repeats. Thus, MMR-IHC and MSI-PCR exhibit high concordance (90–95%),[5–8] but both tests should be performed to assess eligibility for treatment,[12] as a recent report demonstrated that nearly 10% of patients enrolled for immunotherapy in metastatic colorectal cancer (CRC) had false-positive dMMR or MSI-PCR results.[13] Next-generation sequencing (NGS) represents an appropriate alternative molecular test for assessing MSI and tumour mutational burden (TMB), especially in non-Lynch-associated tumours, where the molecular mechanisms driving MSI can be more complex and involve more genes and unusual pathways.[10,12] Nevertheless, NGS can be performed only in selected centres due to the expensive and required expertise.

The hypotheses that have been formulated to explain the biological mechanisms underlying resistance to chemotherapy of MSI-associated diseases primarily concern adjuvant fluorouracil-based treatments in CRC. Often cited in the literature are studies on the antitumor immune response, represented by the lymphocyte infiltrate that characterizes MSI diseases and which would be the basis for the best prognosis of these patients, an advantage contrasted by the immunosuppressive effects of chemotherapy and that explains the lack of benefit of fluoropyrimidine (FP)-based chemotherapy.[14] It is important to contextualize that these studies refer to stage II CRCs. According to some authors, the addition of irinotecan[15] or oxaliplatin[16] counteract resistance to fluorouracil in MSI tumours. However, these hypotheses have not been confirmed in randomized studies. Another interesting hypothesis is provided by an *in vitro* study in which it would seem that mismatch repair proteins are fundamental for establishing the fluorouracil (FU)–DNA complex and that their deficiency reduces the binding of FU to DNA and consequently the antitumor drug effect.[17] Regardless of hypotheses on the potential chemoresistance of MSI diseases, it is clear that this molecular marker plays a fundamental role in clinical oncology practice.

In this review, we will refer to the state of MMR/deficiency or MSI/high using the term MSI to refer more generally to the fingerprint that characterizes these diseases.

Based on the patient's history and familial information, genetic counselling is suggested for suspected Lynch syndrome. Young patient age, familial cases of Lynch syndrome-related cancers (colon cancer, gastric cancer, endometrial cancer) and absence of MMR proteins in cancer

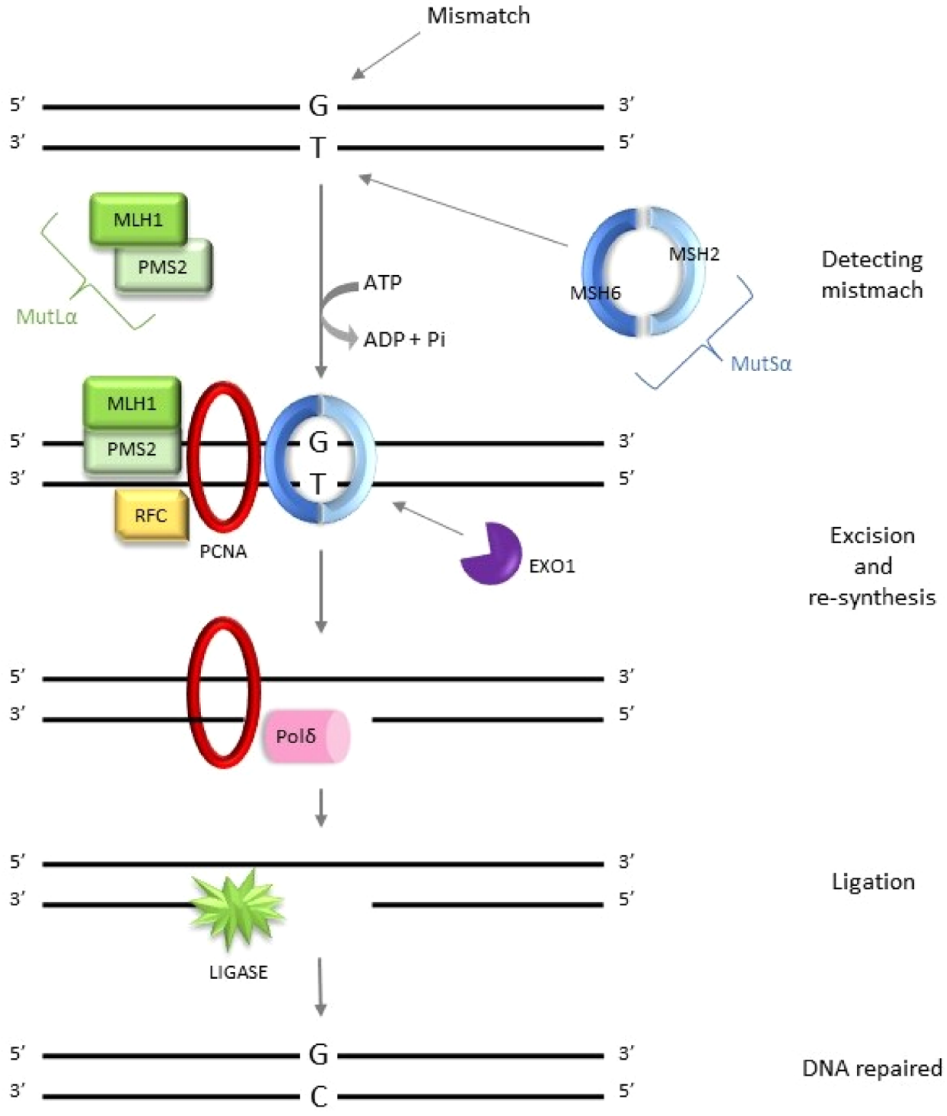

Figure 1. The MMR system consists of a group of proteins encoded by eight genes: MSH2, MSH3, MSH5, MSH6, MLH1, PMS1 (MLH2), MLH3 and PMS2. These proteins interact as heterodimers capable of perceiving and repairing mismatched DNA bases or error from basis insertions or deletions. To correct errors, MSH2 creates two distinct heterodimers, MSH2-MSH6 and MSH2-MSH3 (also called MutSα and MutSβ, respectively), which constitute a clamp that binds to misalignments forming a complex of diffusible ATP-bound proteins that slide the clamps controlling the postreplicated DNA strand and initiating DNA repair. MSH2-MSH6 heterodimers detect single-base mismatches and dinucleotide insertion–deletion distortions, while MSH2-MSH3 identify larger insertion–deletion loops that are ~13 nucleotides long. Specifically, when a G/T mismatch is recognized, the MSH2-MSH6 complex exchanges ADP for ATP, activating the complex. Other molecules, such as proliferating cell nuclear antigen (PCNA), replication factor C (RFC), MutLα (a MLH1-PMS2 heterodimer) and exonuclease 1 (Exo1), are recruited to the complex, leading to the final dissociation of the mismatch. The hMLH1-hPMS2 complex contains endogenous endonuclease activity that impacts the unmethylated strand. Single-stranded DNA breaks generate an entry point for the EXO1 exonuclease, which is required for degradation of the DNA strand–containing mismatched bases.

tissue require genetic testing. NGS techniques allow multiple gene analyses and are useful for resolving doubts regarding hereditary or sporadic cancer origin, leading to tailored familial screening and surveillance.[18]

Currently, in clinical practice, in Europe, determining the status of microsatellites is required only in patients diagnosed with colon and endometrial cancers,[19,20] while in the United States it is also recommended in gastric cancer.

This review aims to provide an overview of the possible use of this molecular fingerprint with particular attention to the chemosensitivity of neoplasms with MSI and the possible implications of this information in the therapeutic decision-making process.

Colorectal cancer

CRC is one of the most common cancers in terms of incidence and mortality, ranking third as a cause of cancer-related death worldwide.[21]

Currently, two different molecular genetic pathways have been distinguished, one involving chromosomal instability (CIN) and the other involving MSI. With respect to CIN, there are a series of genetic changes leading to the activation of proto-oncogenes (e.g. K-RAS, CTNNB1 and PIK3CA) and inactivation of tumour suppressor genes (e.g. p53, APC, SMAD2/4). This genetic pathway represents the most frequent pathogenetic alteration of CRCs, occurring in up to 80% of cases.[22] The second pathway of colorectal carcinogenesis is represented by MSI.

