Published online 13 June 2023

NAR Cancer, 2023, Vol. 5, No. 3 **1**
https://doi.org/10.1093/narcan/zcad031

Critical Reviews and Perspectives

DNA mismatch repair in cancer immunotherapy

Junhong Guan [1,*] **and Guo-Min Li** [2,*]

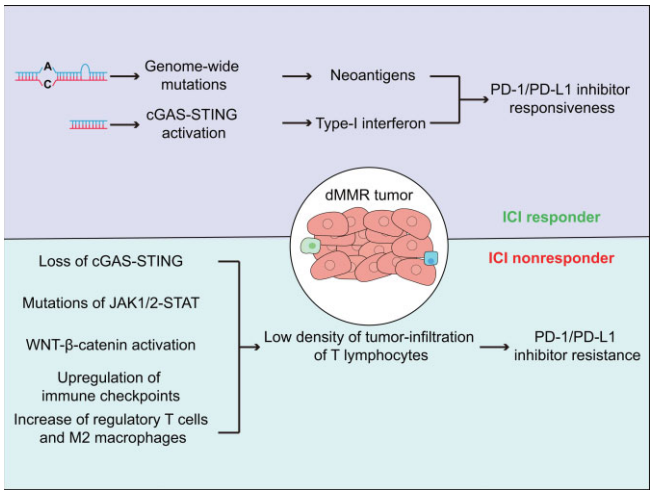
[1]Cuiying Biomedical Research Center, Lanzhou University Second Hospital, Lanzhou, Gansu 730030, China and
[2]Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Received March 17, 2023; Revised May 08, 2023; Editorial Decision May 24, 2023; Accepted June 07, 2023

ABSTRACT

Tumors defective in DNA mismatch repair (dMMR) exhibit microsatellite instability (MSI). Currently, patients with dMMR tumors are benefitted from anti-PD-1/PDL1-based immune checkpoint inhibitor (ICI) therapy. Over the past several years, great progress has been made in understanding the mechanisms by which dMMR tumors respond to ICI, including the identification of mutator phenotype-generated neoantigens, cytosolic DNA-mediated activation of the cGAS-STING pathway, type-I interferon signaling and high tumor-infiltration of lymphocytes in dMMR tumors. Although ICI therapy shows great clinical benefits, ∼50% of dMMR tumors are eventually not responsive. Here we review the discovery, development and molecular basis of dMMR-mediated immunotherapy, as well as tumor resistant problems and potential therapeutic interventions to overcome the resistance.

GRAPHICAL ABSTRACT



INTRODUCTION

DNA mismatch repair (MMR) is an important genome-maintenance system by specifically removing misincorporated nucleotides in the newly synthesized strand during DNA replication. Loss of the MMR function, due to either mutations or promoter hypermethylation of MMR genes, leads to a mutator phenotype and development of various cancers displaying frequent alterations in simple repetitive DNA sequences (1–5), a phenomenon called microsatellite instability (MSI). In human cells, the minimal activities essential for MMR include MutSα (MSH2-MSH6), MutSβ (MSH2-MSH3), MutLα (MLH1-PMS2), proliferating cell nuclear antigen (PCNA), exonuclease 1 (Exo1), replication protein A (RPA), replication factor C (RFC), DNA polymerase δ, and DNA ligase I (6,7).

MMR is nick-directed, and specifically targeted to the newly synthesized DNA strand (8,9). The MMR reaction is carried out in three phases: initiation, excision, and resynthesis (Figure 1). The initiation phase involves mismatch

*To whom correspondence should be addressed. Tel: +86 0931 5190 920; Email: ery_guanjh@lzu.edu.cn
Correspondence may also be addressed to Guo-Min Li. Tel: +1 214 648 9955; Email: Guo-Min.Li@UTSouthwestern.edu

