PATHOLOGICA 2025;117:204-219;
DOI: 10.32074/1591-951X-1110

Review

Lights and shadows of microsatellite status characterization in gastrointestinal cancers in the era of cancer precision therapy

Jessica Gasparello[1], Vittoria Matilde Piva[2], Valentina Angerilli[1], Carlotta Ceccon[1], Marianna Sabbadin[1,3], Claudio Luchini[4], Paola Parente[5], Luisa Toffolatti[3], Federica Grillo[6,7], Francesca Bergamo[2], Umberto Malapelle[8], Sara Lonardi[2], Matteo Fassan[1,2]

[1] Department of Medicine (DIMED), University of Padua, Padua, Italy; [2] Department of Oncology Veneto Institute of Oncology, IOV-IRCCS, Padua, Italy; [3] Surgical Pathology Unit, ULSS2 Marca Trevigiana, Treviso, Italy; [4] Department of Diagnostics and Public Health, Section of Pathology, University and Hospital Trust of Verona, Verona, Italy; [5] Unit of Pathology, Fondazione IRCCS Ospedale Casa Sollievo Della Sofferenza, San Giovanni Rotondo, Foggia, Italy; [6] Pathology Unit, Department of Surgical Sciences and Integrated Diagnostics (DISC), University of Genoa and IRCCS Ospedale Policlinico San Martino, Genoa, Italy; [7] Pathology Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy; [8] Department of Public Health, University of Naples Federico II, Napoli, Italy

Summary

The introduction of immunotherapy has dramatically changed the paradigm of solid tumor treatment with the creation of novel therapeutic opportunities even for tumors that currently lack valid therapeutic options in the advanced or metastatic setting. Initially, the role of deficient mismatch repair status (dMMR)/microsatellite instability (MSI) as a predictive biomarker was confined to colorectal cancer. In 2017, MSI/dMMR became the first true agnostic biomarker to stratify patient response to immune checkpoint inhibitors. MSI/dMMR evaluation is a crucial point in diagnostic-therapeutic decision-making for most gastrointestinal cancer patients and the pathologist must be responsible for the delivery of reliable reporting in this setting. The aim of this review is to summarize the current methods available in routine diagnostics for the evaluation of MSI/dMMR status, their limitations, and potential pitfalls that can be encountered. The authors also give an overview of the role of MSI/dMMR as a prognostic and predictive biomarker in gastrointestinal cancers, with a focus on non-colorectal malignancies.

Key words: microsatellite, microsatellite instability, mismatch repair, gastrointestinal cancer

Received: October 2, 2024
Accepted: October 3, 2024

Correspondence
Matteo Fassan
E-mail: matteo.fassan@unipd.it

How to cite this article: Gasparello J, Piva VM, Angerilli V, et al. Lights and shadows of microsatellite status characterization in gastrointestinal cancers in the era of cancer precision therapy. Pathologica 2025;117:204-219;https://doi.org/10.32074/1591-951X-1110

Introduction

Microsatellites are tandem repeats of short DNA motifs, composed of up to 9 base pairs (bp) and repeated many times throughout the genome[1]. DNA repeated motifs include mononucleotide repeats, dinucleotides, and tetranucleotides, generally rich in adenine (A) or thymine (T)[2]. Microsatellites are widely distributed throughout the entire genome but are predominantly found in noncoding regions including introns, 5' and 3' untranslated regions (UTRs), and intergenic regions[3,4]. Microsatellites spontaneously mutate 10 to 100-fold faster than non-repetitive sequences[5]. The accumulation of mutations at the microsatellite loci level is defined as microsatellite instability (MSI) and is predominantly characterized by the change in the number (gain or loss) of tandem repeats due to DNA slippage during DNA replication[6]. Based on the number of mutated microsatellite loci, microsatellite status (MS) can be classified in high (MSI-H) or low (MSI-L) levels of MSI. Factors affecting microsatellite mutation rate include motif size,

gender, guanine (G) and cytosine (C) content, genomic context, and most importantly the effectiveness of mismatch repair (MMR) enzymes[7].

Role of mismatch repair system in microsatellite stability

The MMR system corrects errors occurring during DNA replication, thus preventing the establishment of mutations during cell division, through the activation of cell cycle arrest and/or mechanisms of programmed cell death (e.g. apoptosis)[8]. Alterations in the MMR system, defined as MMR deficient (dMMR) phenotype, lead to the accumulation of genetic errors, particularly within microsatellites, which are more prone to errors during DNA replication. MMR proteins are encoded by 8 genes (among them: *MSH2, MSH3, MSH6, MLH1, MLH3, PMS2*) heterogeneously located throughout the human genome[9,10].

MMR proteins are involved in the recognition of mismatches and can be classified into two groups: MutS Homolog (MSH) including MSH2, MSH3, and MSH6, and MutL Homolog (MLH) enclosing MLH1, MLH3, and PMS2. MMR acts through an excision and re-synthesis process and can recognize and correct mismatches in both 3′ and 5′ strands. While the heterodimer MSH2-MSH6 detects single base mismatches, MSH2-MSH3 complex identifies larger (2-10bp) mutations[11]. The heterodimer MLH1-PMS2 is the most frequent in the MutL complex[12]. MLH1 and MSH2 are essential for MMR activity, whereas alterations in MSH6 or PMS2 may be, even if partially, replaced by MSH3 and MLH3, respectively (Fig. 1). Deficient MMR phenotype is associated with genetic or epigenetic alterations of genes involved in the MMR pathway, which may be either hereditary or acquired/sporadic. Acquired dMMR phenotype is usually caused by the absence of MLH1 protein expression, due to the hyper-methylation of the *MLH1* gene promot-

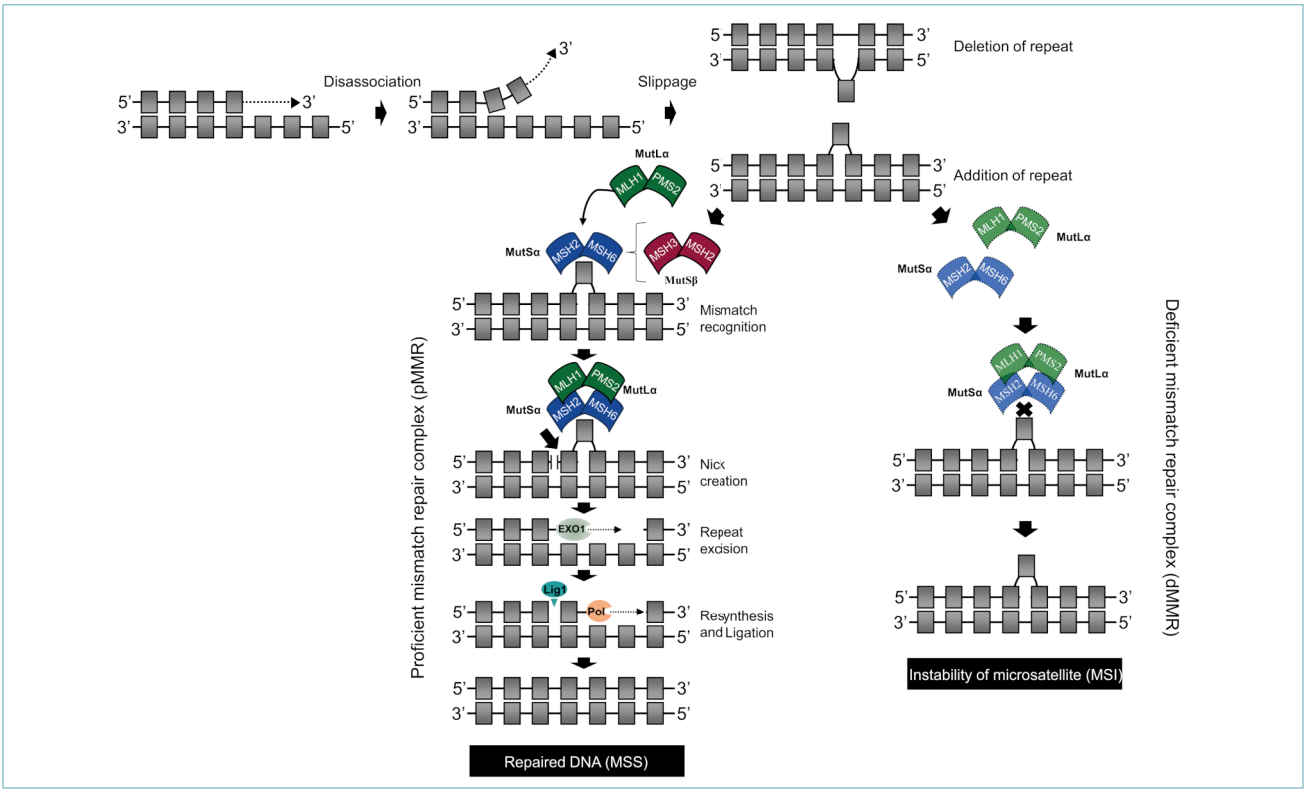

Figure 1. Mechanism of mismatch repair. MSI (microsatellite instability) is characterized by a change in the tandem repeats caused by DNA slippage during DNA replication. DNA slippage is due to temporary dissociations between the DNA polymerase and the DNA-template strand, and results in loss or gain of repeats, when the reassociation occurs ahead or behind where it left off, respectively. The basic process of MMR (mismatch repair) can be divided into four steps: (1) MSHs (MutS homologs) proteins recognize the mismatch and stably bind the double-strand DNA (dsDNA), where the error occurs; (2) MLHs are recruited to bind the MSHs-mismatch complex (MutL homologs: MLHs, act as mediators between the MSHs and the MMR effector enzyme endonuclease MutH); (3) endonuclease MutH cleaves at GATC sites generating a 'nick' in the DNA which allows the excision of the mismatch; (4) the removed DNA sequence is resynthesized by DNA polymerase using the remaining strand as a template.

er and hence silencing of the corresponding gene. Hereditary dMMR phenotype is caused by germline mutations, generally affecting only one allele. Individuals with this mutation are carriers of Lynch syndrome (LS) and present a higher predisposition to certain malignancies, which can develop when a second hit occurs[13]. Among patients diagnosed with LS, those who have germline mutations in *MHL1* and *MSH2*, have a much higher risk of developing cancer and/or develop cancer earlier compared to subjects with *MSH6* or *PMS2* mutations, despite the latter being more frequent.

The role of MSI in current oncology practice

Microsatellite status has been long considered a molecular feature of endometrial (EC) and colorectal (CRC) cancer[14-16]. In the CRC setting, MS is a prognostic and predictive marker. MSI-CRCs have a better prognosis and lower recurrence rate compared to MSS tumors and MSI CRC do not respond well to 5-FU-based monotherapy[17]. The introduction of immune checkpoint inhibitors (ICIs) has dramatically changed the role of MS as a marker. Deficient MMR phenotype results in hypermutated tumors, which produce a high quantity of neoantigens that are recognized by the immune system[18]. Thus, detection of MS (or MMR protein expression) has become paramount for the selection of patients eligible for ICI treatment. Regarding immunohistochemistry, the VENTANA MMR RxDx Panel was approved by FDA as a companion diagnostic to verify the expression of MMR proteins and, to date, represents the only IHC-based assay to identify patients eligible for anti-PD1 immunotherapy[19]. Conversely, a vast array of assays for MSI detection is available, each one with pros and cons (Fig. 2).

