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Review Article

Mismatch repair-based stratification for immune checkpoint blockade therapy

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Abstract: Mismatch repair (MMR) plays a key role in maintaining genomic stability. Mismatch repair deficiency (MMR-D) causes a molecular feature of microsatellite instability (MSI) and contributes to the development of human cancers and genetic diseases with cancer predisposition such as Lynch syndrome. Recent studies have shown that immune checkpoint blockade therapy has a promising response in MMR-D cancers regardless of the tissue of origin. Being able to identify patients with MMR-D cancers is an important challenge in clinical practice. Although immunohistochemistry (IHC) and polymerase chain reaction (PCR)-based MSI analysis combined with a subsequent MMR gene test are used as the standard of care in the clinical setting to identify patients with MMR-D cancers, these methods have limitations as a pan-cancer testing strategy. Next-generation sequencing (NGS) has developed and matured as a clinical option and NGS has advantages for use as a novel testing strategy for MMR-D detection. In this review, we describe the genetic basis of MMR-D, current diagnostic algorithms in the clinical management of MMR-D, the novel NGS approach, and potential detection strategy of anti-cancer immunity biomarkers of MMR-D.

Keywords: Mismatch repair, microsatellite instability, immune checkpoint blockade, programmed cell death protein 1, next-generation sequencing, gene signature

Introduction

The approval in May 2017 of immune checkpoint blockade therapy for treating mismatch repair-deficient (MMR-D) cancers regardless of cancer origin is undoubtedly one of this decade's breakthroughs in cancer treatment. Le and colleagues reported that programmed cell death protein 1 (PD-1) blockade with pembrolizumab achieved responses in 53% of patients with MMR-D cancers [1]; thus, MMR-D may be a biomarker for response to PD-1 blockade in patients with diverse solid tumor types. However, identifying which patients are likely to respond to this cutting-edge therapy remains a challenge to physicians [2]. Questions emerge in clinical practice as to which patients should be given this promising drug treatment and how to identify MMR-D patients with current testing strategies.

The purpose of this review is to describe the current testing strategies for MMR-D, as well as a novel strategy, next-generation sequencing (NGS), and delineate their advantages and limitations in clinical application. We will briefly introduce MMR-D and its relationship to Lynch syndrome, as well as the basic mechanism of MMR-D. Then we will discuss the current diagnostic methods for MMR-D, including the standard-of-care methods and new NGS approaches. Finally, we will describe potential detection strategies of anti-cancer immunity biomarkers of MMR-D. This review is to summarize the current usage of MMR-D detection strategies, meanwhile, to designate the future development of MMR-D detection strategies in the era of immune checkpoint blockade therapy.

What is MMR?

MMR is a highly conserved biological DNA repair pathway in mammalian cells and plays a key role in maintaining genomic stability. Its major function is correcting single-base nucleotide mismatches (insertions or deletions) that

occur during DNA replication and recombination, thereby preventing the mutations from being passed to dividing cells [3]. MMR's other functions include mediating DNA damage signaling and participating in class-switch recombination processes [3, 4].

Three processes are successively involved in the MMR mechanism: recognition, excision, and resynthesis [3, 5]. The major components in MMR include human homologs of MutS, MutL, EXO1, DNA binding protein RPA, proliferating cellular nuclear antigen (PCNA), DNA polymerase delta, and DNA ligase I [3]. Protein MSH2 and MSH6 forms heterodimer MutSα, that performs the functions of recognition of DNA mismatch and small insertion/deletion loops (IDLs), while protein MSH2 and MSH3 forms heterodimer MutSβ, recognizing larger IDLs. Protein MLH1 and PMS2 forms heterodimer MutLα that functions as a regulator of termination of mismatch-provoked excision, as well as plays a critical role in 3' nick-directed MMR involving EXO1 [3, 6]. RPA is involved in all stages of MMR process, includes binding to nicked heteroduplex DNA, stimulating mismatch-provoked excision, facilitating DNA resynthesis [3]. PCNA interacts with MSH2 and MLH1 and plays roles in the initiation and resynthesis steps of MMR [3]. DNA polymerase delta and DNA ligase I participate in the resynthesis process of the excised DNA and ligation [3, 5]. Reconstitution of the MMR process from recombinant proteins was described by Jiricny [4] and Zhang et al [7].