Greater awareness of the diversity of CRC and the implication that this difference has in the pharmacological management of these diseases has been conferred by the discovery of MSI. Currently, 10–25% of colon cancers are estimated to have MSI, of which 3% are hereditary and associated with Lynch syndrome, while the remaining 12% are caused by somatic and acquired hypermethylation of the MLH1 gene promoter. The prevalence of MSI in rectal cancer is less frequent than that in colon cancer, occurring in approximately 10% of cases.[23]

MSI CRCs have a phenotype including a greater onset in the right colon, a better prognosis and a decreased susceptibility to chemotherapy. They are also characterized by a lymphocytic infiltrate with a prominent inflammatory reaction that is poorly differentiated and often presents as the mucinous type.[24] CRC oncogenesis is characterized by a sequential accumulation of genetic alterations that overcome the redundant control mechanisms built into each cell, resulting in gradual tumour development. Several studies have attempted to determine how these alterations develop in an attempt to differentiate and recognize different phenotypes of CRCs. Among these studies, data published by the international multicentre study led by Bert Vogelstein and Albert de la Chapelle elucidated the clinical implications of MSI, demonstrating that deletion mutations in microsatellite sequences were widespread in inherited CRCs and in 13% of sporadic cases, indicating that hereditary cancers and a subset of sporadic cancers share a unique path of tumour development.[25]

These observations have important implications for the clinical practice of CRC, both in localized disease and in the metastatic setting. In this context, rectal cancer will be discussed separately.

Colon cancer

Determining the status of microsatellites has certainly gained in popularity due to its predictive value in directing management with conventional chemotherapy or novel-targeted agents and its prognostic significance for patient.[26] In recent years, the clinical management of CRC has seen important practice changes in both adjuvant and inoperable settings.

The primary clinical trials investigating the predictive role of MSI status on chemotherapy response are shown in both settings in Table 1, which summarizes the primary studies investigating the predictive role of MSI in colon cancer.

Resectable tumours

The first study published on the responsiveness of CRC with respect to MSI by Elsaleh *et al.*[27] concluded that cancers with MSI were more sensitive to adjuvant chemotherapy than tumours without MSI. This conclusion, which was subsequently demonstrated to be incorrect, derives from the design of this study; in particular, the assignment of patients to the treatment groups was at the discretion of oncologists rather than randomized. In fact, a careful evaluation of the study population shows that the patients selected for chemotherapy were characterized by fewer comorbidities and better performance status.

However, subsequent studies investigating the role of MSI as a predictor of response to chemotherapy treatments in the adjuvant setting did not show any benefit for chemotherapy in patients with MSI CRC (Table 1).

In particular, studies conducted by Ribic in 2003 and Sargent in 2010 provided evidence of the lack

Table 1. Predictive and prognostic role of MSI in colorectal cancer.

Author/trial	Study type	No. of MSI/all patients	Year of publication	Stage of disease	Therapy regimen	Results in MSI tumours
Elsaleh *et al.*[27]	Consecutive patients	63/656	2000	III	5-FU-based cht	Benefit of adj cht
Ribic *et al.*[28]	Randomized (specimens from five randomized trials)	95/570	2003	II–III	5-FU-based cht	No benefit of adj cht
De Vos tot Nederveen Cappel *et al.*[29]	Retrospective	47/92	2004	III	5-FU-based cht	No benefit of adj cht
Storojeva *et al.*[30]	Randomized (specimens from trial of SAKK)	21/160	2005	NS	5-FU/mitomycin	No benefit of adj cht, no correlation with OS and DFS in untreated patients
Benatti *et al.*[31]	Consecutive patients	256/1263	2005	I–IV	5-FU-based cht	No benefit of adj cht, better prognosis in stage II and III
Popat *et al.*[32]	Pooled analysis	1277/7642	2005	NS	5-FU	No benefit of adj cht, better prognosis
Lanza *et al.*[33]	Consecutive patients	114/718	2006	II–III	5-FU	Better prognosis, especially among patients without adj cht
Jover *et al.*[34]	Consecutive patients	66/754	2006	II–III	5-FU	No benefit of adj cht
Kim *et al.*[35]	Randomized	98/542	2007	II–III	5-FU/LV	No benefit of adj cht, increased RFS but no difference in OS
Des Guetz *et al.*[36]	Meta-analysis	454/3690	2009	II–III	5-FU-based cht	No benefit of adj cht
Bertagnolli *et al.*[15] Cancer and Leukaemia Group B Protocol 89803	Randomized	96/723	2009	III	5-FU/LV *versus* IFL	Better DFS in MSI patients treated with IFL, no if treated with 5-FU/LV
Sargent *et al.*[37]	Consecutive patients Pooled analysis	70/457 165/1027	2010	II–III	5-FU-based cht	No benefit of adj cht
Klingbiel *et al.*[38] PETACC-3 trial	Randomized	190/1254	2015	II–III	5-FU/LV or FOLFIRI	Stage II: RFS and OS better regardless of cht arm (adding irinotecan no added benefit). Stage III: RFS slightly better but no difference in OS
André *et al.*[39] MOSAIC	Randomized	95/1008	2015	II–III	5-FU/LV *versus* FOLFOX	Better DFS and OS, trends in favour of FOLFOX
Tougeron *et al.*[40]	Retrospective	433/433	2016	II–III	FP +/– oxaliplatin	Adding oxaliplatin improved DFS contrary to FP alone and only in stage III
Sinicrope *et al.*[41]	Pooled analysis NCCTG N0147, NSABP C-08	73/832	2017	Recurrence following adj cht in stage III	FOLFOX +/– bevacizumab or cetuximab	Better SAR Worse SAR in mutant BRAFV600E

(Continued)

Table 1. (Continued)

Author/trial	Study type	No. of MSI/all patients	Year of publication	Stage of disease	Therapy regimen	Results in MSI tumours
Zaanan *et al.*[42]	Pooled analysis NCCTG N0147 and PETACC8	252/2501	2018	III	FOLFOX	Favourable prognostic factor in a multivariate analysis
Chouhan *et al.*[43]	Retrospective cohort study	95/686	2018	III	5-FU-based cht	Neither BRAF nor MSI were individually predictive of OS benefit, benefit of adj cht only if MSI + BRAFmut
Taieb *et al.*[44]	Pooled analysis MOSAIC, NCCTG N0147, PETACC8, PETACC3, NSABP C07, NSABP C08, AVANT	271/2630	2019	Recurrence following adj cht in stage III	5-FU-based cht	Longer SAR regardless of BRAF status (shorter SAR in BRAFmut even if MSI)
Aggarwal *et al.*[45]	Meta-analysis	437/3051	2021	NS Adj setting	5-FU	MSI status does not alter 5-year OS of patients treated with adj 5-FU
Cohen *et al.*[46]	Pooled analysis 12 adjuvant trials	609/5457	2021	III	5-FU/LV +/− oxaliplatin	Adding oxaliplatin to 5-FU improves OS and DFS. Better outcomes in the N1 group but similar OS in the N2 group
Müller *et al.*[47]	Randomized	4/108	2008	IV	FP-oxaliplatin-based first-line cht	Lower rate of response with cht, no correlation with OS and PFS
Overman *et al.*[48] CHECKMATE-142	Phase II	74/74	2017	IV	Nivolumab in subsequent lines	ORR 31% DCR 69% 12-month OS 73%
Overman *et al.*[49] CHECKMATE-142	Phase II	119/119	2018	IV	Nivolumab + ipilimumab in subsequent lines	ORR 55%, DCR 80% 12-month PFS 71% 12-month OS 85%
Lenz *et al.*[50] CHECKMATE-142	Phase II	45/45	2021	IV	Nivolumab + low-dose ipilimumab, first line	ORR 69% DCR 85% (13% CR)
André *et al.*[51] KEYNOTE-177	Randomized	307/307	2020	IV	Pembrolizumab *versus* 5-FU-based cht +/− bevacizumab or cetuximab	PFS 16.5 *versus* 8.2 months