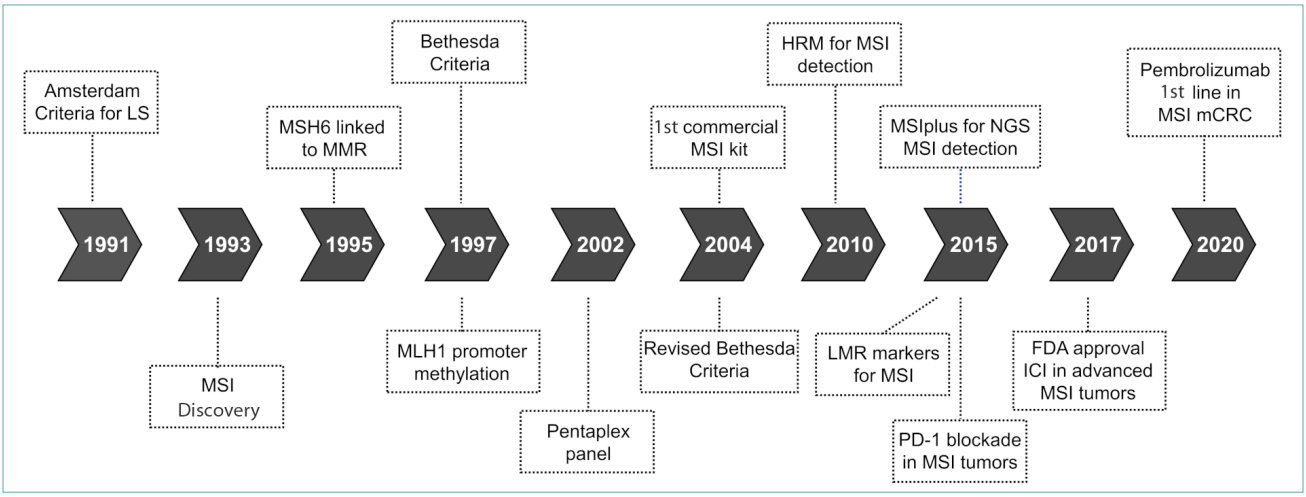

Figure 2. The history of microsatellite status in cancer clinical practice. Since 1993, when three distinct groups reported the presence of repeated sequences in certain tumors, microsatellite instability (MSI) acquired a key role in cancer therapeutics[20-22]. In 1995, Drummond et al. demonstrated that MSH6 is a key protein of the MMR complex and is involved in the repair of single-nucleotide mismatches, while two years later (1997) Kane et al., reported that *MLH1* promoter methylation is one of the major causes of dMMR in sporadic CRCs[23,24]. In the same year, the Bethesda Guidelines reported criteria for the identification of colorectal cancers that needed to undergo testing for MSI. Guidelines also included a list of recommended markers (two mononucleotide and three dinucleotide sequences) defined as Bethesda Panel. Guidelines were revised in 2004. In 2002 Suraweera et al. proposed five quasi-monomorphic mononucleotide markers for MSI detection and two years later the first commercial kit was available[25]. In the following years, several technical improvements were proposed, including high-resolution melting (HRM) to analyze microsatellite PCR products (2010), long mononucleotide repeats (LMR) as possible MSI markers (2015), and finally, the possibility of testing MSI in combination with mutations in genes (*KRAS, NRAS*, and *BRAF*) relevant for CRC patient therapeutic management by NGS. In 2015, Le and colleagues demonstrated that MSI is associated with a higher objective response to PD-1 blockade therapy in colorectal cancers[26]. In 2017 FDA approved pembrolizumab for the treatment of patients with unresectable or metastatic MSI solid tumors that progressed after prior treatment. Three years later (2020), satisfactory results of the KEYNOTE-177 study led the FDA to approve pembrolizumab as first-line treatment for unresectable/metastatic MSI-H colorectal cancers.

Methods for MSI detection

The current practice for MSI detection is based on two major categories of tissue-based testing, an indirect testing strategy based on the evaluation of the MMR complex proteins by immunohistochemistry (IHC) and a direct method based on the detection of alterations in specific microsatellite loci, generally by polymerase chain reaction (PCR) or more recently by next generation sequencing (NGS)[27].

MMR complex assessment by immunohistochemistry

IHC represents the first-line diagnostic test for the detection of dMMR, due to its availability in almost all pathology laboratories. The test is based on the use of specific antibodies against the four MMR proteins and should be evaluated based on the conservation or loss of their nuclear staining[28]. Nuclear expression of all four proteins is indicative of a proficient MMR (pMMR) system. On the contrary, loss of staining of at least one of the MMR proteins is informative of a dMMR phenotype. Typically, due to the proteolytic degradation of the whole heterodimer, loss of MLH1 is associated with loss of PMS2, while MSH2 loss is accompanied by MSH6 loss. Nevertheless, isolated loss of MSH6 or PMS2 can happen. IHC presents several advantages including short testing time, analysis of samples with tumor content lower than 20%, and a direct visual correlation between stained cells and their morphology[29]. Despite being a widespread technique, IHC can be subject to misinterpretation, for example in cases with misrecognized intratumoral heterogeneity for MMR protein expression, or due to staining artifacts[30]. In some cases, missense or in-frame mutations, especially in the context of LS, may produce an inactive protein that is antigenically identical to the wild-type form causing false positive staining[31]. Taking into account the technical variables and the malignancy heterogeneity, IHC detection of MMR presents a sensitivity between 85% and 100% and specificity between 85% and 92%[32]. Based on all these considerations, loss of expression of MMR proteins at IHC indicating dMMR should be confirmed with a direct molecular test (MSI-based PCR or NGS) for eligibility to immunotherapy[27].

MSI detection by polymerase chain reaction (PCR)

MSI testing by PCR-based assays represents the gold standard for molecular detection of MSI. The analysis requires one to a few nanograms of DNA extracted from formalin-fixed paraffin-embedded (FFPE) samples, as template for a PCR reaction, in which fluorescent primers spanning specific marker regions (loci) in microsatellites are employed. The obtained ampli-

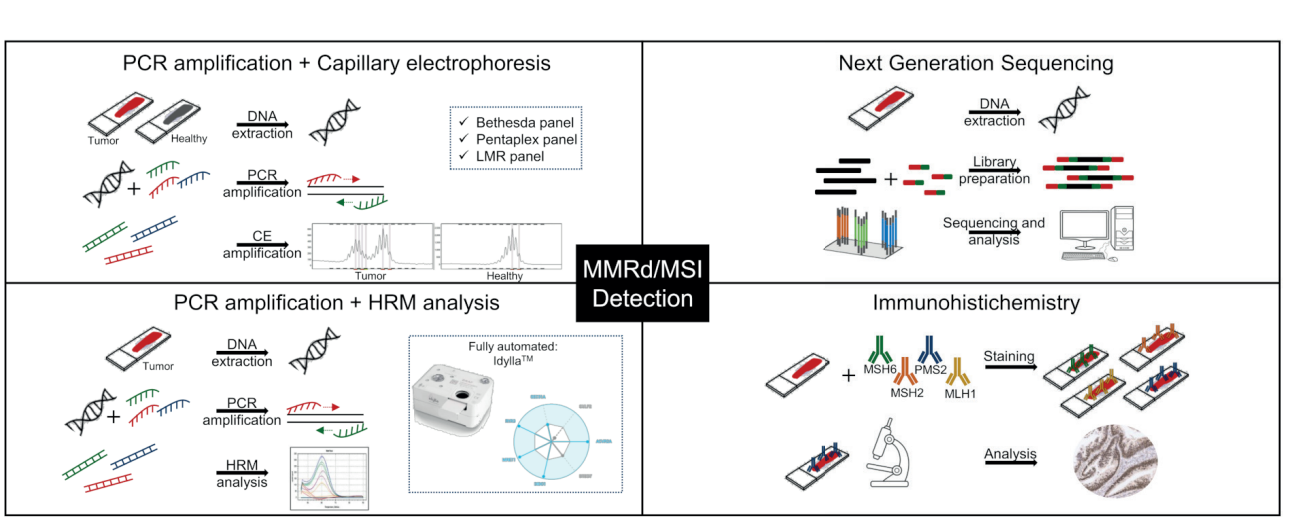

Figure 3. Most common techniques used in clinical practice for mismatch repair deficiency (dMMR)/ microsatellite instability (MSI) identification. Immunohistochemistry for MMR proteins is a fast and widespread method to identify dMMR and is the first-line test to be performed. The longest-standing technique for MSI detection is based on PCR amplification of specific DNA-repeat sequences (loci) using fluorescently labeled primers. Panels of possible loci to be amplified include mononucleotide and dinucleotide short sequences and, more recently, also long mononucleotide repeats (LMR). PCR products can be analyzed by capillary electrophoresis (CE) or high-resolution melting (HRM). The introduction of next-generation sequencing (NGS) techniques has allowed the analysis of several loci simultaneously.

cons are separated based on their size by capillary electrophoresis (CE)[33]. In the analysis, MSI is reported as extra DNA fragment peaks compared with normal tissue. Thus, for punctual MSI analysis, the presence of normal tissue from the same patient is required (Fig. 3).

Marker panels

The first marker panel proposed for MSI detection (Bethesda Panel, 1997) consisted of two mononucleotide (BAT25, BAT26) and three dinucleotide repeats (D5S346, D2S123, D17S250) and presented sensitivity between 67% and 100% and specificity between 61% and 92%. The accuracy of PCR-based MSI analysis was improved after the introduction of panels that included exclusively mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24, MONO-27), with a specificity of 98.7% and sensitivity of 95.8% (revised Bethesda guidelines, 2002)[27,34]. The rationale of this choice is based on the evidence that mononucleotide repeat markers are more specific and sensitive in MSI determination, and, as these markers are quasi-monomorphic in normal DNA, may eliminate the need to employ normal tissue as control[35,36]. Of note, the employment of mononucleotide markers partially overcomes the issue of MSH6-deficient cancers. Due to the partial redundancy of the function of MSH6 and MSH3 proteins, when *MSH6* is mutated the MSH2/MSH3 dimer is still functioning, limiting MSI to mononucleotide tandem repeats[37]. Indeed, a study on ECs has demonstrated that the exclusive employment of mononucleotide marker panels correctly identifies 100% of MSH6-defective cases[37]. Recently, the use of other markers has been suggested. Because sequence instability in microsatellites increases exponentially with the increase of the repeat length, the employment of 52-60 repeated adenine bases defined as long mononucleotide repeats (LMR) has been proposed[38]. At present, a 'mixed' panel consisting of a combination of short mononucleotide repeats (NR-21, BAT-25, BAT-26, MONO-27) and LMRs (BAT-52, BAT-56, BAT-59, BAT-60) is available and has already been tested with good results in pan-cancer cohorts but has yet to be approved as an IVD medical device[39]. Despite no significative improvements related to LMR employment in MSI detection were found in CRC specimens, major advantages were reported in non-CRC specimens. In fact, LMR markers can detect larger shifts in base size, thus improving sensitivity[40]. Considering the wide availability of MSI markers and panels, clear, universally accepted, cut-off values are required. An empirical cut-off of 30% unstable microsatellite loci has been adopted. Accordingly, three microsatellite statuses have been defined: MSI-H, indicating instability in two or more of the 5 analyzed loci (or > 30% of analyzed loci, when more than 5 are analyzed); MSI-L, when instability at one locus of the 5 investigated loci is detected (or in 10%-30% of analyzed loci); and MSS, indicating no loci with instability (or < 10% of analyzed loci)[41]. From previous studies, a large amount of data has been obtained by the analysis of CRC-derived specimens and, consequently, all validated assays are generally calibrated according to CRC samples. This may be an important drawback considering that MSI profiles may differ depending on tumor types. For example, ECs, present smaller mean indels (3 bp vs 6 bp) compared to CRC[42]. Conversely, within the subgroup of gastrointestinal (GI) cancers, nucleotide shifts were the highest in the stomach (mean shift 6.4 nucleotides), followed by colorectum (5.7), small intestine (5.0) and gastroesophageal junction cancers (4.0)[43]. It is also, important to underline that, although more than 20 microsatellite markers have been proposed in the years, PCR-based panels currently in use are generally based on 5 to 8 markers limiting the opportunity to detect MSI, especially in tumors with low rates of MSI.