Mechanisms and manifestations of MMR-D

MMR plays an important role in correcting errors occurring in DNA replication; defects in MMR lead to increased acquisition of mutations, primarily in the form of microsatellites instability (MSI), or alterations in microsatellites, which is a molecular tumor phenotype resulting from the gain or loss of nucleotides from microsatellite tracts [8, 9]. The direct link between MMR-D, MSI, and Lynch syndrome led to investigation of the molecular changes that cause MMR-D. Lynch syndrome, one of the first recognized and most relevant MMR-related cancer-prone syndromes, is defined as the predisposition to a spectrum of cancers, especially colorectal cancer (CRC), that exhibit impaired MMR activity and typically manifest MSI [9].

Soon after MSI was first identified in Lynch syndrome-associated tumors and shown to be due to MMR-D in 1993 [10, 11], the genetic causes of Lynch syndrome were firmly established as germline mutations within four key MMR genes-*MLH1, MSH2, MSH6, or PMS2*-which result in loss of function of the encoded proteins. Alternative mechanisms of MMR-D are heterozygous deletion of epithelial cell adhesion molecule gene (*EPCAM*) that silences MSH2 expression, monoallelic *MLH1* epimutation, or biallelic mutation in any of the four genes [12].

Knudson's two-hit model of carcinogenesis [13] underlies the presence of MSI and the several-hundred-fold increase in mutation frequency observed in MMR-D cells [12]. Overall, MMR-D has been identified in a wide variety of solid tumors, including colorectal, endometrial, ovarian, gastric, pancreatic, ureteral and renal pelvic, brain (usually glioblastoma), and small intestinal cancers [14]. Therefore, a pan-cancer testing strategy is needed to identify patients who are harboring MMR-D or MSI and might benefit from immune checkpoint blockade therapy.

Standard-of-care MMR-D detection strategies

Over the past decades of research on Lynch syndrome, the diagnosis and detection of Lynch syndrome as well as MMR-D and MSI have been standardized with the development of a variety of detection techniques. Two methods are considered the gold standard for detection: IHC and MSI PCR. Subsequent detection strategies such as MMR gene testing, *MLH1* methylation testing, *BRAF* mutation analysis, are performed depending on different clinical situations. The universal screening defining as testing all newly diagnosed colorectal cancers is recommended to determine the colorectal patient population of who should go through the MMR-D or MSI screening tools.

IHC

IHC is the preferred primary screening test for MMR-D and MSI because it is broadly available, less expensive than other methods, and can be followed by targeted confirmatory germline sequencing, therefore saving unnecessary analysis of other MMR genes [15, 16]. For testing of the four MMR proteins (MLH1, MSH2,

MSH6, PMS2) to predict MSI, IHC has a sensitivity about 93% and nearly perfect specificity [15, 17]. On the other hand, in some cases, IHC may also miss MMR-D patients; in the scenario of some missense MMR mutations, the corresponding MMR protein remains intact but is functionally inactivated, resulting in a false-positive MMR result [18]. In addition, cases with *MLH1* promoter methylation may show false-positive nuclear staining for MLH1 protein [17]. Conversely, IHC has a false-negative rate of 5-10% [19, 20]. Because MSI testing has a similar false-negative rate, the two methods are complementary to one another. Thus, MSI PCR is regarded as a parallel method for confirming IHC findings.

MSI PCR testing

Genotyping of microsatellites by using PCR is another standard method of identifying the MSI [15, 20]. The 2004 Bethesda Guidelines for MSI testing recommend a National Cancer Institute-approved standard panel of 5 microsatellites, which is composed of 2 mononucleotidic repeats (BAT-25 and BAT-26) and 3 dinucleotidic repeats (D2S123, D5S346, and D17S250) [20]. It is generally agreed that MSI testing and MMR IHC analysis are almost equally valuable in the detection of Lynch syndrome [17]; they overall have a roughly 94% concordance rate in colorectal and endometrial cancer [21]. However, MSI testing as a single test has been shown to miss a proportion of patients, particularly those harboring *MSH6* and *MSH2* mutations, which account for the majority of Lynch syndrome endometrial cancers [21, 22]. Compared to MSI PCR, IHC has clear advantages as the primary screening modality, because MSI PCR does not enable specifying a target gene on confirmatory germline testing. Therefore, reincorporating MSI testing into universal screening algorithms is now recommended for cases with strong clinical suspicion of MSI but intact MMR protein expression and for confirmation of IHC results [21, 22].