5-FU, 5-fluorouracil; Adj, adjuvant; Cht, chemotherapy; CR, complete response; DCR, disease control rate; DFS, disease-free survival; FOLFIRI, 5-fluorouracil/leucovorin + irinotecan; FOLFOX, 5-FU/LV + oxaliplatin; FP, fluoropirimidine; IFL, weekly bolus irinotecan + 5-FU/LV; LV, leucovorin; MSI, microsatellite instability; MSS, microsatellite stability; NS, not specified; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; SA, survival after recurrence; SAKK, Swiss Group for Clinical Cancer Research.

of survival benefit in stage II MSI CRC patients receiving adjuvant 5-FU.[15,27]

Currently, it is known that the prevalence of the MSI phenotype in stage II CRC is approximately 10–15%, and it is usually associated with lower N stage and poor differentiation. These patients also have an excellent prognosis compared with those with microsatellite stability (90 *versus* 66%) and potential resistance to 5-fluorouracil.[52]

For this reason, current international guidelines recommend that stage II patients with MSI tumours should not receive adjuvant treatment with 5-FU-based chemotherapy.[18] However, this assumption is not always valid, although MSI status represents to date the most reliable molecular prognostic marker for deciding the management for stage II CRCs. In fact, this biomarker cannot be used without considering other prognostic factors. Specifically, in recent guidelines published by the ESMO for patients with nonmetastatic colon cancer, patients having fewer than 12 lymph nodes examined or T4 staging should be considered at high risk regardless of their microsatellite status. The role of MSI in this subgroup is currently considered uncertain.[53]

In stage III patients, the role of MSI as a predictive biomarker of response to chemotherapy is not fully understood,[37] although the MSI phenotype remains a favourable prognostic factor in patients with stage III colon cancer receiving adjuvant 5-FU-based chemotherapy.[41]

Various studies have attempted to clarify these associations, but controversy still exists regarding the utility of several molecular markers for predicting survival in the context of adjuvant chemotherapy for stage III CRCs. For example, the study conducted by Chouhan *et al.* concluded that the MSI test is predictive of the response to adjuvant chemotherapy in stage III colon cancer only when the results are interpreted in combination with BRAF. In particular, neither BRAF status nor MSI was individually predictive of survival benefit, but rather, the association between these two biomarkers was predictive.[42] In fact, BRAF mutation configures a proportion of CRC tumours a poor prognosis, reducing the benefits derived from MSI. In a pooled analysis, survival after relapse in patients with stage III CRC treated with chemotherapy in the adjuvant setting was higher in the MSI phenotype than in the MSS phenotype. The BRAFV600E mutation was a poor prognostic factor in both MSI and MSS patients.[42] Similar results emerged from a study led by Sinicrope *et al.*,[41] underlining the importance of these biomarkers for patient management at recurrence.

To date, adjuvant chemotherapy with FP and oxaliplatin in patients with the stage III CRC MSI phenotype remains the gold standard. In a study conducted by Cohen *et al.* of 5457 patients, the addition of oxaliplatin to 5-fluorouracil improved overall survival (OS) and disease-free survival (DFS) in patients with MSI stage III CC. The better prognosis of patients with MSI compared with MSS was confirmed in patients with N1 stage, while it was comparable in patients with N2 stage.[45]

Given the high efficacy of immunotherapy drugs in patients with metastatic CRC MSI and the uncertain role of chemotherapy in stages II and III in this category of patients, the hypothesis of performing adjuvant treatment with immunotherapy has emerged in recent years. The patients with MSI CRC who hypothetically could benefit most are in the T4 or N2 stages, which is the proportion of patients with the highest risk of relapse. In addition to T and N factors, several future biomarkers could aid in the selection of patients, such as the presence of persistent circulating tumour DNA after surgery.[54] Several clinical trials are currently validating the efficacy of immunotherapy, combined with chemotherapy or monotherapy, in these patient settings. Two phase III randomized trials for resected stage III MSI CRC are ongoing: the ATOMIC study, which is evaluating FOLFOX (5-FU/LV + oxaliplatin) ± atezolizumab for 6 months plus maintenance with atezolizumab or placebo for 6 months (NCT02912559), and the POLEM study (NCT03827044), which evaluates 24 weeks of FP *versus* 12 weeks of FP plus oxaliplatin ± avelumab for MSI or patients with POLE mutation.

Metastatic setting

The use of MSI in clinical practice for metastatic CRCs has become mandatory due to the implications of these data in selecting the best first-line therapy to offer to the patient.[55] In fact, MSI has been configured as a strong predictor of efficacy in blocking the immune checkpoint, leading to the approval of immunotherapy drugs for MSI patients with metastatic CRC. This important

milestone has the most important added value to clinical practice in CRC tumours in recent years. The increased response to immunotherapy drugs of MSI patients is correlated with the high TMB and the burden of neoantigens, favouring the infiltration of immune effector cells and the anti-tumor immune responses within these tumours.[56] The infiltration of specific subgroups of functional immune cells into these tumours is currently considered the cause of the good prognosis and low risk of relapse in surgically treated patients with stage I, II and III disease.[57]

The US Food and Drug Administration (FDA) approval of pembrolizumab, an anti-PD1 monoclonal antibody, in patients with MSI metastatic tumours of any histology is due to the clinical study conducted by Le *et al.*, who enrolled 32 patients comprising 11 and 21 CRC patients with MSI and MSS, respectively. Objective response at 20 weeks and PFS rates were 40% and 78%, respectively, in CRC patients with MSI tumours compared with 0% and 11% for those with MSS tumours.[58] These results were confirmed in a subsequent study by Andrè *et al.*,[49] as reported in Table 1.

Another anti-PD-1 monoclonal antibody, nivolumab, was studied in monotherapy or in combination with ipilimumab (anti-CTLA-4 monoclonal antibody) in a phase II study in MSI CRC patients. The results of this study suggest a superior efficiency of combination therapy of nivolumab + ipilimumab compared with monotherapy with nivolumab (PFS at 12 months 71% *versus* 50%; OS 85% *versus* 73%).[49]

Although these data are very encouraging, the proportion of patients who have access to these new drugs is limited considering that the majority of patients present with MSS cancers for which immune checkpoint blockade fails to achieve better survival. This necessitates greater molecular knowledge of these diseases and the search for additional biomarkers of susceptibility to anti-PD1 antibodies. Ongoing research on MSS tumours with DNA POLE mutations, comprising 2–3% of CRCs, is very interesting. In fact, these enzymes are involved in the mechanisms of DNA repair, and tumours with POLE mutations are characterized by strong immune cell infiltration similar to MSI tumours and similarly respond to immunotherapy drugs.[59] Therefore, despite the important therapeutic breakthroughs in CRC tumours, there is still much to understand. For example, overcoming the mechanisms of primary and secondary resistance to immunotherapy drugs in MSI patients represents an important challenge for CRC MSI disease.[60,61]

Rectal cancer

Although colon cancer (CC) and rectal cancer (RC) are often referred to as a single entity (CRC), these two tumours are different in many regards. In fact, colon and rectal cancers differ from the perspective of molecular carcinogenic alterations, molecular profiles and enzymatic expression models.[62] In particular, MSI is more frequently detected in proximal CC than in RC.[63] As illustrated above, MSI CC tends to have a phenotype associated with proximal location and poor differentiation compared with MSS CC, a phenotype not observed in RC MSI.[64] These two tumours also differ with respect to the therapeutic approach, which is characterized by multiple modifications. However, most studies do not differentiate these two neoplasms but refer to CC and RC tumours as a single entity, especially in the metastatic setting. A separate argument can be made on locally advanced RC disease.