Novel PCR-based MSI detection techniques

At present, PCR amplification followed by CE represents the gold standard, although novel PCR-based MSI testing methods have been developed. For example, the recently introduced droplet digital PCR (ddPCR) can be used for MSI detection. Five common mononucleotide repeat markers can be tested by ddPCR, using competitive probes based on drop-off assay that can distinguish differences as little as 2 bp. Moreover, this type of assay does not require normal tissue as control. Using this technology MSI can be detected in diluted specimens such as circulating free tumor DNA. The use of this technique in the liquid biopsy setting has shown high concordance with FFPE samples in both CRC and non-CRC [44,45]. The major pitfall that can be encountered using a CE approach for PCR amplicon detection is the presence of multiple 'stutter peaks' surrounding the peak of interest that may cause misinterpretation of the electropherogram. For this reason, several other post-amplification analyses were tested in the years. A highly sensitive and specific method for post-amplification microsatellite amplicon analysis is based on high-resolution melting (HRM). The technique analyses the melting (e.g., the transition from double to single-stranded form) proprieties of the obtained amplicons. Changes in amplicon length, due to microsatellite indels, will produce different melting curves[46]. The Idylla MSI test is based on this principle and is a fully automated real-time PCR assay, in which seven microsatellite markers

(ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2) are amplified using a fluorescent-labeled molecular beacon. Obtained PCR amplicons are then analyzed by HRM. The main advantages of the assay are that (i) normal tissue is not required and (ii) the analysis workflow is completely automated, with no additional molecular facilities required. A 5 µm section of FFPE tissue is directly loaded (no dissection is required for cellularity > 20%) on Idylla Cartridge and DNA extraction and PCR amplification are automatically performed in less than 2 hours. Considering that a low DNA input is required and no normal tissue is needed, the assay may be particularly suitable for biopsies. Agreement with IHC is higher than 96% and reaches 100% in samples with optimal tumor cell content (20% or higher)[47]. An alternative testing method is represented by denaturing high-performance liquid chromatography (DHPLC). DHPLC allows amplicon separation based on their size through a solid-phase column. The major limitations of the technology are represented by the need to optimize the column temperature based on the amplicons' melting temperature to achieve optimal amplicon denaturation[48]. Other techniques, even if still at the research use-only stage, have been investigated and include, for example, the possibility to employ a real-time PCR technique based on peptide nucleic acid (PNA) probes. The high sensitivity of PNA-probes allows to detect alterations in specimens with > 5% cellularity (partially solving the cellularity issue) and, at the same time, the combination with melting curve analysis, for amplicon discrimination, avoids artifacts such as 'stutter peaks' typical of CE[49].

MSI detection by next-generation sequencing

NGS has recently emerged as an alternative to PCR for MSI detection, partially overcoming the major limit of PCR-based approaches: the limited number (5 to 10) of the evaluated loci. NGS-based MSI can simultaneously detect dozens to hundreds of microsatellite loci, limiting some of the pitfalls typical of MSI detection, including ethnicity-associated variations or variations related to tumor type. NGS can simultaneously detect multiple genetic alterations; thus, MSI testing can be integrated with the evaluation of tumor mutation burden (TMB), and/or clinically relevant gene alterations[27,50]. ColonCore (MS plus 36 CRC-related genes)[51], MSIPlus (16 microsatellite loci plus *KRAS*, *NRAS*, and *BRAF* genes mutations)[52], ColoSeq (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *MU-*

Table I. Advantages and disadvantages of MS/MMR detection techniques.

Technique	Mechanism	Advantages	Disadvantages
IHC	Analysis of the four proteins of the MMR complex using specific antibodies.	Widespread Short testing time Lower costs Correlation with morphologic features	More prone to subjective interpretation Possible artifacts due to pre-analytical issues (e.g. sample fixation) Possible false positive staining in case of missense or in-frame mutations
PCR amplification + CE	Amplification of specific loci by PCR followed by capillary electrophoresis of the amplified sequences	Gold standard for molecular MSI detection Low DNA input Direct detection of alterations in DNA caused by dMMR	Requires specific instrumentation Longer turnaround times Results can be influenced by the loci analyzed in the panel Low number of analyzed loci (5 to 10) Healthy tissue is required for each tumor as controls
PCR amplification + HRM	Amplification of specific loci by PCR followed by high-resolution melting analysis of the amplified sequences	Possibility of a fully automated protocol Only one FFPE section is required Low turnaround times Healthy tissue is not required as control samples.	Higher costs Cellularity of the specimen may affect final results Only 7 loci are analyzed
NGS	Sequencing of dozens of loci using NGS-based techniques	Possibility to analyze dozens of loci Possibility to analyze simultaneously MS and other tumor-specific genetic alterations Possibility to identify novel loci in MSI non well characterized tumors	Higher cost Specific bioinformatic tools for computational analysis are required Possibility to introduce errors during the sequencing-by-synthesis Higher DNA input is required

IHC: immunohistochemistry; MMR: mismatch repair; PCR: polymerase chain reaction; CE: capillary electrophoresis; MSI: microsatellite instability; HRM: high resolution melting; MS: microsatellite status; NGS: next generation sequencing.

TYH)[53] are some examples of integrated panels, available for NGS-based MSI profiling in CRC-specimens. The use of NGS does not require healthy tissue as a control, thanks to the use of appropriate bioinformatic tools. NGS platforms present several advantages, although n unmet issue is still represented by computational analysis. These kinds of platforms require dedicated computational tools and the creation of specific algorithms to determine the percentage of unstable loci. Computational algorithms for MSI score definition can work in three different ways: comparing indels distribution of microsatellites in paired tumor vs healthy specimens (MSIsensor, MANTIS), comparing indels distribution in tumor tissue with a defined reference set on targeted gene panels (mSINGS, MSIFinder), or analyzing the number of single nucleotide variants and indels throughout the genome to detect the hypermutated status (MSIseq)[54]. NGS-based MSI detection is burdened by the drawbacks of NGS-based analysis, including the possibility of introducing errors during the sequencing-by-synthesis. The error rate typically ranges from 0.002% to 2% depending on the repeat region length[51]. A number of repeats lower than seven is unlikely to be related to MSI status, but longer mononucleotide repeats (> 15 bp) may be more prone to artifacts due to the sequencing procedure. NGS-based protocols are more expensive in comparison with IHC or PCR and must be carried out in hub centers. The 2022 guidelines for MSI detection from the College of American Pathologists strongly recommended the use of MMR-IHC or MSI by PCR in non-CRC neoplasms, while NGS-MSI assay is allowed only in the CRC setting[56] (Tab. I).

Agreement between different MMR/MS status detection methods

Several studies have evaluated the concordance rate between IHC and molecular assays, but most have been performed in CRC or EC specimens. The majority of studies reported a discordance rate between PCR-based assays and IHC ranging from 1% to 10%, in the CRC setting[57]. For example, Chen and colleagues analyzed a series of 855 CRC patients, comparing IHC with MSI molecular definition using PCR followed by CE (a panel of 5 mononucleotide loci was considered). The analysis reported 45 discordant cases and a level of concordance equal to 88.8%[58]. Similarly, in a smaller cohort of endometrial carcinomas (333 patients) a concordance between IHC and PCR of 98.8% was reported (Cohen's K: 0.98), however, in this case, a panel of both mono and di-nucleotide loci was employed[59]. Regarding the concordance between different molecular assays, Dedeurwaerdere and colleagues compared the three main molecular techniques: PCR followed by CE (five mononucleotide loci plus three dinucleotide loci), Idylla MSI assay and NGS (custom panel including 15 loci), in a mixed CRC/EC (57%/43%) cohort[60]. The three molecular techniques were compared with IHC used as a gold standard. In the CRC cohort, all three molecular assays achieved 100% sensitivity and specificity in comparison with IHC in samples with cellularity of 30% or higher, but PCR and NGS failed in a sample with cellularity of 20%, which was sufficient for Idylla MSI assay. In the EC cohort, all three molecular assays achieved a specificity of 100% but showed lower sensitivity. In fact, in this setting, the most sensitive method was NGS (75%) followed by PCR (67%) and Idylla MSI (58%). Limited information is available regarding the concordance among different techniques in gastric cancer. A recent work by Marino et al. reported very low discordance (0.57%; K = 0.957, 95% CI, p = 0.000) between IHC and PCR in gastric cancer specimens[61].

Preanalytical factors affecting MMR/MS evaluation

Regardless of the assay employed, sample adequacy must be always taken into account. Several pre-analytical factors may affect MSI evaluation. An important factor influencing both MMR-IHC and PCR- or NGS-based MSI detection is sample fixation. In IHC, hypo-fixation or hyper-fixation may result in a reduction of immunostaining intensity[62]. On the other hand, fixation issues may result in a reduction of DNA quality in MSI testing due to high DNA fragmentation[63]. As demonstrated by Malapelle and colleagues, DNA integrity number (DIN)≥ 4 is required for a good concordance between IHC and molecular detection of MSI[64]. In this context, biopsy specimens do not generally have fixation issues compared with surgical specimens[65]. However, surgically obtained tissue presents higher neoplastic cellularity, which is another key point to evaluate in sample adequacy determination. Tumor cellularity equal or higher than 20% is generally required in CRC, while non-CRC specimens may need even higher parameters[42,66]. Therefore, small biopsies, especially from metastases, may result in insufficient tumor-DNA content. In the same way, an abundant mucinous component in certain neoplasms may affect DNA yield and consequently MSI determination[67,68]. Several clinical and technical aspects must be taken into consideration to avoid erroneous interpretations. Hypoxic conditions may decrease MLH1 expression

while neoadjuvant chemo- or radio-therapy may induce the artifactual loss of MSH6 expression in IHC[69,70]. In metastatic CRC, Crisafulli and colleagues demonstrated that resistance to alkylating agents (e.g. temozolomide) is accompanied by the onset of MSH6 mutations decreasing the efficiency of MMR machinery[71]. If available, pre-operative biopsies should be used for MMR evaluation, otherwise caution must be used when interpreting IHC[72].

Molecular and biological factors underlying MMR/MS discordance

As previously said, discordance between MMR and MS status can be encountered in clinical practice. The first step is the exclusion of misinterpretation of the IHC and/or molecular assay and intratumor heterogeneity as underlying causes of the discordance. However, other causes have to be taken into account. MSI phenotype is not only associated with dMMR, but also with the ultramutated phenotype due to *POLE* or *POLD1* pathogenetic gene mutations, in the absence of MMR protein alteration[73]. Furthermore, tumors with MSH6 isolated deficiency are often MSS at molecular analysis by PCR, due to the partial redundancy of the function of MSH6 and MSH3 proteins, as previously described[37]. These cases can be correctly labeled as MSI only by using a panel with a sufficient number of mononucleotide markers, instead of the classic Bethesda Panel. As reported by Chen et al, pathogenic mutations of MMR genes may lead to the expression of a full-length but nonfunctional protein with retained antigenicity, and thus to a strongly positive staining or in some cases to a positive staining, but weaker than internal control[74]. This phenomenon has been reported in LS cases with missense mutations and in somatic MMR genes double mutation cases.