MMR gene testing and MLH1 methylation testing

For CRC, tumors with normal results for either the IHC or MSI PCR test will need no further testing because they are regarded as MMR proficient and not indicative of Lynch syndrome. For tumors that show IHC abnormality, to further confirm the sporadic or Lynch-related tumors, MMR germline gene testing or *MLH1* methylation testing are recommended as the subsequent screening processes. For MSH2, MSH6, or PMS2 abnormality identified by IHC, corresponding gene testing should be performed. In addition, heterozygous deletions of the terminal end of the adjacent gene, *EPCAM*, leads to epigenetic silencing of *MSH2* in some Lynch syndrome cases, thus screening for *EPCAM* deletions is a routine genetic testing for Lynch syndrome as well [12, 17, 23].

For MLH1/PMS2 IHC abnormalities, *MLH1* methylation testing should serve as the next procedure. *MLH1* epimutation is the underlying defect in the vast majority of sporadic MSI CRCs manifesting MLH1 abnormality and accounts for up to 10% of Lynch syndrome cases that are negative for MMR gene mutation [12]. As a result of *MLH1* hypermethylation, the *BRAF* V600E hotspot mutation is also recommended to be tested because it is associated with sporadic MSI-high (MSI-H) CRCs [17, 24]. If *MLH1* methylation and *BRAF* mutation are both positive, it indicates sporadic MSI without further testing. In cases one of these two criteria exists and if Lynch syndrome or MMR-D is suspected, germline mutation testing is recommended [17]. Notably, *BRAF* mutation is uncommon in endometrial cancer; thus, BRAF testing cannot distinguish endometrial cancers with underlying sporadic MMR-D or Lynch syndrome [17, 25]. Therefore, the current standards of care of MMR-D testing strategies in CRC and endometrial cancer remain in question as to their optimal use in different cancer types.

Universal screening

Discussion of algorithms for whom should be screened for MMR-D and MSI is ongoing. Both the Amsterdam criteria (relying solely on family history to diagnose Lynch syndrome) [26] and the Bethesda Guidelines (combining MSI testing with family history and clinical factors) [20] fail to identify all Lynch syndrome mutation carriers; for example, one study found the Bethesda Guidelines missed approximately 28% of carriers [27]. Currently, the NCCN guidelines recommend universal testing for MMR-D and MSI in all colorectal cancers, or selective testing of those diagnosed younger than age

Table 1. Summary of NGS approaches applied to study MSI or MMR-D in solid cancers in the past 5 years

Year	Cancer type	No. of patients	No. positive for MMR-D/MSI	Sequencing approach	Platform	Sensitivity	Specificity	Ref
2015	CRC	50	34%	TS	Illumina MiSeq	100%	100%	[31]
2015	CRC	142	20.4%	TS by AmpliSeq Cancer Hotspot Panel	Personal Genome Machine	ND	ND	[63]
2015	CRC	78	ND	ES, TS	MSIplus	97%	100%	[40]
2016	CRC	224	13%	ES, selective introns for 410-gene panel	MSK-IMPACT assay	100%	100%	[35]
2016	CRC	243	11.7%	ES, RNAseq	Illumina HiSeq 2500	91-92%	98-100%	[64]
2017	CRC	138	1.4%	ES, introns	Foundation One (Foundation Medicine)	ND	ND	[65]
2017	CRC	91	ND	TS by ColonCore NGS panel	MSI-ColonCore	97.9%	100%	[36]
2017	CRC	68	48.5%	TS by 111 loci smMIP panel	Illumina NextSeq 500	100%	100%	[66]
	Prostate	33	33.3%			100%	100%	
	EC	43	55.8%			95.8%	100%	
2014	GC	295	22%	WGS, RNA sequencing	Six platforms	ND	ND	[67]
2018	COUP	389	1.8%	592-gene panel	Illumina NextSeq	ND	ND	[68]
2018	Pancreatic cancer	833	0.8%	ES, selective introns for 468-gene panel	MSK-IMPACT assay	ND	ND	[69]
2018	Prostate cancer	91	29.7%	TS	mSINGS, MSIplus, large-panel NGS	96.6% (MSIplus)	100% (MSIplus)	[41]
						93.1% (large-panel NGS)	98.4% (large-panel NGS)	
2014	EC	242	28.9%	ES	MSIsensor	ND	ND	[30]
2014	BC	656	ND	ES	NA	88.4%	77.1%	[70]
2017	BC	640	1.7%	WGS	Illumina GAIIx, HiSeq 2000, or 2500	ND	ND	[48]
2013	Across cancer types	551	5.8%	TS	NA	ND	ND	[71]
2014	Across cancer types	324	ND	ES, TGS	mSINGS, ColoSeq, UW-OncoPlex	97.8%	98.32%	[28]
2014	Across cancer types	ND	ND	WGS	Complete Genomics Illumina short-read sequencing	98%	99%	[72]
2016	23 cancer types	7197	ND	WGS, ES	Sputnik algorithm	ND	ND	[47]
2016	18 cancer types	ND	EC 30% CRC 19% GC 19%	ES	MISA, mSINGS, MOSAIC classifier	95.8%	97.6%	[9]
2017	6 cancer types	458	ND	WES	MANTIS	97.18%	99.68%	[29]
2018	26 cancer types	11348	3%	592-gene NGS panel	Illumina NextSeq	95.8% (compared to PCR)	99.4% (compared to PCR)	[34]
						87.1% (compared to IHC)	99.6% (compared to IHC)	