To date, the biological markers identifying patients with locally advanced RC who would benefit from neoadjuvant chemotherapy treatment are unknown. Several studies have investigated the utility of MSI data in selecting patients for induction chemotherapy by studying its correlation with different outcomes, such as complete pathological response (pCR), downstaging, posttreatment stage or regression grade. pCR is currently considered the most important prognostic factor. A study by Hasan *et al.* enrolled a total of 5086 patients with locally advanced RC recorded in the American College of Surgeons National Cancer Database. This retrospective analysis found that MSI is independently associated with a reduction in pCR for locally advanced RC after neoadjuvant chemoradiation.[65] However, these data on the reduced response to induction chemotherapy of MSI patients compared with MSS patients are not consistent across all published studies. Specifically, some studies have hypothesized an association between MSI and increased pCR rates,[66,67] while other studies found no association between MSI and the pCR rate.[68,69] A meta-analysis of the published literature revealed no significant differences between MSI and MSS patients treated with induction chemotherapy for locally advanced RC.[70]

However, today, it is common practice to use total neoadjuvant therapy consisting of neoadjuvant chemotherapy followed by chemoradiotherapy. Based on these data, the role of MSI status remains to be clarified and to date cannot be used as a selection factor or a predictor of response to neoadjuvant chemotherapy/chemoradiotherapy. Furthermore, neoadjuvant strategies using immunotherapy and chemoradiotherapy are being evaluated in clinical trials, and they could advance understanding regarding the most appropriate therapeutic management of these patients.

Gastric cancer

Gastric cancer (GC) is one of the world's leading causes of cancer morbidity and mortality, ranking fifth in incidence and third in mortality.[71] The 5-year survival rate remains very low because most patients are diagnosed with metastatic disease. Chemotherapy drugs used in the metastatic setting have slightly improved the life expectancy of these patients. A potential improvement in the survival of these patients could be achieved by more precise medicine. A potential improvement for a more targeted treatment could be provided by a more specific classification that considers the molecular characteristics of the disease.

The classification used since 1965 is the Lauren classification, in which gastric adenocarcinoma is basically divided into two distinct types, the intestinal subtype, which is the most common, and the diffuse subtype, involving a third of patients and having a worse prognosis.[72]

Recently, a classification based on the molecular profile was defined by The Cancer Genome Atlas (TCGA), identifying four distinct gastric cancer subtypes: Epstein–Barr virus (EBV)-positive, microsatellite unstable tumours (MSI), genomically stable tumours (GS) and chromosomal tumour instability (CIN).[73] This molecular classification does not currently impact clinical practice; however, it helps to identify a number of patients potentially susceptible to immunotherapeutic treatments. In particular, EBV-positive and MSI tumours are associated with signatures suggesting potential responsiveness to immunotherapy treatments.[74] Data published in a meta-analysis that included 34 studies reported a prevalence of MSI in 10.7% of intestinal type *versus* 2.9% and 0.9% for diffuse and mixed type, respectively.[75] For sporadic and hereditary tumours, there do not seem to be significant differences in the frequency of the MSI signature;[76] instead, it appears to be linked to tumour staging.

In fact, the percentage of patients with MSI appears to be related to the stage of the disease, occurring more often in patients with low N0 stage (approximately 20%) and significantly less frequently in metastatic disease (<5%).[73] The incidence of MSI in nonmetastatic GC tumours makes the potential use of this biomarker very intriguing, particularly in the neoadjuvant and adjuvant settings, with the aim of improving the results obtained from neoadjuvant treatment, where chemotherapy still provides too low of a benefit in terms of pCR and DFS (see Table 2).

Perioperative/adjuvant chemotherapy

The first choice for therapy in patients with GC with operable T2 or higher or N+ includes perioperative chemotherapy treatment.[98] In patients fit for polychemotherapy, the standard of care is represented by the FLOT scheme (fluorouracil plus leucovorin, oxaliplatin and docetaxel), which has shown a benefit in terms of OS superior to previously used chemotherapy regimens, such as EOF (epirubicin, oxaliplatin, fluorouracil) and ECX (epirubicin, cisplatin, xeloda).[99] This indication is currently independent of the molecular profile of the disease, including MSI. However, in the current literature, several findings indicate that MSI has relevance in therapeutic decision-making in this disease setting.

These observations were suggested by subpopulation data from two major studies. In particular, in the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) study, where non-Asian patients with GC were enrolled as candidates for perioperative chemotherapy, patients with the MSI molecular profile exhibited worse survival outcomes in the chemotherapy plus surgery arm [hazard ratio (HR), 2.22; 95% confidence interval (CI), 1.02–4.85; $p=0.04$].[88] Similar results emerged from the CLASSIC study in the adjuvant setting in patients with surgical GC, where once again patients with MSI seem to experience no advantage from chemotherapy, in this case in terms of 5-year DFS (DFS for chemotherapy *versus* surgery-only groups: 83.9% *versus* 85.7%; $p=0.93$).[87]

A meta-analysis conducted by Pietrantonio *et al.*,[89] which included the aforementioned MAGIC and CLASSIC trials and an additional

Table 2. Predictive and prognostic role of MSI in gastric cancer.

Author/trial	Study type	MSI GC/all patients (no.)	Stage of disease	Therapy regimen	Results in MSI tumours
An *et al.*[77]	Retrospective	170/1990	Radically resected	5-FU-based cht	No benefit in DFS in stage II–III with adj cht, no significant prognostic value
Kim *et al.*[78]	Retrospective	105/1276	Radically resected stage II–III	Adj cht or surgery alone	Good prognosis with surgery alone, benefits attenuated by cht
Marrelli *et al.*[79]	Retrospective	111/472	Radically resected	–	Positive prognostic value but limited to noncardiac intestinal type cancer. No prognostic value in diffuse-mixed type and signet-ring cell/mucinous histotypes
Sohn *et al.*[80]	Retrospective (data from a TCGA cohort)	57/262	I–IV	–	Poorer OS than those with EBV subtype but better than those with GS subtype
Polom *et al.*[75]	Meta-analysis	1718/18,612	I–IV	–	Positive prognostic value (HR for OS MSI *versus* MSS: 0.69)
Hashimoto *et al.*[81]	Retrospective	28/285	Resectable	Preoperative cht	Negative predictive value for response to neoadj cht. If no neoadj cht RFS longer in MSI (HR 0.30), if neoadj cht no significant difference in RFS
Haag *et al.*[82]	Retrospective	9/101	Resectable	Neoadj cht platinum-based	Positive prognostic value No significant benefit of neoadj cht
Di Bartolomeo *et al.*[83]	Randomized (Translational Analysis from the ITACA-S Trial)	23/256	Radically resected stage II–III	5-FU/LV *versus* sequential FOLFIRI and cisplatin-docetaxel	Independent positive prognostic factor (better DFS and OS)
Kim *et al.*[84]	Retrospective	2 cohorts: 41/359 162/162	Radically resected stage IB-III	FP-based cht	Better DFS and OS Adjuvant cht improved DFS
Kim *et al.*[85]	Retrospective	88/881	Radically resected stage IB-III	5-FU CRT *versus* surgery alone	No benefit of adj CRT Independent prognostic factors
An *et al.*[86]	Retrospective	64/790	Recurrence of resected stage II–III	FP-based cht	MSI and adj cht were not associated with OS after recurrence. MSI patients who did not receive adj cht had better response to cht after recurrence
Choi *et al.*[87] CLASSIC	Randomized (post hoc analysis)	40/592	Radically resected stage II–III	CAPOX *versus* observation	No benefit of adj cht Independent positive prognostic factors
Smyth *et al.*[88] MAGIC	Randomized (post hoc analysis)	20/303	Resectable stage II–III	Perioperative ECF *versus* surgery alone	Positive prognostic effect in patients treated with surgery alone, negative in patients treated with cht
Pietrantonio *et al.*[89]	Meta-analysis (MAGIC, CLASSIC, ARTIST and ITACA-S trials)	121/1556	Radically resected	Depending on the trial: perioperative ECF, adj CAPOX, 5-FU/LV, sequential FOLFIRI and cisplatin + docetaxel, cisplatin + capecitabine, CRT	MSI is a robust prognostic marker: 5-years DFS 71.8% *versus* 52.3% 5-years OS 77.5% *versus* 59.3% No benefit of adj cht