MSI detection by liquid biopsy

Tissue biopsies present several limitations including the inability to capture tumor heterogeneity and the impossibility to perform longitudinal disease monitoring[75,76]. Current European guidelines recommend the use of liquid biopsy for MSI and other predictive biomarkers in the metastatic setting only if an adequate tissue sample is not available and re-biopsy is not possible[77]. Future scenarios include the use of liquid biopsy to cherry-pick patients who benefit from adjuvant therapy and to monitor response and resistance onset to immunotherapy[78]. Circulating tumor DNA (ctDNA) is considered a good analyte to assess MS. Currently, the most robust assays to detect MS in liquid biopsy specimens are droplet digital PCR (ddPCR) and NGS[78]. While ddPCR-based assays may be used both for tissue and liquid biopsies but analyze a limited number of loci (3 to 5 loci), NGS-based panels allow the simultaneous analysis of hundreds of loci but require a specific set-up for plasma-derived specimens. In fact, due to the fragmentation of ctDNA, assays traditionally used for tissue specimens are not suitable for the analysis of ctDNA[79]. Nakamura and colleagues analyzed a cohort of advanced GI cancers using an NGS-based assay (Guardant360), obtaining an overall percent agreement of 98.2% between tissue specimens and ctDNA[80]. It is important to underline that the aforementioned study, as most of the currently available studies aimed at detecting MSI in ctDNA, was focused only on advanced malignancies, that are expected to have high ctDNA shedding. In fact, the fraction of ctDNA among total circulating free DNA (cfDNA) represents a key factor in obtaining a robust concordance between liquid biopsy and tissue specimens.

MSI as a predictive biomarker in GI cancers

MSI was considered for many years as a prerogative of CRC and EC, although about 4% of solid tumors can present MSI-H phenotype and LS has been demonstrated to increase the risk of developing a wide variety of cancers. The approval by the FDA of the anti-PD-1 antibody pembrolizumab for the treatment of any solid tumor with MSI-H/dMMR phenotype has dramatically increased the efforts to identify MS status in those tumors that previously were not routinely screened for MS[81,82]. Overall, MSI is very common in GI cancers with a frequency of about 15%[83].

Gastroesophageal cancer (GC)

The percentage of GCs presenting MSI ranges from 8 (Asiatic population) to 25% (Western population) depending on ethnicity[84]. In general, MSI phenotype is associated with older age at diagnosis, female gender, intestinal histotype, and earlier stage at diagnosis (stages I-II). MSI GC is considered a distinct GC subtype with specific histopathologic features (mucinous and medullary histotypes and high tumor-infiltrating lymphocytes) and genetic profile (high tumor mutational burden and frequent mutations in *PIK3CA*, *ERBB3*, *ERBB2*, and *EGFR*)[85]. Most MSI GC are sporadic forms associated with *MLH1* promoter hypermethylation, while only 1.6% are LS-related[86,87]. MSI GCs are generally characterized by better prognosis with significantly longer overall survival if compared with MSS

tumors. The seminal KEYNOTE-059 trial evaluated the efficacy of pembrolizumab in previously heavily pretreated advanced gastric and gastroesophageal junction (GEJ) cancer patients. An objective response rate (ORR) based on radiologic assessment was obtained in 11.6% of the entire pembrolizumab-treated cohort while a notable ORR of 57% was observed in the MSI-H subgroup[88]. Similarly, the trial CheckMate 032, conducted in metastatic chemotherapy-refractory gastric/GEJ cancer patients, evaluated the efficacy of the anti-PD-1 nivolumab and reported an ORR of 29% in the MSI-H group against 11% in the MSI-L/MSS group[89]. Subsequently, the association of ICIs to chemotherapy was tested in the first-line setting. The phase III CheckMate-649 study, which investigated the efficacy of adding nivolumab to chemotherapy in previously untreated advanced or metastatic HER2-negative gastric/GEJ adenocarcinoma, showed the benefit of adding nivolumab to chemotherapy for patients with a combined positive score (CPS) > 5 and it is at present, the study with the longest follow-up survival data. Three-year follow-up data demonstrated a greater benefit of adding immunotherapy to chemotherapy in the MSI-H subgroup compared to the MSS group in both the overall population (38.7 vs 12.3 months, [HR 0.34, 0.16 to 0.74] and 13.8 vs 11.5 months [HR 0.79, 0.70 to 0.89]) and even greater in the patients with PD-L1 CPS≥5[90].

Moving to the earlier setting, some studies explored the role of immunotherapy in the neoadjuvant/adjuvant setting with no convincing results in the all comers. The KEYNOTE-585 was the first phase III study that demonstrated a significant benefit of association of the anti-PD1 pembrolizumab to standard chemotherapy in locally advanced gastric/GEJ cancer. The benefit was impressive in the MSI-H group with a reduction in the risk of death of 40% compared to 10% in the MSS group[91]. Based on the observation by Pietrantonio and colleagues that locally advanced MSI-H GEJ/GC could benefit less from standard perioperative chemotherapy, dedicated trials to this immunosensitive GC/GEJ population were conducted[92]. In the phase II NEONIPIGA trial, patients with locally advanced MSI-H gastric/GEJ adenocarcinomas receiving the combination of nivolumab and the anti-CTLA4-ipilimumab showed an impressive high rate of pathological complete response (pCR: 59%)[93]. Similar results were achieved in the proof-of-concept INFINITY phase II trial where the activity and safety of neoadjuvant short-term tremelimumab combined with durvalumab was tested in MSI-H/dMMR, EBV-negative resectable gastric/GEJ cancer. A notable pCR rate of 60% and major-complete pathological response (< 10% viable cells) of 80% were reported[94]. In the same setting, the PANDA study tested one cycle of atezolizumab monotherapy followed by four cycles of atezolizumab plus docetaxel, oxaliplatin and capecitabine, showing a pCR in both the two patients presenting dMMR phenotype who underwent to surgery, compared to 39% in the pMMR group[95]. Globally these encouraging results support the use of immunotherapy in the neoadjuvant/perioperative setting in locally advanced resectable MSI-H/dMMR gastric/GEJ cancers and suggest the possibility to omit surgery in highly selected patients. However, further exploration and validation in larger cohorts of patients are necessary.

Colorectal cancer

The frequency of MSI in sporadic CRC is estimated to be about 10-15% and MSI is generally associated with right colon tumor localization, mucinous histotype, lymphocytic infiltration, and advanced stage (IV)[96]. MSI phenotype is a good prognostic factor in early-stage (II-III) CRC, while in stage IV it is associated with worse prognosis. MS in CRC is also considered an important predictive factor of response to chemotherapy and ICI treatment. MSI patients do not benefit from 5-FU adjuvant monochemotherapy but are responsive to combination treatment. As for immunotherapy, the first studies conducted in non-selected CRC demonstrated very limited effects, but the selection of patients based on MSI dramatically changed the response to treatment, obtaining unprecedented high and durable response rates, and impressive survival improvements in the MSI-H mCRC[97,98]. The first study that highlighted the predictive role of MSI-H/dMMR was the study by Le DT and colleagues in which no response was observed in pMMR compared to 40% in MSI mCRC[26]. These promising results were then confirmed by more extensive studies, including the phase II KEYNOTE-164 in pretreated mCRC and KEYNOTE-177 in the first line which defined a new standard of treatment in this setting[99,100]. The multi-cohort study CheckMate142 explored the use of monotherapy with nivolumab and the combination of ipilimumab plus nivolumab, highlighting the benefit of this combination in both previously treated and untreated mCRC. Recently, the results of the phase III trial CheckMate 8HW confirmed the impressive benefit of ipilimumab plus nivolumab compared to investigators' choice standard treatment with a median progression-free survival that was not reached in the ICI arm and a 12-month PFS rate of 79% compared to 21% in the chemotherapy arm.

The remarkable results obtained in MSI-H mCRC patients have led to a growing interest in the possible applications of immunotherapy in earlier settings[101-103]. In the NICHE study, a combination of nivolumab and ipil-

imumab was employed in stage I-III resectable CRCs. The study enrolled 35 patients, including 20 who presented a dMMR phenotype and 15 pMMR CRCs. A pathological response after surgery was observed in all dMMR patients, compared to 27% among the pMMR CRC patients. On this basis, the NICHE-2 study enrolled 115 high-risk stage II or stage III MSI-H/dMMR CRCs to receive one cycle of nivolumab plus ipilimumab and a second cycle of nivolumab followed by surgery. The primary endpoint was met with an amazing 98% of pathological response, 95% major (< 10% residual viable tumor), and 68% of pCR respectively, with a very favorable safety profile[104]. At the ESMO Congress, the primary endpoint of 3-year disease-free survival was presented and none of the patients relapsed[105]. Comparable results in terms of pCR were observed in smaller studies. The first employed the anti-PD1 toripalimab alone or in combination with celecoxib and a pCR rate of 65%, 59%, and 52.8%, respectively[106-109]. The UNICORN study (NTC05845450), an academic window-of-opportunity umbrella platform trial of short-course pre-operative targeted treatments in patients with highly molecularly selected and resectable CRC, is ongoing. In this study the two cohorts of dMMR/MSI-H cancers, treated with the anti-CTLA4 botensilimab and botensilimab plus the anti-PD1 balstilimab respectively, have recently completed the planned accrual.

Locally advanced rectal cancer (LARC) requires a multimodal treatment with a combination of chemotherapy, radiotherapy, and surgery in different sequences[110,111]. However, dMMR/MSI-H LARC may not share the same benefits from neoadjuvant induction chemotherapy as their MSS counterpart[112]. The phase II study conducted by Cercek and colleagues defined a new standard. Six months of neoadjuvant therapy with the anti-PD1 dostarlimab reached 100% of clinical complete in MSI-H/dMMR stage II-III rectal adenocarcinomas[113]. No patients underwent radio-chemotherapy or surgery and no relapse was observed at the data cut-off. Finally, promising data derived from the combination of botensilimab with balstilimab in the NEST-1 trial[114]. MMR/MSI assessment is mandatory and crucial on all CRC specimens regardless of stage and should be primarily conducted on biopsy specimens at first diagnosis.