Note: BC, Breast cancer; CRC, Colorectal cancer; GC, Gastric cancer; EC, Endometrial cancer; COUP, Cancer of unknown primary; WGS, Whole-genome sequencing; ES, Exome sequencing; TS, Targeted sequencing; NA, not applicable; ND, not determined.

70 and older patients who meet the Bethesda criteria or in those with endometrial cancer younger than 50 years old [14, 17, 19]. In fact, as more solid data emerge showing a promising effect of immune checkpoint blockade therapy targeted to MMR-D patients regardless of their tumors' origin, universal screening may have the potential to be carried out in a broader population of patients with advanced cancers who currently lack chemotherapy or targeted therapy options and are searching for novel treatment opportunities. However, the cancer specificity of this standard of care for MMR-D detection strategies may become a main obstacle as to apply it across the different cancer types besides Lynch syndrome-related cancers.

A new detection approach: next-generation DNA sequencing

With the development and maturation of next-generation DNA sequencing (NGS), this technology is emerging as a new pan-cancer approach for MSI testing. NGS is a massively parallel or deep DNA sequencing technology that has been widely used in human genomic research. RNA sequencing, whole-genome sequencing, whole-exome sequencing, or targeted sequencing assays can be employed in cancer research or as clinical diagnostic methods [28]. With different platforms (MANTis, MSIsensor, mSING, MIseq, Illumina, etc), many studies have conducted MSI genotyping in colorectal, gastric, endometrial, and other cancers using both blood DNA samples and formalin-fixed, paraffin-embedded samples [28-33]. Studies have shown that NGS is 95.8-100% concordant with the MSI PCR-based method [31, 34]. **Table 1** summarizes the NGS-based studies of MSI and MMR-D in solid cancers that have been reported in the past 5 years searched by the Pubmed database.

Major advantages of NGS

NGS provides a pan-cancer approach to MMR-D/MSI testing and provides a highly informative full mutational signature as an output; these benefits are described in detail in the next subsections. In addition, the NGS technology has several other advantages over MSI screening testing. First, it can detect point mutations and other sequence variants (such as single nucleotide variations and copy number variations) and can yield gene signatures for targeted therapeutics as well as identify the mutation load for immunotherapy. Second, it allows a large number of genes to be sequenced for each patient within a short period of time and thus highly increasing the efficiency of tests [28]. Third, the initial assessment for the MMR protein via either IHC or MSI PCR analysis is not needed by the use of a multi-gene tumor panel [35]. Fourth, MSI assessment and mutation detection are combined into the same NGS process, this can decrease the demand for tissue samples [36], and in some cases, a matched germline control from the same individual is not even needed, thus simplifying sample collection in the clinic.