(Continued)

Table 2. (Continued)

Author/trial	Study type	MSI GC/all patients (no.)	Stage of disease	Therapy regimen	Results in MSI tumours
Kohlruss *et al.*[90]	Retrospective	Before neoadj cht 15/143; in resected tumours 59/617	Resectable	Platinum/5-FU-based neoadj cht	MSI and EBV are not predictive of response to neoadj cht (better response in MSS), but indicative of a good prognosis, in particular MSI irrespective of treatment with cht.
Fuchs *et al.*[91] KEYNOTE-059	Phase II	7/259	IV, advanced line	Pembrolizumab	ORR 57.1%
Marabelle *et al.*[92] KEYNOTE-158	Phase II	24/233	IV, advanced line	Pembrolizumab	ORR 45.8%, PFS 11 months
Janjigian *et al.*[93] CHECKMATE-032	Phase I–II	11/160	IV, advanced line	Nivolumab or nivolumab plus ipilimumab	ORR 44.4%, DCR 77.8%
Pietrantonio *et al.*[94]	Meta-analysis (KEYNOTE-062, CheckMate-649, JAVELIN Gastric 100, KEYNOTE-061)	123/2545	IV: first line, maintenance or second line according to the trial	Anti PD1/PD-L1 therapy, according to the trial	HR for OS 0.34 (*versus* 0.85 in MSS) HR for PFS 0.57
Andre *et al.*[95] GARNET	Phase I	8/106	IV, advanced line	Dostarlimab	ORR 37.5
Janjigian *et al.*[96] CHECKMATE 649	Phase III	44/1581	IV, first line	Nivolumab + cht *versus* cht alone (cht: XELOX or FOLFOX)	HR for OS 0.33 (*versus* 0.73 MSS)
Chao *et al.*[97]	Post hoc analysis of: KEYNOTE-059	7/174	IV: third line or higher	Pembrolizumab	OS and PFS NR ORR 57.1%
	KEYNOTE-061	27/514	Second line	Pembrolizumab *versus* paclitaxel	PFS 17.8 months (*versus* 3.5 months) ORR 46.7% (*versus* 16.7%)
	KEYNOTE-062	50/682	First line	Pembrolizumab +/− cisplatin and 5-FU/capecitabine *versus* cht alone	PFS 11.2 months (*versus* NR *versus* 6.6 months) ORR 57.1% (*versus* 64.7% *versus* 36.8%)

5-FU, 5-fluorouracil; Adj, adjuvant; CAPOX, capecitabine + oxaliplatin; Cht, chemotherapy; CRT, chemoradiotherapy; DCR, disease control rate; DFS, disease-free survival; EBV, Epstein–Barr virus; ECF, epirubicin + cisplatin + fluorouracil; FOLFIRI, 5-fluorouracil/leucovorin + irinotecan; FOLFOX, 5-FU/LV + oxaliplatin; FP, fluoropirimidine; GC, gastric cancer; GS, genomically stable; HR, hazard ratio; LV, leucovorin; MSI, microsatellite instability; MSS, microsatellite stability; Neoadj cht, neoadjuvant chemotherapy; NR, not reached; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; TCGA, The Cancer Genome Atlas.

two trials (ARTIST and ITACA-S), confirmed the favourable prognosis of patients with MSI GC compared with MSS patients, confirming the lack of survival benefit from perioperative chemotherapy. These data are currently changing the clinical practice of MSI-operable GC patients.

Conversely, data on the benefit of adjuvant chemotherapy treatment in patients with MSI GC after surgery are discordant. Some data, in line with stage II colon cancers, seem to confirm a lack of benefit or even a detrimental effect of chemotherapy in this setting,[100] while other data exhibit the opposite trend with a benefit to adjuvant chemotherapy treatments in MSI patients with gastric cancer.[84] At present, MSI data cannot be used in the therapeutic decision-making process in the adjuvant setting.

Metastatic treatment

As previously reported, published data suggest that patients with an MSI signature or EBV could benefit from the use of immunotherapy drugs. The first data to demonstrate the efficacy of immunotherapy drugs, in this case pembrolizumab, was the KEYNOTE-012 study. In particular, patients with PD-L1+ advanced GC presented a response to treatment in 22% of cases. Of these patients, a substantial percentage (17%) had MSI. Furthermore, among MSI patients, the response to treatment was surprising, reaching 50% of patients.[101] In a second phase II study involving a cohort of patients with gastric/gastroesophageal junction cancer treated with pembrolizumab, patients with MSI status experienced an objective response rate (ORR) of 57.1%, markedly distinguishing themselves from patients with MSS, who had a lower ORR (9%).[91]

Data on the efficacy of immunotherapy drugs in advanced gastric MSI patients were extrapolated from the results of the Garnet study, which evaluated the efficacy of dostarlimab in patients with pretreated MSI solid tumours. The study was originally designed for patients with MSI advanced endometrial cancers; however, study cohort F also enrolled patients with other tumour histologies, particularly gastrointestinal tract cancers (93% of the total). The confirmed ORR in MSI patients was 38.7% (95% CI: 29.4–48.6), with a complete response rate of 7.5%, demonstrating a remarkable response to treatment in this pretreated patient setting.[95]

A meta-analysis conducted by Pietrantonio *et al.* enrolled a consistent number of patients, for a total of 2545 patients, of whom 4.8% had MSI status. From this meta-analysis, it emerged that MSI GC patients treated with pembrolizumab experienced a benefit in terms of higher OS than MSS patients (HR for OS of 0.34 *versus* 0.82).[94]

In fact, the literature significantly confirms the utility of information on MSI in patients with GC, both in the perioperative and metastatic settings, to identify the most effective treatment for this group of patients.[74]

Endometrial cancer

Endometrial cancer (EC) is the most common gynaecologic malignancy in the United States.[102] In most cases (approximately 67%), it presents in the early stage with an 81% 5-year OS rate, while survival decreases in the advanced stage to only 17–15%.[103] Among modifiable risk factors associated with the development of endometrial cancer, the strongest correlation is observed with obesity and metabolic syndrome, while approximately 2–5% of endometrial cancers have a germline mutation of the mismatch repair genes that leads to Lynch syndrome.[104]

Historically, EC was classified into two histopathologic categories: types 1 and 2.[105] Type 1 cancers are the most frequent, being low-grade oestrogen-driven endometrioid tumours, while type 2 cancers are high-grade nonendometrioid tumours. Recently, this classification has been refined, thanks to TCGA, with a genomic classification that proposes four groups: POLE ultramutated, MSI hypermutated (MSI-H), copy-number low and copy-number high, which represent prognostic predictors of response to therapy.[106]

Approximately 30% of localized and 13–30% of recurrent ECs are MSIs.[107] In this review, we focused on the prognostic role of MSI status on the response to chemotherapy and the possible benefit of immunotherapy. Indeed, a dramatic response to immune checkpoint inhibitors (ICIs) was observed in MSI EC. However, the prognostic value of TMB, PD-L1 expression, tumour-infiltrating lymphocytes (TILs) and Janus kinase 1 (JAK1) and β2-microglobulin (B2M) mutations in MSI EC patients needs to be clarified.[108–111]

Table 3 reports the primary studies on MSI EC.

Table 3. Predictive and prognostic role of MSI in endometrial cancer.