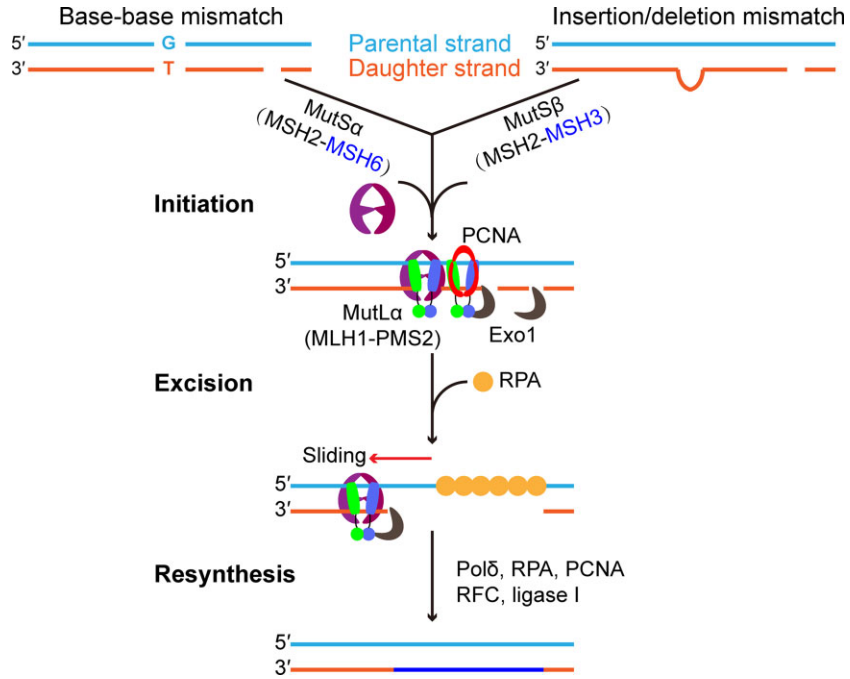


Figure 1. MMR reaction. MMR is targeted to the newly-synthesized (nicked) strand and can be divided into three steps: initiation, excision, and resynthesis. The initiation reaction involves mismatch recognition by MutSα or MutSβ and subsequent interactions/communications among MutSα/β, MutLα and PCNA, which results in Exo1 recruitment at a nick generated by MutLα. In the excision phase, the nicked DNA strand is excised from the MutL-generated nick up to and beyond the mismatch in a manner depending on MutSα/β, MutLα, and RPA. Once the Exo1-catalyzed excision approches the mismatch, MutSα or the MutSα-MutLα complex slides away from the mismatch, providing Exo1 the opportunity to excise the mismatch. The excision is then terminated by the interaction between MutLα and Exo1. The DNA gap is filled by DNA polymerase δ in concerted reactions with RFC, PCNA and RPA, and the nick is ligated by ligase I.

recognition by MutSα or MutSβ, assembly of the initiation complex containing MutSα (or MutSβ), MutLα and PCNA, localization of the strand discrimination signal (i.e. a single strand nick) that can be several hundred base pairs away from the mismatch. This large protein-DNA complex activates the MutLα endonuclease activity (10) to make a single strand break ∼20 bp away 5′ to the mismatch on the nicked strand (11). Exo1 is then recruited to the MutLα-created nick to initiate the excision reaction (Figure 1). During the excision phase, Exo1 excises the nascent DNA strand from the MutLα-created nick toward the mismatch to generate a single-strand DNA gap, which is protected by RPA from nuclease digestion (7,12). Once the Exo1-catalyzed excision reaches the MutSα- or MutSα-MutLα-occupied mismatch, MutSα or the MutSα-MutLα complex slides away from the mismatch, yielding the right of way to Exo1 so that the mispaired base can be excised (11). Upon mismatch removal, the Exo1-catalyzed excision is terminated by MutLα through the physical interaction between these two proteins (7), as depleting MutLα or disrupting the MutLα-Exo1 interaction leads to uncontrolled Exo1 excision (7,13). During the resynthesis phase of MMR, DNA polymerase δ fills in the single-strand DNA gap left by DNA excision, in a concerted reaction that requires RFC, PCNA and RPA, followed by DNA ligase I-catalyzed nick ligation (Figure 1).

The mutator phenotype renders MMR-deficient cells highly resistant to many commonly used chemotherapeutic drugs such as cisplatin and alkylating agents (14,15). This is because an active MMR system recognizes chemically-modified DNA lesions located on the template DNA strand, but its targeted repair on the newly synthesized strand fails to remove the chemical adducts, which triggers a new round of lesion recognition and processing (16). This results in a futile repair cycle that can provoke apoptosis (Figure 2A) (17,18). Alternatively, binding of DNA lesions by MutSα can directly trigger ATR- or ATM-mediated apoptosis (Figure 2A) (17). However, MMR deficient cells have lost their ability to recognize and/or process chemically induced lesions, and therefore fail to induce apoptosis. Thus, they become tolerant to chemotherapeutic drugs. This resistant feature posts great therapeutical challenges to patients defective in MMR (dMMR). Strikingly, recent studies have shown that dMMR (also referred to as high frequency of MSI, MSI-H) tumors, regardless of tumor types, are highly responsive to immune checkpoint inhibitors (ICIs), particularly antibodies against the programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) (19,20). Therefore, immunotherapy has become an important direction for cancer treatment, being regarded as the fourth leading cancer treatment technology after surgery, radiotherapy, and chemotherapy. In this review, we will focus on the molecular mechanisms by which dMMR facilitates immunotherapy.