Pancreatic ductal adenocarcinoma (PDAC)

Approximately 1-2% of PDAC are MSI-H/dMMR[115-117]. MSI status is more often associated with medullary and mucinous/colloid histology and the absence of *KRAS* and *TP53* mutations[115,118]. Recently, also signet-ring variant of PDAC has been described as an additional histology potentially enriched in MSI[119]. According to preliminary data, the percentage of MSI-H/dMMR rises to 6.9%, when intraductal papillary mucinous neoplasm (IPMN)-associated PDACs are considered[120]. PDACs are part of LS-associated spectrum cancers. In fact, LS patients present a 9-fold higher risk of developing pancreatic cancer compared with the general population[121]. Unfortunately, the role of MSI as a predictive marker in this cancer is still highly debated: on the one hand, Riazy and colleagues demonstrated that no survival advantage is appreciable in MSI-H/dMMR patients treated with 5-FU or gemcitabine-based therapies; on the other hand, Cloyd and colleagues demonstrated that in the metastatic setting, MSI-H/dMMR patients had a better outcome compared with their MSS counterpart when treated with FOLFIRINOX (16.5 months vs 11.1 months median OS)[122,123]. NCCN guidelines (2020), recommend molecular MSI definition or MMR testing for ICI treatment in advanced PDAC[124]. Although limited, preliminary data on ICI-based treatment in MSI-H/dMMR PDAC are promising. In the expanded proof-of-concept study by Le and colleagues 12 different MSI-H/dMMR cancer patients, including some PDACs, received pembrolizumab with a notable benefit in term of ORR and survival, supporting the hypothesis that MSI-H/dMMR cancers could be sensitive to ICIs, regardless the origin of tumor's tissue. The KEYNOTE-158, phase II study investigated the efficacy of pembrolizumab in patients with previously treated, advanced non-colorectal MSI-H/dMMR cancers. The study enrolled 233 patients with 27 tumor types including 22 patients (9.7%) with pancreatic cancer. In the entire population, the ORR was 34.3% (95% CI, 28.3% to 40.8%), the median PFS was 4.1 months (95% CI, 2.4 to 4.9 months) and the median overall survival was 23.5 months (95% CI, 13.5 months to not reached). These results supported the tumor-agnostic approval of pembrolizumab for the treatment of advanced MSI-H/dMMR cancers. A clinical benefit was observed even in PDAC but was less evident (ORR 18.2%, 5.2 to 40.3; mPFS 2.1 month, 1.9 to 3.4; mOS 4.0 months, 2.1 to 9.8; and median duration of response 13.4 months, 8.1 to 16.0+) probably due to the intrinsic scarce immunogenicity in the pancreatic microenvironment and taking into account the later lines setting[125]. Moreover, Taieb and colleagues investigated a cohort of 31 advanced MSI-H/dMMR PDACs and achieved an ORR of 48.4% and stable disease in 19.4% of patients[126]. In another retrospective cohort, 55 MSI-H/dMMR PDACs cases were described with a high rate of LS (58%) and a durable control disease in 17 patients who received ICIs (median ICI duration 27.7 months (95% CI, 11.5 to not reached); disease control rate 80%)[127]. Some studies focused in the identification of potential issues that may cause resistance to ICI in MSI-H/dMMR PDAC, among them the enrichment in *B2M* and *JAK* gene mutations

in tumors and the presence of intratumor heterogeneity were identified[128].

Biliary tract cancers

Biliary tract cancers (BTCs) are a heterogeneous class of malignancies with different histopathological and genetic features, resulting in different clinical behaviors. The class encompasses gallbladder cancers (GBCs) and cholangiocarcinomas (CCAs), which are classified as intrahepatic (iCCA) and extrahepatic cholangiocarcinoma (eCCA), depending on the origin site of the malignancy. According to revised Bethesda Guidelines for LS, BCTs are included within LS-associated tumors. Data on MS status in BTCs are limited by their heterogeneity and rarity[129]. When considering the whole BTC group, MSI/dMMR prevalence in the Caucasian population is less than 3%, although dramatically higher rates (up to 30%) are reported in certain regions, such as Thailand. Indeed, MSI status in CCA appears to be associated with liver fluke infection[130]. A study by Eluri and colleagues analyzed the largest cohort of iCCA (7565 cases) available in the literature and reported a prevalence of MSI status of 1.8%, while another study conducted exclusively on resection specimens and considering perihilar CCA (pCCA) reported a prevalence of 2.4%[131,132]. A higher MSI rate was found in GBCs in which MSI is seen in 7.8% of cases, generally associated with MSH2 loss[133]. Considering the low rate of resectable BTCs in daily practice, the work of Kai and colleagues is of great interest. Surgical resection, biopsy, and fine needle aspiration (FNA) specimens from BTCs were compared demonstrating that MSI determination can be performed successfully in 98.3% of samples, even in cases in which tissue availability is limited[134]. In the abovementioned KEYNOTE-158 study, a notable ORR of 40.9% and a mOS of 24.3 months were reached in MSI-H/dMMR previously treated advanced BTC patients in which the survival from diagnosis of advanced disease is about 12 months, pointing out the need for upfront MMR/MSI status evaluation at diagnosis[125]. Moreover, in another multicenter observational study focused patients with hepatobiliary (HBP) and pancreatic cancer, pembrolizumab led to an ORR of 73% for BTCs compared to 30% for PDACs[135].

Other gastrointestinal malignancies

Unfortunately, in other rare gastrointestinal malignancies data are still lacking regarding the predictive role of MSI status.

Tumors arising in the ampulla of Vater (AVC) are rare but occur relatively frequently in patients with LS and are generally associated with MSH6 germline mutations[136]. Across different studies, the prevalence of MSI in AVC is approximately 18% and a strong association with the intestinal AVC subtype has been reported[116,137,138]. MSI/dMMR is associated with better prognosis and MSI patients who undergo resection present a lower rate of recurrent disease[139]. Interestingly, in AVC, MSI is not restricted to the intestinal phenotype but can be found also in pancreatobiliary and mixed histologies[116,140].

Characterization of MS in hepatocellular carcinoma (HCC) is still an open issue and available data are heterogeneous and highly inconclusive. MSI-H/dMMR cases in HCC seem to be rare, with a prevalence of less than 3%[141]. However, different prevalence values are often reported in the Asiatic and European populations, probably due to the different etiologies of HCC[142]. Inflammatory events (e.g. cirrhosis and viral infections such as HCV, HBV) may induce dysfunctions of the MMR complex[143]. Moreover, as reported in a study comparing mononucleotide repeat alterations in HCC and in CRC, variations observed in HCC are less extensive than in CRC [144].

Finally, data on MSI-H/dMMR in neuroendocrine neoplasms (NENs) are limited. Studies performed on gastric and colorectal neuroendocrine carcinomas (NECs) and mixed neuroendocrine-non neuroendocrine neoplasms (MiNeNs) reported a prevalence of up to 15%, mainly due to *MHL1* promoter methylation. In these tumors, MSI was associated with a more favorable prognosis[145,146]. Conversely, MSI-H/dMMR is rare in neuroendocrine tumors (NETs) of the small intestine and pancreas and cases of NETs in patients with LS are mostly anecdotal[147,148].

Conclusions

Since the FDA approval of pembrolizumab for the treatment of patients with unresectable or metastatic MSI-H/dMMR solid tumors, who progressed following previous treatment, MSI has become the first true agnostic biomarker, and its evaluation has become a milestone of tumor molecular characterization. IHC represents the first-line diagnostic test for dMMR detection, due to its availability in almost all pathology laboratories and its low costs. If dMMR status is unclear, MSI evaluation by molecular analysis must be performed. In this setting, the pathologist plays a central role in therapeutic decision-making. The pathologist must perform an accurate assessment of MMR protein expression and should be aware of potential pitfalls and misinterpretations to guarantee the patient's access to the best treatment. The pathologist in charge of molecular diagnostics should also be responsible for the evaluation

of the adequacy of the specimen for molecular analysis, for choosing the best assay and panel, and for providing the clinicians with a clear and concise report and additional comments to facilitate its interpretation. Among GI cancers, MSI-H/dMMR status and its detection methods have been widely studied and developed in the CRC setting. However, there are still open issues and questions to be addressed in non-CRC GI malignancies. Future perspectives include: i) the optimization of MMR/MSI detection in biopsy specimens for the use of ICIs in the neoadjuvant setting; ii) the use of liquid biopsy as a non-invasive tool for MSI evaluation; and iii) the development of NGS panels for a reliable definition of MSI status in non-CRC malignancies.

Funding

MF and CL are supported by European Union - Next Generation EU, Mission 4 Component 1 CUP: C53D23002200006. MF is also supported by European Union - Next-Generation EU, PNRR M4C2-Investimento 1.4- CN00000041, and by the Department of Medicine (BIRD_2024).

Conflicts of interest statement

MF reports research funding (to institution) from Roche, Astellas, and Diaceutics; personal honoraria as an invited speaker from Roche, Astellas, AstraZeneca, Lilly, Incyte, Bristol Myers Squibb, Agilent, Merck Serono, Pierre Fabre, GlaxoSmithKline, Novartis, and Amgen; and for participation in advisory boards of Amgen, Astellas, Roche, Merck Serono, GlaxoSmithKline, Novartis, and Janssen.

Authors contributions

All authors equally contributed in the writing and conceptualization of the manuscript. All authors have read and agreed to the published version of the manuscript.

References

1 Depienne C. Tandem repeat expansions: the good, the bad and the hidden. Med Genet 2022;33:291-292. https://doi.org/10.1515/medgen-2021-2097

2 Eckert KA, Hile SE. Every microsatellite is different: Intrinsic DNA features dictate mutagenesis of common microsatellites present in the human genome. Mol Carcinog 2009;48:379-388. https://doi.org/10.1002/mc.20499.

3 Bagshaw ATM. Functional Mechanisms of Microsatellite DNA in Eukaryotic Genomes. Genome Biol Evol 2017;9:2428-243. https://doi.org/10.1093/gbe/evx164

4 Imai K, Yamamoto H. Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. Carcinogenesis 29:673-80. https://doi.org/10.1093/carcin/bgm228

5 Kristmundsdottir S, Jonsson H, Hardarson MT, et al. Sequence variants affecting the genome-wide rate of germline microsatellite mutations. Nat Commun 2023;14:3855. https://doi.org/10.1038/s41467-023-39547-6

6 Li YC, Korol AB, Fahima T, et al. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review: MICROSATELLITE EVOLUTION. Mol Ecol 2002;11:2453-265. https://doi.org/10.1046/j.1365-294x.2002.01643.x

7 Gemayel R, Vinces MD, Legendre M, et al. Variable Tandem Repeats Accelerate Evolution of Coding and Regulatory Sequences. Annu Rev Genet 2010;4:445-477. https://doi.org/10.1146/annurev-genet-072610-155046

8 Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res 2008;18:85-98. https://doi.org/10.1038/cr.2007.115

9 Liu D, Keijzers G, Rasmussen LJ. DNA mismatch repair and its many roles in eukaryotic cells. Mutat Res Mutat Res 2017;773:174-187. https://doi.org/10.1016/j.mrrev.2017.07.001

10 Martín-López JV, Fishel R. The mechanism of mismatch repair and the functional analysis of mismatch repair defects in Lynch syndrome. Fam Cancer 2013;12:159-168. https://doi.org/10.1007/s10689-013-9635-x

11 Pe□ina-Šlaus N, Kafka A, Salamon I, et al. Mismatch Repair Pathway, Genome Stability and Cancer. Front Mol Biosci 2020;7:122. https://doi.org/10.3389/fmolb.2020.00122

12 Peltomäki P. Update on Lynch syndrome genomics. Fam Cancer 2016;15:385-393. https://doi.org/10.1007/s10689-016-9882-8

13 Schöniger S, Rüschoff J. Mismatch Repair Deficiency and Microsatellite Instability. Encyclopedia 2022;2:1559-1576. https://doi.org/10.3390/encyclopedia2030106

14 Santoro A, Bragantini E, Castiglione F, et al. Biomarker characterization in endometrial cancer in Europe: first survey data analysis from 69 pathological academic and hospital labs. Pathologica 2024;116:32-45. https://doi.org/10.32074/1591-951X-926

15 Jain E, Prasad S, Dhar A, et al. The utility of evaluating mismatch repair proteins in endometrial carcinoma: an experience from a tertiary referral centre in North India. Pathologica 2021;113:115-120. https://doi.org/10.32074/1591-951X-129

16 Remo A, Fassan M, Lanza G. Immunohistochemical evaluation of mismatch repair proteins in colorectal carcinoma: the AIFEG/GIPAD proposal. Pathologica 2016;108:104-109.