Author/trial	Study type	No. MSI EC/all patients	Year of publication	Stage of disease/ setting	Therapy regimen	Results in MSI tumours
Resnick *et al.*[112]	Retrospective	155/477	2010	Adjuvant	RT or Cht	No difference in OS and PFS in overall patients; increase in OS and PFS if nonendometrioid tumours treated with RT
Kim *et al.*[113]	Retrospective	162/535	2018	Adjuvant	RT and Cht	Lower rate of recurrence, no difference in PFS or OS in a multivariable analysis
Loukovaara *et al.*[114]	Retrospective	287/795	2021	Adjuvant	VBT WPRT +/– Cht	No effect on disease-specific survival
León-Castillo *et al.*[115] PORTEC-3	Phase III	137/410	2020	Adjuvant	CTRT *versus* RT alone	5-year RFS: 72% 5-year RFS with CTRT *versus* RT: 68% *versus* 76%
Reijnen *et al.*[116]	Retrospective multicentre cohort study	57/128	2019	IB/II, grade 3, endometrioid; adjuvant	RT	Adjuvant RT improved survival
Marabelle *et al.*[92] KEYNOTE-158	Phase II	49/233	2020	IV advanced line	Pembrolizumab	ORR 57.1%, PFS 25.7%, OS NR
Oaknin *et al.*[117] GARNET	Phase I	71/71	2020	IV, after platinum-based cht	Dostarlimab	ORR 42.3% (12.7% CR), DOR NR
Makker *et al.*[118]	Phase IB-II	11/108	2020	IV advanced line	Lenvatinib + Pembrolizumab	ORR 63.6% (*versus* 36.2% in MSS), DCR 91%, DOR 21.2 months, PFS 18.9 months (*versus* 7.4 in MSS), OS NR (*versus* 16.4 in MSI)
Azad *et al.*[119] NCI-MATCH (EAY131)	Phase II	13/42	2020	IV advanced line	Nivolumab	ORR 68% (15% CR, 53% PR)
Konstantinopoulos *et al.*[120]	Phase II	16/33	2019	IV advanced line	Avelumab	ORR 26.7%. MSS cohort closed because of futility
Antill *et al.*[121] PHAEDRA	Phase II	36/71	2019	IV (first-line therapy in 58% in MSI group)	Durvalumab	ORR 47%, PFS 8.3 months (*versus* 1.8 in MSS), 12-month OS 71% (*versus* 51% in MSS), OS NR

Adj, adjuvant; Cht, chemotherapy; CR, complete response; CTRT, chemoradiotherapy; DCR, disease control rate; DFS, disease-free survival; DOR, duration of response; EC, endometrial cancer; HR, hazard ratio; MSI, microsatellite instability; MSS, microsatellite stability; NR, not reached; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; RFS, recurrence-free survival; RT, radiotherapy; VBT, vaginal brachytherapy; WPRT, whole pelvic radiotherapy.

Resectable tumours

Treatment for early-stage disease has historically been total hysterectomy and bilateral salpingo-oophorectomy with or without adjuvant radiotherapy. Adjuvant chemotherapy is indicated based on risk factors for relapse [histological subtype such as nonendometrioid type, grade 3 histology, myometrial invasion ⩾50%, lymphovascular space invasion (LVSI), lymph node metastases and tumour diameter >2 cm].[122] Maggi *et al.*[123] found no difference in PFS or OS comparing five cycles of cisplatin, doxorubicin and cyclophosphamide to external pelvic radiation in high-risk patients. Furthermore, a Japanese multicentre randomized trial reached the same conclusion, with no difference in OS, relapse rate or PFS between whole-pelvic irradiation with three or more courses of cyclophosphamide, doxorubicin and cisplatin chemotherapy in patients with high-risk EC.[124] However, in the NSGO/EORTC trial, a statistically significant improvement in PFS and cancer-specific survival was observed for combined chemoradiotherapy (CTRT) compared with radiotherapy alone.[125]

In these studies, doxorubicin/platinum-based regimens were used for four to six cycles, and the regimen most commonly used was paclitaxel plus carboplatin (TC). Radiotherapy (RT) is variably used before or after chemotherapy or in a 'sandwich' modality, with three cycles of chemotherapy administered before and after radiotherapy. However, when chemotherapy was given after pelvic radiotherapy, there was a higher rate of cytopenia that required granulocyte-colony stimulating factor use during chemotherapy.[126] Recently, the PORTEC-3 trial investigated the role of pelvic radiotherapy *versus* whole pelvic radiotherapy with two concomitant doses of cisplatin followed by four courses of CP in high-risk women.[127] The trial reported a benefit of 5% for 5-year OS and 7% for 5-year failure-free survival (FFS) with CTRT, with a greater benefit observed in serous cancer subgroups.[128] A subsequent analysis investigated the prognostic relevance of molecular classification in the benefit of adjuvant treatment. Out of 410 EC samples analysed, 22.7% exhibited abnormal expression of p53 (p53abn), 12.4% had an exonuclease domain of DNA Polymerase Epsilon (POLE) mutation (POLEmut), 33.4% had a protein loss MMR (MSI) and 31.5% displayed no specific molecular profile (NSMP). The strongest negative prognostic factor was p53abn, and this subgroup had a worse prognosis but benefitted the most from CTRT treatment (22.4% benefit in relapse-free survival and 23.1% advantage in OS). Furthermore, 71% of serous tumours were found to have a p53 mutation, and their prognosis was equal to that of the nonserous subtypes that had the mutation.[115] This suggests that p53 status allows the identification of a larger and more selected subgroup of patients who may benefit from adjuvant treatment with CTRT. Ultramutated EC with POLEmut subgroups results in a proofreading dysfunction during DNA replication that elicits a cytolytic immune response and impairs the function of cancer cells by decreasing their metastatic potential. POLEmut ECs are associated with a good prognosis with any treatment,[109,116] suggesting that the prognosis is independent of adjuvant therapy.[115] MSI ECs also have a deficit in DNA damage repair with an accumulation of mismatches, insertions and deletions that elicit a strong immune response that gives this subgroup of ECs an intermediate prognosis.[114] The MSI and NSMP subgroups did not benefit from CTRT *versus* RT alone in the PORTEC-3 trial. Thus, RT remains an effective, well-tolerated and appropriate adjuvant treatment in high-risk early-stage MSI EC.[116,128]

The best adjuvant strategy is under investigation in the PORTEC-4a trial (NCT03469674), which stratifies patients into three prognostic profiles (favourable, intermediate and unfavourable) based on the four TCGA molecular subtypes and integrates them with other prognostic factors, such as substantial invasion of the lymphovascular space, expression of L1-cell adhesion molecules and expression of CTNNB1. Women with high-risk EC are randomized to vaginal brachytherapy or adjuvant treatment (no treatment for a favourable profile, brachytherapy for an intermediate profile and pelvic RT for an unfavourable profile). More selectively, the TransPORTEC clinical trial plans seek to refine adjuvant treatment based on the Molecular Profile Program (RAINBO). Stage II/III MMRd EC patients are randomized to RT *versus* RT plus an ICI (GreenMMRd trial). The ADELE trial aims to evaluate the efficacy of ICI tislelizumab plus chemotherapy in the adjuvant setting after chemoradiation in high-risk MMRd EC.[129]

Metastatic setting

In advanced or recurrent EC, hormonal therapy or chemotherapy can be used. Progestinic agents are indicated for low-grade tumours with

endometrioid histology, while TC is the standard first-line chemotherapy with a median PFS of 13 months and a median OS of 37 months.[130]

In the first line, the comparison between TC and the paclitaxel-doxorubicin-cisplatin (TAP) regimen showed noninferiority of TC with a more manageable toxicity profile than TAP.[131] The MITO END-2 study evaluated the addition of bevacizumab to first-line TC or recurrent EC but failed to demonstrate an increase in PFS.[130] However, the study did report an increased ORR at 6 and 12 months.[130] A limitation of this study is the absence of an assessment of predictive biomarkers for the response to bevacizumab in the four EC subtypes. Indeed, p53-mutated patients have been recently observed to exhibit improved PFS and OS when treated with TC plus bevacizumab *versus* temsirolimus.[132]

Unfortunately, no randomized study has currently performed a subgroup analysis considering the four TCGA molecular groups. ICI monotherapy demonstrated antitumour activity in MSI EC pretreated with an ORR of 42.3% for dostarlimab, 26.7% for avelumab, 40% for durvalumab, 38% for nivolumab and 57% for pembrolizumab.[92,117,119–121] Unfortunately, the mechanisms of primary resistance to immunotherapy are unknown, and predictive indicators of ICI response are not available. To stimulate a greater response, several phase III trials are investigating the role of ICI combination therapy in first-line MSI EC: dostarlimab (RUBY; NCT03981796), atezolizumab (AtTEnd; NCT03603184) and pembrolizumab (GY018', NCT02549209) in combination with TC chemotherapy against TC, lenvatinib with pembrolizumab *versus* TC (NCT03572478)[105] and durvalumab plus olaparib plus TC *versus* durvalumab plus TC *versus* placebo plus TC (NCT04269200).