Benefit of a pan-cancer MMR-D and MSI detection

One of the most important benefits of NGS testing is its lack of specificity to tumor site and tumor type. To date, the MSI PCR testing method (the 5-marker Bethesda panel) has traditionally had the highest clinical relevance in Lynch syndrome-related cancers, such as CRC, endometrial cancer [17], and gastric cancer [37]. Its sensitivity and specificity have been shown reliable in these cancer types through decades of research on Lynch syndrome. However, a small set of loci in MSI-PCR testing panel were selected based on markers from CRC, potentially excluding loci that would predict other cancer types [29, 38]. For MMR-D and MSI can predict the effect of immune checkpoint blockade regardless of primary tumor site, the US Food and Drug Administration (FDA) granted accelerated approval to anti-PD-1 antibody for patients with unresectable or metastatic, MSI-H or MMR-D solid tumors that have progressed on prior therapy and have no satisfactory treatment options [39]. Thus, studies are needed to confirm and validate MSI PCR's sensitivity and specificity across cancer types other than CRC and endometrial cancer.

In contrast, a study by Hempelmann and colleagues revealed that two NGS MSI-detection methods, MSIplus [40] and MSI by Large Panel NGS [28], both had higher sensitivity and specificity than the 5-marker Bethesda panel (MSI-PCR) in colorectal cancer and had higher sensitivity and similar specificity in prostate cancer as well [41]. In addition, the NGS method has been intensively studied across cancer types,

demonstrating its wide usage spectrum in solid tumors [29, 34]. In Vanderwalde and colleagues' study [34], MSI was measured by NGS through counting insertions or deletions of 2-5 nucleotides in specific areas of the genome with broader coverage of microsatellites, demonstrating good performance compared with MSI PCR testing across 26 cancer types. Therefore, NGS analysis as a pan-cancer MSI testing method has been technically validated across different cancer types.

Benefit of a gene mutational signature

NGS can also provide more elaborate genomic information from each sample's readout, including MSI variant events and a mutational signature, than is obtained with the current standard testing methods. MMR-D enhances the mutation frequency in cancer cells, accumulates downstream genetic mutations, and increases the chances of mutations in important oncogenes or tumor suppressor genes, forming a specific mutational signature for each tumor. Similarly, in lung cancer and melanoma, the mutational signatures related to smoking or UV light, respectively, influence both mutational and immune profiles of tumors, and thereby can predict the immune response to immune checkpoint blockade [42-44]. We expect that the NGS method can provide the most reproducible immune-predictive signatures for MMR-D tumors by combining both MSI variants and mutational signatures. In addition, a multigene somatic genomic profiling NGS study for Lynch syndrome-associated CRC tumors indicated that the mutational signature may help to shed light on the inherent biologic pathogenesis among MMR-D tumors [35].

The Cancer Genome Atlas (TCGA) colon cancer study showed that hundreds to thousands of somatic point mutations are accumulated in MMR-deficient tumors compared with MMR-proficient tumors [12, 45]. Currently there are four mutational signatures associated with MMR-D cataloged in the COSMIC database [46]. Hause and colleagues [9] examined 5930 cancer exomes from 18 cancer types by using NGS, constructed a genomic classifier for MSI, and identified a specific instability signature without regard to cancer types. They utilized the most informative and independent classification features-average gain of novel microsatellite alleles and locus instability within DEFB105A/B, created a weighted-tree classifier (MOSAIC) for predicting MSI status, which showed concordance with MSI PCR testing. They also summarized the most significant genes with MSI-H cancers and illustrated the utility of NGS MSI analysis data as a primary approach for identifying cancer-driving mutations [9].

Likewise, Cortes-Ciriano and colleagues [47] utilized the whole-exome and whole-genome sequencing data from TCGA across 23 cancer types to create a predictive model for the MSI phenotype. The highlight of that study is that it ranked the frequency of frameshift MSI across tumor types and generated a cancer-type-specific frameshift MSI loci catalogue, thereby enabling a random forest classification model for MSI detection incorporating with a conformal prediction pipeline. In addition, the study also created a mutational signature analysis for MMR-D and uncovered new genes showing predictive power for MSI-H status [47]. Therefore, NGS MSI-detection methods, such as the MOSAIC or the random forest classification model may serve as good strategies for pan-cancer MSI determination and MSI-specific mutational signature exploration.