MMR-deficient tumors display a mutator phenotype

It is well established that dMMR induces an elevated mutation frequency. The mutations include various base-base substitutions and small insertions/deletions in any region

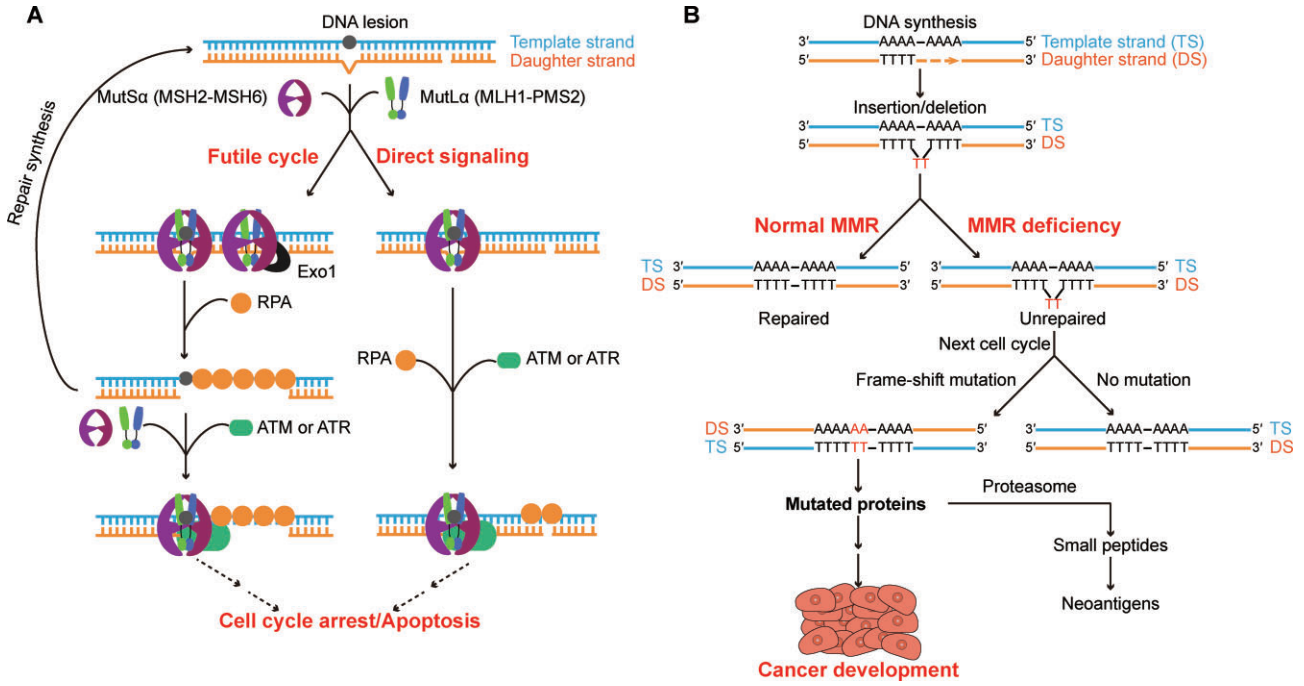


Figure 2. Models for MMR-induced DNA damage response and MMR deficiency-induced tumorigenesis. (**A**) Schematic model for MMR-mediated damage response. In the futile repair cycle model, the MMR system recognizes chemically-modified DNA lesions located on the template DNA strand, but it fails to remove the DNA adducts, because the repair only targets on the newly synthesized strand. This leads to a new round of lesion recognition and processing, inducing a futile repair cycle. The futile cycle activates ATM or ATR signaling and induces cell cycle arrest or apoptosis. In the direct signaling, MutS and MutL complexes recognize the DNA lesion and recruit ATM or ATR to directly induce downstream signaling. (**B**) Model for MMR deficiency-induced tumorigenesis. Microsatellite sequence represents a DNA fragment with simple repetitive nucleotides like single nucleotide or dinucleotide repeats. During DNA replication, microsatellite sequence is frequently associated with insertion or deletion errors (TT insertion on daughter strand is shown). These errors can be readily corrected by the MMR system. However, in cells defective in MMR, these errors remain unpaired and cause frame-shift and other mutations in the next cell cycle. Mutations in genes critical for cellular functions, such as DNA damage response, cell cycle checkpoints, apoptosis and proliferation, lead to cancer development.

of a gene. Many of these mutations result in a truncated polypeptide or a loss of function full-length protein. Given the importance of MMR in genome maintenance, it is expected that loss of the MMR function causes human diseases. However, this was not confirmed until 1993, when MSI was first identified in certain colorectal cancers (21–23) and these MSI tumor cells were found to be defective in MMR (24). Subsequently, MSI was also detected in a wide variety of non-colonic tumors (25), including bladder, breast, cervical, endometrial, gastric, lung, head and neck squamous cell carcinoma, prostate, ovarian, and skin tumors, as well as glioma, leukemia, and lymphoma (26). Biochemical and genetic studies have revealed that these MSI tumors have lost their MMR activity (27–31), contained mutations in key MMR genes *MLH1*, *MSH2* and *MSH6* (32–40) or had a hypermethylated *MLH1* promoter (41–43), which silences the *MLH1* expression.