Pembrolizumab plus lenvatinib has shown efficacy in advanced EC that progresses after previous treatment.[133] The final primary efficacy analysis reports an ORR of 38% at 24 weeks, 63.6% (30.8–89.1%) in patients with MSI tumours ($n = 11$) and 36.2% (26.5–46.7%) in patients with MSS tumours ($n = 94$). Regardless of MSI status, the median duration of response was 21.2 months, the median PFS was 7.4 months and the median OS was 16.7 months. Several combination therapies are being studied in previously treated EC patients: nivolumab plus ipilimumab (NCT02982486), nivolumab plus indoleamide 2,3-dioxygenase inhibitor (BMS986205; NCT04106414), lenvatinib plus pembrolizumab plus doxorubicin or weekly paclitaxel (NCT03517449), rucaparib plus nivolumab (NCT03572478)[107] and IMGN853 plus pembrolizumab (NCT03835819).

MSI prognostic value across other cancer types

MSI status has assumed increasing importance as a prognostic and predictive factor, in some cases modifying clinical practice, including in colorectal, gastric and endometrial adenocarcinomas. The clinical guidelines currently recommend the MSI testing be performed only for colorectal and endometrial cancers, but it is understandable that, given its importance in therapeutic decision-making in these pathologies, an increasing interest in research has arisen over the years about the prevalence and extent of MSI among other cancers.

In this chapter, we describe the primary studies in the literature investigating the incidence and possible therapeutic implications of MSI status in different tumour types.

With respect to incidence, a study published by Bonneville *et al.*[131] investigated these data in different tumour histotypes, leading to a very interesting general picture. The authors extrapolated data on the full exomes of 11,139 tumour-normal pairs from TCGA and Therapeutically Applicable Research for a total of 39 cancers. From these data, it emerged that MSI status was present in 27 of the 39 cancer histotypes evaluated, for an overall positivity of 3.8%. The important incidence of MSI detected in cancers such as adrenocortical carcinoma (ACC, 4.3%), cervical cancer (2.6%) and mesothelioma (2.4%) is very interesting, suggesting a potential role of MSI data in these cancer types. In particular, it could pave the way for new therapeutic options, given the known sensitivity of MSI tumours to ICIs and therefore to the broadening of the indication for the MSI test.

A recent review suggests that ACC could be associated with Lynch syndrome.[134] Indeed, the prevalence of Lynch syndrome in ACC is 3.2%, which is higher than that in the general population.[135] However, when treatment with ICIs has been investigated in ACC patients, the efficacy is scarce.[136,137] The majority of patients were heavily pretreated, and the number of MSI patients was very low. Pembrolizumab resulted in an ORR of

23% and disease control rate (DCR) of 52% in 39 ACC patients, with a median PFS of 2.1 months and OS of 24.9 months. Nine patients achieved a partial response, and two of them had MSI.[138] Considering the modest results achieved, ICIs are evaluated in combination with immune stimulators or tyrosine kinase inhibitors: nivolumab plus ipilimumab (NCT02834013, NCT03333616), nivolumab plus EO2401 (NCT04187404) and camrelizumab plus apatinib (NCT04318730).[134] We hope the outcomes of these trials will provide more precise answers to the clinical application of MSI in ACC cancer.

Concerning cervical cancer, there are few data in the literature; in particular, the prognostic role of MSI in these patients is not clear.[139,140] In a cohort of patients with HPV + cervical cancer ($n = 95$), 12% of MSI patients were identified.[141] In some studies that included women with HPV + cervical cancer treated with immunotherapy, there were no promising results;[142] however, MSI data for patient selection were not used in these studies. In a phase II study that evaluated the efficacy and safety of nivolumab in patients with advanced or recurrent uterine cervical cancer, uterine corpus cancer, or soft tissue sarcoma (STS), a potential predictive role of MSI in response to nivolumab was hypothesized.[143] These data remain to be confirmed; to date, there is no clinical study that has selected these patients based on MSI data.

Regarding mesothelioma, despite the data published by Bonneville *et al.* where the incidence of MSI in mesothelioma seems significant, there are very few data in the literature. In a large retrospective study, data were collected from 335 patients with malignant pleural mesothelioma. The study did not identify any patients with malignant pleural mesothelioma with MSI, for which it concluded that the response to anti-programmed cell death 1-based immunotherapy may be driven by other mechanisms.[144] Another study conducted in Spain reached similar conclusions.[145]

Following the evolution of the use of the MSI signature in CC, much interest has aroused a possible similar use in other pathologies of significant oncological impact for incidence, such as breast cancer. Unfortunately, the percentage of MSI breast cancers is very low. Currently, the indication for immunotherapy for breast tumours concerns only the triple-negative phenotype, and PDL1 positivity is used as a predictor of response.[146] In a study that included MSI triple-negative breast cancer patients, MLH1 protein expression was inversely correlated with PD-L1 expression. Therefore, the study concluded that MMR protein testing may warrant further study, as these proteins could provide information to predict which patients might benefit from ICIs.[147] Consistently, from data published in the literature, the loss of MMR proteins in breast tumours appears to be a more common event than MSI and exhibits intratumour heterogeneity. In particular, in a study that included breast cancer patients with both hormone-positive (HR+) and oestrogen-receptor-negative (HR−) phenotypes, patients with HR− breast cancers treated with chemotherapy lived longer in cases of MMR-deficient ($n = 9$) than MMR-proficient ($n = 33$) or MMR-heterogeneous ($n = 7$) tumours.[148]

Concerning prostate cancer (PC), the prevalence of MSI is approximately 3%, and approximately 20% of patients present with Lynch syndrome.[149] However, data regarding the incidence of MSI come only from studies using immunotherapy, while the possible prognostic role of MSI in chemotherapy- and hormonal therapy-naive patients has not been investigated. Undoubtedly, some features of prostatic tumours, such as aggressive histology, ductal type, homologous recombination deficiency (HRD) mutations, high TMB and MSI, can affect subgroups of patients potentially sensitive to ICIs.[150] Of the 32 MSI PC patients from the Memorial Sloan Kettering Cancer Center database, 11 were treated with ICIs, 6 (54.4%) exhibited a half decline in prostate-specific antigen levels, and 4 had a pathological response.[151] In particular, pembrolizumab showed some efficacy in small studies with advanced MSI PC with an ORR of 60%.[152] Other studies have tested ICIs alone or in combination with endocrine therapy in an unselected PC population with an ORR less than 20%.[150] Considering the limited data on PC immunotherapy, we must await the results of ongoing studies with several combination approaches that will hopefully clarify the subgroup of patients who most benefit from ICIs.