Further, mutational signatures can conversely be developed as a strategy to distinguish MMR-D from pool sequencing data. Davies and colleagues [48] utilized mutational signatures known as substitution signatures, which are imprints of the mutagenic processes associated with MMR-D, to identify MMR-D breast tumors from a whole-genome sequencing dataset; they successfully identified the 11 MMR-D patients out of 640 patients and found that they had highly distinctive whole-genome profiles. This study suggested that genomic signatures reflect the direct pathophysiology of MMR abrogation and could outperform current biomarkers of MMR-D [48]. Similarly, Tian and colleagues [49] developed and validated a 64-gene MSI signature identifying MSI CRC patients. This signature could be linked to a deficient MMR phenotype and translated to a diagnostic microarray technically and clinically.

Limitations

Nevertheless, MSI NGS testing also has limitations. First, the availability of a larger cohort with whole-genome sequencing data requires systematic bioinformatics support to pipeline,

delineate, analyze, and interpret the raw data, support that is not available in all clinical laboratories. Second, NGS has become a key technology in the basic science setting, but its rapid development as an established tool in translational research raises many concrete questions about results interpretation and patient counseling. Clinical guidelines for researchers, pathologists, genomic counselors, and physicians are needed. Third, with the production of increased amounts of sequencing data, data storage and data confidentiality will become important problems that can't be neglected.

Anti-cancer immunity biomarkers promoted by MMR-D

More and more evidence indicates that MMR-D induces hyper-cancer immunity leading to the promising effect of immune checkpoint blockade. Intensive studies have suggested that the response to immune checkpoint blockade is highly related to mutation-associated neoantigens (MANAs) and response of specific T lymphocyte cells [50, 51]. Thus, combining related anti-cancer immunity biomarkers with MMR-D detection is a prospective direction for predicting the efficacy of immune checkpoint blockade and may facilitate precise identification of candidate patients.

MMR-D triggers hypermutation status and neoantigen generation

Le and colleagues' genomic analysis of whole-exome sequences [1, 52] revealed a mean of 1782 somatic mutations per tumor in MMR-D neoplasms, compared to 73 mutations per tumor in MMR-proficient neoplasms. Germano et al's exome sequencing data of MLH1-knockout cancer cells [53] indicated that MMR-D presented an augmented mutation burden resulting in increased neoantigens, which are calculated from the mutant peptide RNA sequencing data. Evidence suggests that MMR-D triggers hypermutation status and generates a very large number of MANAs that might be recognized by the immune system [1].

Likewise, other types of tumors (e.g., melanoma, lung cancer) characterized by high mutation burden were found to have a high neoantigenic targets of tumor-specific immune response [50]. Rizvi's study also confirmed that the mutation burden as well as smoking molecular signature may perform as additional biomarkers to predict response to immune checkpoint blockade in lung cancer [54]. Large-scale analyses of neoantigen-specific T cell reactivity carried out in melanoma patients provide evidence as to how the immune system recognizes MANAs to control malignancies [50]. Therefore, hypermutation status and neoantigen generation on the one hand are the consequences of MMR-D or UV or smoking exposure but on the other hand trigger hyper immunity and predict the effect of immune checkpoint blockade.

MMR-D tumors harbor functional MANA-specific cytotoxic T cells

Two decades ago, studies showed that MSI high colon cancer tissue carried significantly higher numbers of cytotoxic lymphocytes infiltrating within neoplastic epithelial structures compared with MSI low colon cancer tissues [55]. MSI was considered the major determinant of the presence of activated cytotoxic intraepithelial lymphocytes [55] and tumor-infiltrating lymphocytes, and their molecular subsets may be predictive markers for MSI [56]. In 2015, Llosa and colleagues [57] found that MMR-D CRC displayed high infiltration with activated CD8+ cytotoxic T lymphocytes as well as activated Th1 cells and that MSI CRC tumors selectively demonstrated highly upregulated expression of multiple immune checkpoints, including PD-1, PD-L1, CTLA-4, LAG-3 and IDO, which suggested that the immune environment of MSI CRC tumors may link to blockade of specific checkpoints [57]. Recently, a whole-genome transcriptomic analysis found that premalignant lesions in patients with Lynch syndrome displayed a distinct immune profile characterized by CD4 T cells and proinflammatory and checkpoint molecules [58]. Lal and colleagues [59, 60] showed that MSI-H CRC cancer is associated with high-level expression of a coordinated immune response cluster (CIRC) characterized by T helper cells and immune genes together.