17 Zeinalian M, Hashemzadeh-Chaleshtori M, Salehi R, et al. Clinical Aspects of Microsatellite Instability Testing in Colorectal Cancer. Adv Biomed Res 2018;7:28. https://doi.org/10.4103/abr.abr_185_16

18 Wang QX, Qu CH, Gao YH, et al. The degree of microsatellite instability predicts response to PD-1 blockade immunotherapy in mismatch repair-deficient/microsatellite instability-high colorectal cancers. Exp Hematol Oncol 2021;10:2. https://doi.org/10.1186/s40164-020-00193-z

19 Alkholifi FK, Alsaffar RM. Dostarlimab an Inhibitor of PD-1/PD-L1: A New Paradigm for the Treatment of Cancer. Medicina (Mex) 2022;58:1572. https://doi.org/10.3390/medicina58111572

20 Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin JP, et al. Clues to the Pathogenesis of Familial Colorectal Cancer. Science 1993;260:812-816. https://doi.org/10.1126/science.8484121

21 Ionov Y, Peinado MA, Malkhosyan S, et al. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993;363:558-561. https://doi.org/10.1038/363558a0

22 Thibodeau SN, Bren G, Schaid D. Microsatellite Instability in Cancer of the Proximal Colon. Science 1993;260:816-819. https://doi.org/10.1126/science.8484122

23 Drummond JT, Li GM, Longley MJ, et al. Isolation of an hMSH2-p160 Heterodimer That Restores DNA Mismatch Repair to Tumor Cells. Science 1995;268:1909-1912. https://doi.org/10.1126/science.7604264

24 Kane MF, Loda M, Gaida GM, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic

colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res 1997;57:808-811.

25 Suraweera N, Duval A, Reperant M, et al. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. Gastroenterology 2002;123:1804-1811. https://doi.org/10.1053/gast.2002.37070

26 Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 2015;372:2509-2520. https://doi.org/10.1056/NEJMoa1500596

27 Luchini C, Bibeau F, Ligtenberg MJL, et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. Ann Oncol 2019;30:1232-1243. https://doi.org/10.1093/annonc/mdz116

28 Grant M, Haydon A, Au L, et al. Immunohistochemistry testing for mismatch repair deficiency in Stage 2 colon cancer: A cohort study of two cancer centres. Int J Surg. 2018;51:71-75. https://doi.org/10.1016/j.ijsu.2018.01.020

29 Wang C, Zhang L, Vakiani E, et al. Detecting mismatch repair deficiency in solid neoplasms: immunohistochemistry, microsatellite instability, or both? Mod Pathol 2022;35:1515-1528. https://doi.org/10.1038/s41379-022-01109-4

30 Denčić T, Petrović A, Jovičić Milentijević M, et al. The Importance of Immunohistochemical Heterogeneous Expression of MMR Protein in Patients with Colorectal Cancer in Stage II and III of the Disease. Medicina (Mex) 2023;59:489. https://doi.org/10.3390/medicina59030489

31 Aiyer KTS, Doeleman T, Ryan NA, et al. Validity of a two-antibody testing algorithm for mismatch repair deficiency testing in cancer; a systematic literature review and meta-analysis. Mod Pathol 2022;35:1775-1783. https://doi.org/10.1038/s41379-022-01149-w

32 Zhang X, Li J. Era of universal testing of microsatellite instability in colorectal cancer. World J Gastrointest Oncol 2013;5:12. https://doi.org/10.4251/wjgo.v5.i2.12

33 Vatrano S, Pettinato A, Randazzo V, et al. Validation study of an alternative system to detect microsatellite instability in colorectal carcinoma. Pathologica 2020;112:178-183. https://doi.org/10.32074/1591-951X-113

34 Guyot D'Asnières De Salins A, Tachon G, Cohen R, et al. Discordance between immunochemistry of mismatch repair proteins and molecular testing of microsatellite instability in colorectal cancer. ESMO Open 2021;6:100120. https://doi.org/10.1016/j.esmoop.2021.100120

35 Goel A, Nagasaka T, Hamelin R, et al. An Optimized Pentaplex PCR for Detecting DNA Mismatch Repair-Deficient Colorectal Cancers. PLoS ONE 2010;5:e9393. https://doi.org/10.1371/journal.pone.0009393

36 Campanella NC, Berardinelli GN, Scapulatempo-Neto C, et al. Optimization of a pentaplex panel for MSI analysis without control DNA in a Brazilian population: correlation with ancestry markers. Eur J Hum Genet 2014;22:875-880. https://doi.org/10.1038/ejhg.2013.256

37 You JF, Buhard O, Ligtenberg MJL, et al. Tumours with loss of MSH6 expression are MSI-H when screened with a pentaplex of five mononucleotide repeats. Br J Cancer 2010;103:1840-1845. https://doi.org/10.1038/sj.bjc.6605988

38 Bacher JW, Sievers CK, Albrecht DM, et al. Improved Detection of Microsatellite Instability in Early Colorectal Lesions. PLOS ONE 2015;10:e0132727. https://doi.org/10.1371/journal.pone.0132727

39 Bacher JW, Udho EB, Strauss EE, et al. A Highly Sensitive Pan-Cancer Test for Microsatellite Instability. J Mol Diagn 2023;S1525157823001757. https://doi.org/10.1016/j.jmoldx.2023.07.003

40 Lin JH, Chen S, Pallavajjala A, et al. Validation of Long Mononucleotide Repeat Markers for Detection of Microsatellite Instability. J Mol Diagn 2022;24:144-157. https://doi.org/10.1016/j.jmoldx.2021.10.011

41 Funkhouser WK, Lubin IM, Monzon FA, et al. Relevance, Pathogenesis, and Testing Algorithm for Mismatch Repair-Defective Colorectal Carcinomas. J Mol Diagn 2012;14:91-103. https://doi.org/10.1016/j.jmoldx.2011.11.001

42 Wang Y, Shi C, Eisenberg R, et al. Differences in Microsatellite Instability Profiles between Endometrioid and Colorectal Cancers. J Mol Diagn 2017;19:57-64. https://doi.org/10.1016/j.jmoldx.2016.07.008

43 Shinozaki-Ushiku A, Kunita A, Iwasaki A, et al. Microsatellite instability profiles of gastrointestinal cancers: comparison between NON-COLORECTAL and colorectal origin. Histopathology 2023;82:466-477. https://doi.org/10.1111/his.14830

44 Klouch KZ, Stern MH, Trabelsi-Grati O, et al. Microsatellite instability detection in breast cancer using drop-off droplet digital PCR. Oncogene 2022;41:5289-5297. https://doi.org/10.1038/s41388-022-02504-6

45 Silveira AB, Bidard FC, Kasperek A, et al. High-Accuracy Determination of Microsatellite Instability Compatible with Liquid Biopsies. Clin Chem 2020;66:606-613. https://doi.org/10.1093/clinchem/hvaa013

46 Janavicius R, Matiukaite D, Jakubauskas A, et al. Microsatellite Instability Detection by High-Resolution Melting Analysis. Clin Chem 2010;56:1750-1757. https://doi.org/10.1373/clinchem.2010.150680

47 Ukkola I, Nummela P, Kero M, et al. Diagnostic performance of Idylla MSI test in colorectal cancer biopsies. Diagn Pathol 2023;18:39. https://doi.org/10.1186/s13000-023-01328-6

48 Soong R, Anuar D, Liu Y, et al. Denaturing High Performance Liquid Chromatography for the Detection of Microsatellite Instability Using Bethesda and Pentaplex Marker Panels. Diagn Mol Pathol 2008;17:127-133. https://doi.org/10.1097/PDM.0b013e3181577daf

49 Jang M, Kwon Y, Kim H, et al. Microsatellite instability test using peptide nucleic acid probe-mediated melting point analysis: a comparison study. BMC Cancer 2018;18:1218. https://doi.org/10.1186/s12885-018-5127-6

50 Nowak JA, Yurgelun MB, Bruce JL, et al. Detection of Mismatch Repair Deficiency and Microsatellite Instability in Colorectal Adenocarcinoma by Targeted Next-Generation Sequencing. J Mol Diagn 2017;19:84-91. https://doi.org/10.1016/j.jmoldx.2016.07.010

51 Zhu L, Huang Y, Fang X, et al. A Novel and Reliable Method to Detect Microsatellite Instability in Colorectal Cancer by Next-Generation Sequencing. J Mol Diagn 2018;20:225-231. https://doi.org/10.1016/j.jmoldx.2017.11.007

52 Hempelmann JA, Scroggins SM, Pritchard CC, et al. MSIplus for Integrated Colorectal Cancer Molecular Testing by Next-Generation Sequencing. J Mol Diagn 2015;17:705-714. https://doi.org/10.1016/j.jmoldx.2015.05.008

53 Pritchard CC, Smith C, Salipante SJ, et al. ColoSeq Provides Comprehensive Lynch and Polyposis Syndrome Mutational Analysis Using Massively Parallel Sequencing. J Mol Diagn 2012;14:357-366. https://doi.org/10.1016/j.jmoldx.2012.03.002

54 Swaerts K, Dedeurwaerdere F, De Smet D, et al. DeltaMSI: artificial intelligence-based modeling of microsatellite instability scoring on next-generation sequencing data. BMC Bioinformatics 2023;24:73. https://doi.org/10.1186/s12859-023-05186-3

55 Styk J, Pös Z, Pös O, et al. Microsatellite instability assessment is instrumental for Predictive, Preventive and Personalised Medicine: status quo and outlook. EPMA J. 2023;14:143-165. https://doi.org/10.1007/s13167-023-00312-w

56 Vikas P, Messersmith H, Compton C, et al. Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy: ASCO Endorsement of College of American Pathologists Guideline. J Clin Oncol 2023;41:1943-1948. https://doi.org/10.1200/JCO.22.02462

[57] Huang W, Ho CL, Lee CT, et al. High concordance rate of capillary electrophoresis workflow for microsatellite instability analysis and mismatch repair (MMR) immunostaining in colorectal carcinoma. PLOS ONE 2023;18:e0284227. https://doi.org/10.1371/journal.pone.0284227

[58] Chen J, Yan Q, Sun J, et al. Microsatellite Status Detection of Colorectal Cancer: Evaluation of Inconsistency between PCR and IHC. J Cancer 2023;14:1132-1140. https://doi.org/10.7150/jca.81675

[59] Wang C, Kuang W, Zeng J, et al. A retrospective study of consistency between immunohistochemistry and polymerase chain reaction of microsatellite instability in endometrial cancer. PeerJ 2023;11:e15920. https://doi.org/10.7717/peerj.15920

[60] Dedeurwaerdere F, Claes KB, Van Dorpe J, et al. Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer. Sci Rep 2021;11:12880. https://doi.org/10.1038/s41598-021-91974-x

[61] Zito Marino F, Amato M, Ronchi A, et al. Microsatellite Status Detection in Gastrointestinal Cancers: PCR/NGS Is Mandatory in Negative/Patchy MMR Immunohistochemistry. Cancers 2022;14:2204. https://doi.org/10.3390/cancers14092204

[62] Libard S, Cerjan D, Alafuzoff I. Characteristics of the tissue section that influence the staining outcome in immunohistochemistry. Histochem Cell Biol 2019;151:91-96. https://doi.org/10.1007/s00418-018-1742-1

[63] Ottestad AL, Emdal EF, Grønberg BH, et al. Fragmentation assessment of FFPE DNA helps in evaluating NGS library complexity and interpretation of NGS results. Exp Mol Pathol 2022;126:104771. https://doi.org/10.1016/j.yexmp.2022.104771

[64] Malapelle U, Parente P, Pepe F, et al. Impact of Pre-Analytical Factors on MSI Test Accuracy in Mucinous Colorectal Adenocarcinoma: A Multi-Assay Concordance Study. Cells 2020;9:2019. https://doi.org/10.3390/cells9092019

[65] Jain N. Essentials Before Sending Biopsy Specimens: A Surgeon's Prespective and Pathologists Concern. J Maxillofac Oral Surg 2011;10:361-364. https://doi.org/10.1007/s12663-011-0234-9