Regarding cholangiocarcinoma, MSI has been reported in 5–10% of cases.[153] The eight MSI biliary tract cancers (BTCs) included in the basket trial with pembrolizumab achieved an ORR of 25% with two complete responses and four stable diseases.[153] Similarly, the 22 MSI BTCs in Keynote 158 displayed an ORR of 40.9% with 2

complete responses and 7 partial responses, an mPFS of 4.2 months and an mOS of 24.3 months.[92] Unfortunately, all other immunotherapy studies in BTC included a population with no stratification for MMR genes and did not report significant benefits from ICI treatment.[154] Less than 2% of pancreatic cancers exhibit MSI.[155] The majority of them arise from the head and are associated with medullary and mucinous/colloid histology.[156] Keynote 158 included 22 patients with MSI pancreatic cancer, one who achieved a complete response and three who achieved a partial response with an ORR of 18.2%, mPFS of 2.1 months and mOS of 4 months.[93] Although the frequency of MSI in pancreatic cancer is very limited, in these patients where the therapeutic lines are scarce and the prognosis is poor, immunotherapy can represent a valid therapeutic option.

New studies with ICIs in the previous line or in combination therapy are needed to draw conclusions on their efficacy in MSI BTC patients.

Together, these findings highlight the need to more deeply understand the potential role of dMMR and MSI in cancer types.

Discussion

Microsatellites are regions of 10–60 base pairs that contain repetitions of 1–5 base pair motifs. The repetition of these loci along the genome is verified and maintained during cell division by the MMR system, which is involved in cellular DNA repair mechanisms. The compromise of this system, known as MMRd, can lead to microsatellite instability, called MSI. There are several mechanisms by which MMRd can occur, including both somatic and hereditary mutation of the germinal MMR pathway. MSI has aroused increasing interest, as it has been studied and well described in colorectal and gastric adenocarcinoma and in endometrial tumours, while little is known regarding the implications of this signature in other tumour types. The ramifications of these findings are both prognostic and predictive of response to cancer treatments.

Regarding the prognostic implications, the first known data on the prognosis of MSI tumours concern early colon cancer. In addition to the favourable prognostic data, these tumours are characterized by resistance to chemotherapy and do not benefit from adjuvant chemotherapy treatment with FPs.[15] These data of good prognosis and chemoresistance therefore seem to be mutually inclusive in stage II CRCs, with the exceptions mentioned above. This convergence, however, is lost in stage III colon cancer, where the good prognosis of MSI tumours is maintained compared with MSS but not the reduced chemosensitivity; in fact, adjuvant chemotherapy according to the CAPOX (capecitabine plus oxaliplatin) or FOLFOX (5-fluorouracil plus oxaliplatin) scheme remains the therapeutic standard for this stage to date. It is also important to emphasize that MSI at this stage alone cannot guide prognostic evaluation, and other factors must also be considered. For example, BRAF mutation seems to predict a worse prognosis in patients with MSI stage III CRC.[157,158] Concerning rectal cancer, which also exhibits a lower incidence of MSI tumours than CC, the role of this prognostic and predictive signature is less clear.[159] To date, MSI data cannot be used when deciding up the choice of neoadjuvant chemoradiotherapy treatment compared with surgery alone in the potentially resectable setting.

Furthermore, data regarding the prognostic and predictive value of response to chemotherapy of MSI status in metastatic CC are discordant;[160,161] however, a significant impact on patient survival in this setting has been achieved with the introduction of first-line immunotherapy in MSI patients.[50,162]

For gastric adenocarcinoma, the role of the MSI status has been widely recognized in recent years, leading to a new classification of GC that, compared with the well-known Lauren classification, in use since 1965, recognizes a subtype of MSI GC with well-defined characteristics and an expected response to immunotherapy treatments.[72] The MSI phenotype has a higher incidence in the early stages than after metastasis[72] and defines – within the potentially operable stages (I-III) – a subgroup of patients with better prognosis than the MSS counterpart in which there does not seem to be a benefit of neoadjuvant chemotherapy treatment compared with surgery alone, treatment that could be detrimental.[89] To date, there are no randomized clinical trials, but data in the literature, albeit derived from retrospective studies and meta-analyses, are modifying current clinical practice in this category of patients, where in recent years, perioperative chemotherapy has been the gold standard in clinical practice. For the metastatic setting, in Europe,

the use of immunotherapy in advanced gastric tumours is not yet clinical practice outside clinical trials, while the FDA has approved the use of pembrolizumab in patients with MSI in this setting.[18] Another monoclonal anti-PD1 antibody, dostarlimab, has obtained approval for patients with MSI solid tumours who have progressed on or following prior treatment and who have no satisfactory alternative treatment options, adding another step towards precision medicine.[95]

A very fitting example of the importance of the molecular characterization of the disease for the best therapeutic choice with the addition or elimination of chemotherapy, with its related toxicities, is given by the paradigm of adjuvant treatment in endometrial tumours, which in recent years has seen an ever greater refinement in the decision-making process. The choice of adjuvant treatment in these tumours is based on the evaluation of the presence of clinicopathological risk factors that allow the identification of low-, intermediate-, intermediate- and high-risk diseases with a range of therapeutic possibilities ranging from surgery alone to brachytherapy, pelvic radiotherapy and radiochemotherapy. In recent years, it has been understood that it is essential to add molecular information to this clinicopathological characterization, as molecular subgroups, including MSI tumours, have a greater prognostic impact than the histopathological characteristics of the tumours taken individually. In fact, the POLE mutated category seems to have a good prognosis regardless of the adjuvant chemotherapy treatment that could therefore be spared in this category of patients, and the MSI patients do not seem to benefit from the addition of chemotherapy to radiotherapy, while instead the p53 mutation recognizes a category of high-risk patients who benefit from adjuvant chemotherapy treatment in terms of recurrence-free survival.[163]

These data lead to the rationale on which ongoing trials are based, designing new therapeutic possibilities such as the use of checkpoint inhibitors in patients with POLE mut or MSI. Currently, molecular classification is not used in the metastatic setting, nor is it used by ongoing clinical trials in patient selection. However, this information is useful, as clinical trials evaluating the activity of pembrolizumab (KEYNOTE-158) and dostarlimab (GARNET) have shown high response rates in patients with MSI tumours.[164] The data published in the literature on the discrepancy of MSI on relapsed disease compared with primary operated disease are interesting, suggesting the usefulness of rebiopsia on relapsed disease to redefine the molecular profile.[165]

We can therefore conclude that at present, MSI is used in the setting of localized disease for prognostic purposes and with the aim of refining adjuvant and neoadjuvant therapies in colon, gastric and endometrial cancers, as well as a marker predictive of response to treatment with checkpoint inhibitors in the metastatic setting.

Unfortunately, not all ongoing clinical trials concerning these cancer types include MSI data among the characteristics of the study patients. However, considering what has been said thus far and the literature, this information could be invalidating, regarding not only immunotherapy trials but also chemotherapy trials, considering the potential chemoresistance or even detrimental role of chemotherapy in some categories of patients due to toxicity linked to avoidable treatment. For that reason, the collection of information from clinical trials could provide a key to understanding what is still not completely clear regarding the presence of MSI and the response to cancer treatments, whether they are chemotherapy or immunotherapy.

Finally, the acquisition of this information has led to a growing interest in the potential use of this molecular marker in other cancer histotypes where the incidence of MSI status could be higher than expected.

Conclusion

MSI has acquired increasing importance in recent years, so microsatellites have been included in new molecular classifications of diseases affecting colon, gastric and endometrial cancers. These classifications will lead to increasingly personalized cancer treatment. Achieving this goal cannot be exempt from the collection of information on the conditioning of the mutational status of microsatellites in the various disease settings and in the various tumour histologies, valuable information that will hopefully be provided by ongoing clinical research.

Author contribution(s)

Sara Cherri: Conceptualization; Writing – original draft.

Ester Oneda: Writing – original draft.

Silvia Noventa: Data curation.

Laura Melocchi: Supervision; Validation.

Alberto Zaniboni: Conceptualization; Supervision; Validation.

ORCID iD

Sara Cherri https://orcid.org/0000-0001-7062-8723

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