Checkpoint blockade boosts cytotoxic T cell activity in MMR-D tumors

Immunotherapies boost the ability of endogenous T cells to destroy cancer cells, therapeutic efficacy in a variety of human malignancies in

basic and clinical research have demonstrated this hypothesis.

Germano and colleagues [53] genetically inactivated MLH1 in colorectal, breast, and pancreatic mouse cancer cells. The mutational burden was found increased with the inactivation of MMR, and persistent renewal of neoantigens was found in vitro and in vivo. Furthermore, when transplanted tumors were treated with anti-PD-1 and/or anti-CTLA-4, the growth of MMR-D tumors was markedly impaired compared with that of MMR-proficient tumors, and increased levels of cytotoxic CD8+ T cells were found in MMR-D tumors. These results strongly suggest that inactivation of MMR triggers neoantigen generation and impairs tumor growth; this effect could be further boosted by checkpoint blockade therapy [53]. Likewise, in Gubin and colleagues' in vivo study of mice bearing sarcomas [61], they found mutant tumor-antigen-specific T cells are reactivated following treatment with anti-PD-1 and/or anti-CTLA-4, revealing that checkpoint blockade cancer immunotherapy targets tumor-specific mutant antigens.

Based on pretherapy T cell infiltrates and response to PD-1 blockade in cancers, cytotoxic T cell activity appears to play a central role in cancer immunotherapy [50, 62]. Le and colleagues [1] performed deep sequencing of T cell receptor (TCR) CDR3 regions (TCRseq) in MMR-D tumors and peripheral blood from patients who were responding to immunotherapy to assess T cell clonal representation, the study showed that the clones peaked rapidly after PD-1 blockade. The investigators also proved that these clones are specific for mutated peptides and these MANA-related TCRs peaked soon after PD-1 treatment and corresponded with tumor marker and radiographic response. This study gives strong evidence that in MMR-D tumors harboring functional MANA-specific cytotoxic T cells, which play a critical role in response to PD-1 blockade and kill the cancer cells [1].

Conclusion and perspective

In summary, the stand of care detection strategies for MMR-D and MSI include IHC, MSI PCR testing, genetic MMR testing, methylation testing, etc. Universal screening or selective screening are recommended in clinic setting for CRC, endometrial cancer patients. However, in the era of immune checkpoint blockade therapy, among different cancer types, a pan-cancer detection strategy is currently needed. In addition, more valued gene information consisting of gene variants and gene signature, with combination of anti-cancer immunity profiles is needed to furthest predict the efficiency of immune checkpoint blockade. Thus, a few key questions may guide further research.

What are new molecular determinants/mechanisms in MMR-D?

MMR-D promotes cancer immunity and inspires the immune system to fight against cancer cells. Besides MMR gene mutation and epigenetic regulation, new immunity biomarkers such as MANA and functional MANA-specific cytotoxic T cells, as well as cytotoxic T cell activity, can also serve as biomarkers of MMR-D and determinants of immune checkpoint blockade efficiency.

In recent years, with the NGS research on MMR-D tumors, e.g., using the MOSAIC or the random forest classification model, the novel gene profiles and gene signature of MMR-D have shown us a broad portrait of MMR-D. This information helps to illustrate the MMR-D genomic alteration spectrum and explore the mutational signature across tumor types. These advances will improve our understanding of the genomic drivers and consequences of MMR-D and MSI.

Can we identify tumors with MMR-D among different cancer types?

Currently, standard-of-care testing methods, can only identify selected patients with MMR-D in limited cancer types. Nevertheless, with the FDA approval of immune checkpoint blockade therapy in MMR-D solid tumors, MMR-D detection strategies are in high demand across a variety of tumor types. In fact, NGS testing meets the requirement for pan-cancer testing, even though the specific sequencing type, pipeline type, and bioinformatics support still need to be comprehensively considered and optimized. Furthermore, NGS testing can provide mutation data and gene signature for individual cancer patients who may benefit from immune checkpoint blockade therapy. Therefore, NGS has great potential as a promising method for

MMR-D or MSI detection in the near future, and with the combination of immune profiles, to help us precisely identifying candidates for immune checkpoint blockade therapy.

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Disclosure of conflict of interest

None.

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