[66] Shi C, Washington K. Molecular Testing in Colorectal Cancer: diagnosis of Lynch syndrome and personalized cancer medicine Am J Clin Pathol 2012;137:847-859. https://doi.org/10.1309/AJCPI83DINULUJNI

[67] Goswami RS, Luthra R, Singh RR, et al. Identification of Factors Affecting the Success of Next-Generation Sequencing Testing in Solid Tumors. Am J Clin Pathol 2016;145:222-237. https://doi.org/10.1093/ajcp/aqv023

[68] Parente P, Malapelle U, Angerilli V, et al. MMR profile and microsatellite instability status in colorectal mucinous adenocarcinoma with synchronous metastasis: a new clue for the clinical practice. J Clin Pathol 2023;76:492-496. https://doi.org/10.1136/jclinpath-2022-208143

[69] Lu Y, Wajapeyee N, Turker MS, et al. Silencing of the DNA Mismatch Repair Gene MLH1 Induced by Hypoxic Stress in a Pathway Dependent on the Histone Demethylase LSD1. Cell Rep 2014;8:501-513. https://doi.org/10.1016/j.celrep.2014.06.035

[70] Bao F, Panarelli NC, Rennert H, et al. Neoadjuvant Therapy Induces Loss of MSH6 Expression in Colorectal Carcinoma. Am J Surg Pathol 2010;34:1798-1804. https://doi.org/10.1097/PAS.0b013e3181f906cc

[71] Crisafulli G, Sartore-Bianchi A, Lazzari L, et al. Temozolomide Treatment Alters Mismatch Repair and Boosts Mutational Burden in Tumor and Blood of Colorectal Cancer Patients. Cancer Discov 2022;12:1656-1675. https://doi.org/10.1158/2159-8290.CD-21-1434

[72] Grillo F, Paudice M, Gambella A, et al. Evaluating mismatch repair deficiency in colorectal cancer biopsy specimens. Histochem Cell Biol 2023;160:113-125. https://doi.org/10.1007/s00418-023-02202-8

[73] Parente P, Grillo F, Vanoli A, et al. The Day-To-Day Practice of MMR and MSI Assessment in Colorectal Adenocarcinoma: What We Know and What We Still Need to Explore. Dig Dis 2023;1-11. https://doi.org/10.1159/000531003

[74] Chen W, Hampel H, Pearlman R, et al. Unexpected expression of mismatch repair protein is more commonly seen with pathogenic missense than with other mutations in Lynch syndrome. Hum Pathol 2020;103:34-41. https://doi.org/10.1016/j.humpath.2020.07.001

[75] Sisodiya S, Kasherwal V, Khan A, et al. Liquid Biopsies: Emerging role and clinical applications in solid tumours. Transl Oncol 2023;35:101716. https://doi.org/10.1016/j.tranon.2023.101716

[76] Malapelle U, Rolfo C, on behalf of the International Society of Liquid Biopsy (ISLB). Liquid biopsy as a follow-up tool: Comment on longitudinal monitoring of somatic genetic alterations in circulating cell-free DNA during treatment with epidermal growth factor receptor-tyrosine kinase inhibitors. Cancer 2020;126:22-25. https://doi.org/10.1002/cncr.32482

[77] Russo A, Incorvaia L, Del Re M, et al. The molecular profiling of solid tumors by liquid biopsy: a position paper of the AIOM-SIAPEC-IAP-SIBioC-SIC-SIF Italian Scientific Societies. ESMO Open 2021;6:100164. https://doi.org/10.1016/j.esmoop.2021.100164

[78] Ceccon C, Angerilli V, Rasola C, et al. Microsatellite Instable Colorectal Adenocarcinoma Diagnostics: The Advent of Liquid Biopsy Approaches. Front Oncol 2022;12:930108. https://doi.org/10.3389/fonc.2022.930108

[79] Willis J, Lefterova MI, Artyomenko A, et al. Validation of Microsatellite Instability Detection Using a Comprehensive Plasma-Based Genotyping Panel. Clin Cancer Res 2019;25:7035-7045. https://doi.org/10.1158/1078-0432.CCR-19-1324

[80] Nakamura Y, Okamoto W, Denda T, et al. Clinical Validity of Plasma-Based Genotyping for Microsatellite Instability Assessment in Advanced GI Cancers: SCRUM-Japan GOZILA Substudy. JCO Precis Oncol 2022;e2100383. https://doi.org/10.1200/PO.21.00383

[81] Twomey JD, Zhang B. Cancer Immunotherapy Update: FDA-Approved Checkpoint Inhibitors and Companion Diagnostics. AAPS J 2021;39. https://doi.org/10.1208/s12248-021-00574-0

[82] Callea M, Pedica F, Doglioni C. Programmed death 1 (PD-1) and its ligand (PD-L1) as a new frontier in cancer Immunotherapy and challenges for the Pathologist: state of the art. Pathologica 2016;108:48-58.

[83] Corso G. Microsatellite instability in gastrointestinal cancers. Eur J Hum Genet 2022;30:996-997. Https://doi.org/10.1038/s41431-022-01134-6

[84] Ratti M, Lampis A, Hahne JC, et al. Microsatellite instability in gastric cancer: molecular bases, clinical perspectives, and new treatment approaches. Cell Mol Life Sci 2018;75:4151-4162. https://doi.org/10.1007/s00018-018-2906-9

[85] The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202-209. https://doi.org/10.1038/nature13480

[86] Capelle LG, Van Grieken NCT, Lingsma HF, et al. Risk and Epidemiological Time Trends of Gastric Cancer in Lynch Syndrome Carriers in The Netherlands. Gastroenterology 2010;138:487-92. https://doi.org/10.1053/j.gastro.2009.10.051

[87] Hu G, Qin L, Zhang X, et al. Epigenetic Silencing of the MLH1 Promoter in Relation to the Development of Gastric Cancer and its use as a Biomarker for Patients with Microsatellite Instability: a Systematic Analysis. Cell Physiol Biochem 2https://doi.org/10.1016/S0140-6736(21)00797-2018;45:148-62. https://doi.org/10.1159/000486354

[88] Fuchs CS, Ohtsu A, Tabernero J, et al. Preliminary safety data from KEYNOTE-059: Pembrolizumab plus 5-fluorouracil (5-FU) and cisplatin for first-line treatment of advanced gastric can-

cer. J Clin Oncol 2016;34:4037-4037. Https://doi.org/10.1200/JCO.2016.34.15_suppl.403

89 Ott PA, Le DT, Kim JW, Aet al. Nivolumab (NIVO) in patients (pts) with advanced (adv) chemotherapy-refractory (CT-Rx) esophagogastric (EG) cancer according to microsatellite instability (MSI) status: checkmate 032. Ann Oncol 2017;28:229-230. Https://doi.org/10.1093/annonc/mdx369.058

90 Janjigian YY, Shitara K, Moehler M, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. The Lancet 2021;398:27-40. https://doi.org/10.1016/S0140-6736(21)00797-2

91 Shitara K, Rha SY, Wyrwicz LS, et al LBA3 Final analysis of the phase III KEYNOTE-585 study of pembrolizumab plus chemotherapy vs chemotherapy as perioperative therapy in locally-advanced gastric and gastroesophageal junction cancer. Ann Oncol 2024;35:S213. Https://doi.org/10.1016/j.annonc.2024.06.007

92 Pietrantonio F, Miceli R, Raimondi A, et al. Individual Patient Data Meta-Analysis of the Value of Microsatellite Instability As a Biomarker in Gastric Cancer. J Clin Oncol 2019;37:3392-3400. https://doi.org/10.1200/JCO.19.01124

93 André T, Tougeron D, Piessen G, et al. Neoadjuvant Nivolumab Plus Ipilimumab and Adjuvant Nivolumab in Localized Deficient Mismatch Repair/Microsatellite Instability-High Gastric or Esophagogastric Junction Adenocarcinoma: The GERCOR NEONIPIGA Phase II Study. J Clin Oncol 2023;41:255-265. https://doi.org/10.1200/JCO.22.00686

94 Pietrantonio F, Raimondi A, Lonardi S, et al. INFINITY: A multicentre, single-arm, multi-cohort, phase II trial of tremelimumab and durvalumab as neoadjuvant treatment of patients with microsatellite instability-high (MSI) resectable gastric or gastroesophageal junction adenocarcinoma (GAC/GEJAC). J Clin Oncol 2023;41:358-358. Https://doi.org/10.1200/JCO.2023.41.4_suppl.358

95 Verschoor YL, Van De Haar J, Van Den Berg JG, et al. Neoadjuvant atezolizumab plus chemotherapy in gastric and gastroesophageal junction adenocarcinoma: the phase 2 PANDA trial. Nat Med 2024;30:519-530. https://doi.org/10.1038/s41591-023-02758-x

96 Corso G, Pascale V, Flauti G, et al. Oncogenic mutations and microsatellite instability phenotype predict specific anatomical subsite in colorectal cancer patients. Eur J Hum Genet 2013;21:1383-1388. https://doi.org/10.1038/ejhg.2013.66

97 Manca P, Corti F, Intini R, et al. Tumour mutational burden as a biomarker in patients with mismatch repair deficient/microsatellite instability-high metastatic colorectal cancer treated with immune checkpoint inhibitors. Eur J Cancer 2023;187:15-24. https://doi.org/10.1016/j.ejca.2023.03.029

98 Loupakis F, Maddalena G, Depetris I, et al. Treatment with checkpoint inhibitors in a metastatic colorectal cancer patient with molecular and immunohistochemical heterogeneity in MSI/dMMR status. J Immunother Cancer 2019;7:297. https://doi.org/10.1186/s40425-019-0788-5

99 Le DT, Diaz LA, Kim TW, et al. Pembrolizumab for previously treated, microsatellite instability-high/mismatch repair-deficient advanced colorectal cancer: final analysis of KEYNOTE-164. Eur J Cancer 2023;186:185-195. https://doi.org/10.1016/j.ejca.2023.02.016

100 André T, Shiu KK, Kim TW, et al. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. N Engl J Med 2020;383:2207-2218. https://doi.org/10.1056/NEJMoa2017699

101 Lenz HJ, Van Cutsem E, Luisa Limon M, et al. First-Line Nivolumab Plus Low-Dose Ipilimumab for Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: The Phase II CheckMate 142 Study. J Clin Oncol 2022;40:161-170. https://doi.org/10.1200/JCO.21.01015

102 André T, Lonardi S, Wong KYM, et al. Nivolumab plus low-dose ipilimumab in previously treated patients with microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: 4-year follow-up from CheckMate 142. Ann Oncol 2022;33:1052-1060. https://doi.org/10.1016/j.annonc.2022.06.008

103 Andre T, Elez E, Van Cutsem E, et al. Nivolumab (NIVO) plus ipilimumab (IPI) vs chemotherapy (chemo) as first-line (1L) treatment for microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC): First results of the CheckMate 8HW study. J Clin Oncol 2024;42:LBA768-LBA768. Https://doi.org/10.1200/JCO.2024.42.3_suppl.LBA768

104 Chalabi M, Verschoor YL, Tan PB, et al. Neoadjuvant Immunotherapy in Locally Advanced Mismatch Repair-Deficient Colon Cancer. N Engl J Med 2024;390:1949-1958. https://doi.org/10.1056/NEJMoa2400634

105 Chalabi M. Neoadjuvant Nivolumab Plus Ipilimumab Leads to a Pathological Response in a High Proportion of Patients with Locally Advanced dMMR Colon Cancer. Neoadjuvant Nivolumab Plus Ipilimumab Leads Pathol Response High Proportion Patients Local Adv DMMR Colon Cancer. Ann Oncol 35 suppl 2:1-72. Https://doi.org/10.1016/annonc/annonc1623

106 Hu H, Kang L, Zhang J, et al. Neoadjuvant PD-1 blockade with toripalimab, with or without celecoxib, in mismatch repair-deficient or microsatellite instability-high, locally advanced, colorectal cancer (PICC): a single-centre, parallel-group, non-comparative, randomised, phase 2 trial. Lancet Gastroenterol Hepatol 2022;7:38-48. https://doi.org/10.1016/S2468-1253(21)00348-4

107 Ludford K, Ho WJ, Thomas JV, et al. Neoadjuvant Pembrolizumab in Localized Microsatellite Instability High/Deficient Mismatch Repair Solid Tumors. J Clin Oncol 2023;41:2181-2190. https://doi.org/10.1200/JCO.22.01351

108 Shiu kk. Novel IO Approaches Demonstrate High pCR Rates in the Neoadjuvant Setting, Questioning the Need for Surgery in Certain Patients With CRC. Nov IO Approaches Demonstr High PCR Rates Neoadjuvant Setting Quest Need Surg Certain Patients CRC. J Clin Oncol 2024;42 suppl 17; abstr LBA3504.

109 de la Fouchardiere C. IMHOTEP Phase II trial of neoadjuvant pembrolizumab in dMMR/MSI tumors: Results of the colorectal cancer cohort. IMHOTEP Phase II Trial Neoadjuvant Pembrolizumab DMMRMSI Tumors Results Colorectal Cancer Cohort. Ann Oncol 2024;35 (suppl_2):S428-S481. Https://doi.org/10.1016/annonc/annonc1588

110 Glynne-Jones R, Wyrwicz L, Tiret E, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2017;28:22-40.

111 Cervantes A, Adam R, Roselló S, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 2023;34:10-32.

112 Cercek A, Dos Santos Fernandes G, Roxburgh CS, et al. Mismatch Repair-Deficient Rectal Cancer and Resistance to Neoadjuvant Chemotherapy. Clin Cancer Res 2020;26:3271-3279. https://doi.org/10.1158/1078-0432.CCR-19-3728

113 Cercek A, Lumish M, Sinopoli J, et al. PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. N Engl J Med 2022;386:2363-2376. https://doi.org/10.1056/NEJMoa2201445

114 Kasi PM, Jafari MD, Yeo H, et al. Neoadjuvant botensilimab plus balstilimab in resectable mismatch repair proficient and deficient colorectal cancer: NEST-1 clinical trial. J Clin Oncol 2024;42:117-117. https://doi.org/10.1038/s41388-023-02835-y

115 Luchini C, Brosens LAA, Wood LD, et al. Comprehensive characterisation of pancreatic ductal adenocarcinoma with microsatellite instability: histology, molecular pathology and clinical implications. Gut 2021;70:148-156. https://doi.org/10.1136/gutjnl-2020-320726

116 Luchini C, Scarpa A. Microsatellite instability in pancreatic and ampullary carcinomas: histology, molecular pathology, and

clinical implications. Hum Pathol 2023;132:176-182. https://doi.org/10.1016/j.humpath.2022.06.009

[117] Lawlor RT, Mattiolo P, Mafficini A, et al. Tumor Mutational Burden as a Potential Biomarker for Immunotherapy in Pancreatic Cancer: Systematic Review and Still-Open Questions. Cancers 2021;13:3119. https://doi.org/10.3390/cancers13133119

[118] Luchini C, Paolino G, Mattiolo P, P et al. KRAS wild-type pancreatic ductal adenocarcinoma: molecular pathology and therapeutic opportunities. J Exp Clin Cancer Res 2020;39:227. https://doi.org/10.1186/s13046-020-01732-6

[119] Simbolo M, Silvestris N, Malleo G, et al. Clinical and Genomic Characterization of Pancreatic Ductal Adenocarcinoma with Signet-Ring/Poorly Cohesive Cells. Mod Pathol 2023;36:100251. https://doi.org/10.1016/j.modpat.2023.100251

[120] Lupinacci RM, Goloudina A, Buhard O, et al. Prevalence of Microsatellite Instability in Intraductal Papillary Mucinous Neoplasms of the Pancreas. Gastroenterology 2018;154:1061-1065. https://doi.org/10.1053/j.gastro.2017.11.009

[121] Kastrinos F. Risk of Pancreatic Cancer in Families With Lynch Syndrome. JAMA 302(16):1790. https://doi.org/10.1001/jama.2009.1529

[122] Riazy M, Kalloger SE, Sheffield BS, et al. Mismatch repair status may predict response to adjuvant chemotherapy in resectable pancreatic ductal adenocarcinoma. Mod Pathol 2015;28:1383-1389. https://doi.org/10.1038/modpathol.2015.89

[123] Cloyd JM, Katz MHG, Wang H, et al. Clinical and Genetic Implications of DNA Mismatch Repair Deficiency in Patients With Pancreatic Ductal Adenocarcinoma. JAMA Surg 2017;152:1086. https://doi.org/10.1001/jamasurg.2017.2631

[124] Mukherji R, Debnath D, Hartley ML, et al. The Role of Immunotherapy in Pancreatic Cancer. Curr Oncol 2022;29:6864-6892. https://doi.org/10.3390/curroncol29100541

[125] Marabelle A, Le DT, Ascierto PA, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. J Clin Oncol 2020;38:1-10. https://doi.org/10.1200/JCO.19.02105

[126] Taïeb J, Sayah L, Heinrich K, et al. Efficacy of immune checkpoint inhibitors in microsatellite unstable/mismatch repair-deficient advanced pancreatic adenocarcinoma: an AGEO European Cohort. Eur J Cancer 2023;188:90-97. https://doi.org/10.1016/j.ejca.2023.04.012

[127] O'Connor CA, Harrold E, Lin D, et al. Lynch Syndrome and Somatic Mismatch Repair Variants in Pancreas Cancer. JAMA Oncol 2024, in press. https://doi.org/10.1001/jamaoncol.2024.3651

[128] Luchini C, Mafficini A, Chatterjee D, et al. Histo-molecular characterization of pancreatic cancer with microsatellite instability: intra-tumor heterogeneity, B2M inactivation, and the importance of metastatic sites. Virchows Arch 2022;480:1261-1268. https://doi.org/10.1007/s00428-021-03205-3

[129] Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) and Microsatellite Instability. JNCI J Natl Cancer Inst 2004;96:261-268. https://doi.org/10.1093/jnci/djh034

[130] Goeppert B, Roessler S, Renner M, et al. Mismatch repair deficiency is a rare but putative therapeutically relevant finding in non-liver fluke associated cholangiocarcinoma. Br J Cancer 2019;120:109-114. https://doi.org/10.1038/s41416-018-0199-2

[131] Eluri M, Danner De Armas A, Ross JS, et al. Clinical and mutational profiles of microsatellite instability (MSI-H) in intrahepatic cholangiocarcinoma (iCCA). J Clin Oncol 2023;41:607-607. Https://doi.org/10.1200/JCO.2023.41.4_suppl.607

[132] Suto T, Habano W, Sugai T, et al. Infrequent microsatellite instability in biliary tract cancer. J Surg Oncol 2001;76:121-126. https://doi.org/10.1002/1096-9098(200102)76:2<121::aid-jso1022>3.0.co;2-7

[133] Moy AP, Shahid M, Ferrone CR, et al. Microsatellite instability in gallbladder carcinoma. Virchows Arch 2015;466:393-402. https://doi.org/10.1007/s00428-015-1720-0

[134] Kai Y, Ikezawa K, Takada R, et al. Success rate of microsatellite instability examination and complete response with pembrolizumab in biliary tract cancer. JGH Open 2021;5:712-716. https://doi.org/10.1002/jgh3.12576

[135] Maruki Y, Ikezawa K, Sasaki M, et al. Clinical characteristics and the course of hepatobiliary and pancreatic malignant tumors with high microsatellite instability after pembrolizumab administration: A multicenter observational study. J Clin Oncol 2024;42:459-459. https://doi.org/10.1200/JCO.2024.42.3_suppl.459

[136] Silva VWK, Askan G, Daniel TD, et al. Biliary carcinomas: pathology and the role of DNA mismatch repair deficiency. Chin Clin Oncol 2016;5:62-62. https://doi.org/10.21037/cco.2016.10.04

[137] Agaram NP, Shia J, Tang LH, et al. DNA Mismatch Repair Deficiency in Ampullary Carcinoma: A Morphologic and Immunohistochemical Study of 54 Cases. Am J Clin Pathol 2010;133:772-780. https://doi.org/10.1309/AJCPGDDE8PLLDRCC

[138] Liang H, Zhu Y, Wu Y et al. Ampulla of Vater carcinoma: advancement in the relationships between histological subtypes, molecular features, and clinical outcomes. Front Oncol 2023;13:1135324. https://doi.org/10.3389/fonc.2023.1135324

[139] Harthimmer MR, Stolborg U, Pfeiffer P, et al. Mutational profiling and immunohistochemical analysis of a surgical series of ampullary carcinomas. J Clin Pathol 2019;72:762-770. https://doi.org/10.1136/jclinpath-2019-205912

[140] Xue Y, Balci S, Aydin Mericoz C, et al. Frequency and clinicopathologic associations of DNA mismatch repair protein deficiency in ampullary carcinoma: Routine testing is indicated. Cancer 2020;126:4788-4799. https://doi.org/10.1002/cncr.33135

[141] Zhu M, Jin Z, Hubbard JM. Management of Non-Colorectal Digestive Cancers with Microsatellite Instability. Cancers 2021;13:651. https://doi.org/10.3390/cancers13040651

[142] Barzi A, Zhou K, Wang S, et al. Etiology and Outcomes of Hepatocellular Carcinoma in an Ethnically Diverse Population: The Multiethnic Cohort. Cancers 2021;13:3476. https://doi.org/10.3390/cancers13143476

[143] Eso Y, Shimizu T, Takeda H, et al. Microsatellite instability and immune checkpoint inhibitors: toward precision medicine against gastrointestinal and hepatobiliary cancers. J Gastroenterol 2020;55:15-26. https://doi.org/10.1007/s00535-019-01620-7

[144] Chiappini F. Microsatellite instability mutator phenotype in hepatocellular carcinoma in non-alcoholic and non-virally infected normal livers. Carcinogenesis 2003;25:541-547. https://doi.org/10.1093/carcin/bgh035

[145] Sahnane N, Furlan D, Monti M, et al. Microsatellite unstable gastrointestinal neuroendocrine carcinomas: a new clinicopathologic entity. Endocr Relat Cancer 2015;22:35-45. https://doi.org/10.1530/ERC-14-0410

[146] Furlan D, Sahnane N, Mazzoni M, et al. Diagnostic utility of MS-MLPA in DNA methylation profiling of adenocarcinomas and neuroendocrine carcinomas of the colon-rectum. Virchows Arch Int J Pathol 2013;462:47-56. https://doi.org/10.1007/s00428-012-1348-2

[147] Arnason T, Sapp HL, Rayson D, et al. Loss of expression of DNA mismatch repair proteins is rare in pancreatic and small intestinal neuroendocrine tumors. Arch Pathol Lab Med 2011;135:1539-1544. https://doi.org/10.5858/arpa.2010-0560-OA

[148] Sorscher S, Saroya B. A molecularly confirmed neuroendocrine tumor resulting from Lynch Syndrome. J Gastrointest Oncol 2013;4:95-96. https://doi.org/10.3978/j.issn.2078-6891.2012.040