In addition to causing frequent alterations in long-stretch simple repetitive sequences, dMMR also leads to increased rates of insertion/deletion mutations in gene coding regions with short repetitive nucleotide sequences, which drives frame-shift mutations. In the presence of an active MMR system, insertion/deletion mispairs can be readily corrected using the parental strand sequence as the template during the repair DNA synthesis (Figure 2B, left). However, in dMMR cells, the insertion/deletion heteroduplex stays. In the next cycle of DNA replication, the error-containing strand (the previous daughter strand) now serves as the template strand for DNA replication, leading to inserting two nucleotides in the new daughter stand (Figure 2B, right). Markowitz and coworkers found that colorectal cancer cells with MSI harbor mutations at short microsatellite sequences $(GT)_3$ and $(A)_{10}$ in the gene coding transforming growth factor-β type II receptor (TGFβR2), which cause frame-shift mutations that lead to truncated products of TGFβR2 (44). Similarly, frame-shift mutations in coding microsatellite sequences of other important genes, including MBD4, BLM, RAD50, MLH3, CHK1, MSH3, MSH6, PTEN, BAX and caspase-5, were also identified (reviewed in (45)). Thus, tumors with defective MMR genes harbor increased genome-wide mutations, leading to a mutator phenotype and cancer development. These frameshift mutation-induced proteins can be degraded into small peptides by the proteasome system, thereby generating neoantigens (Figure 2B, right).

The principle and requirement of cancer immunotherapy

As an effective defense mechanism, the human immune system prevents infections and protects us from many diseases including cancer (46). By distinguishing self and non-self substances through the molecular interaction between T cell receptors (TCRs) and large peptide complexes with major histocompatibility complex (MHC) class I molecules

located on the surface of cells (47,48), the immune system is tolerance to its own components, but removes non-self elements (49). Interestingly, although there are obvious differences between normal cells and cancer cells, the immune system fails to eliminate cancer cells from our bodies. This is because (i) cancer cells are derived from normal cells (50,51), and have therefore 'inherited' all systems from normal cells; (ii) because of mutations, cancer cells usually adopt altered signaling pathways and/or a defective immune system; (iii) cancer cells also produce many factors (e.g. immunosuppressive cytokines) that suppress the immune system (52,53); (iv) cancer cells are often associated with exhausted T cells that express multiple inhibitory receptors and have lost the anti-tumor function. This may explain why immunotherapy is effective to some but not all patients. However, the use of ICIs, which disrupt the communications between T cells and cancer cells, has been a revolutionary strategy in cancer treatment (54). Immune checkpoint proteins, such as PD-L1 on tumor cells and PD-1 on T cells, help keep immune responses under control. When PD-1 on the surface of T cells recognizes and bind to PD-L1 on the surface of tumor cells, they send an 'off' signal to the T cells to prevent the immune system from destroying the tumor cells. However, ICIs pembrolizumab and nivolumab (PD-1-specific monoclonal antibodies) block PD-1 from binding with PD-L1. This prevents the 'off' signal from being sent, allowing the T cells to kill tumor cells.

Tumor microenvironment (TME) is infiltrated with various immune cells, including T cells, B cells, macrophages, NK cells and other monocytes, which constitute the immune system against tumor progression (55). However, tumors have emerged multiple strategies to evade immune surveillance, including promoting T cell exhaustion by overexpressing inhibitory receptors PD-1, cytotoxic T lymphocyte antigen-4 (CTLA-4), LAG-3 and/or TIM-3 in T cells, while expressing PD-L1 in tumor cells or other immune cells (56).These inhibitory receptors can recognize and bind to their ligands, which prevents T cell from destroying tumors. T cell exhaustion occurs when T cells are exposed to persistent tumor antigens, but the exhausted T cells can be revitalized with ICIs, which is regarded as a common mechanism to rejuvenate anti-tumor immunity (57,58). For instance, CTLA-4 competes with CD28 to bind to CD80/86 on the surface of dendritic cells, which blocks antigen-presenting cell-mediated T-cell priming and activation. Thus, using anti-CTLA-4 antibody can inhibit the interaction between CTlA-4 and CD80/86, recovering T-cell priming and promoting anti-tumor immunity (59–61). In addition, the inhibitory signaling activated by PD-1 or PD-L1 binding also limits T cell activity and promotes T-cell exhaustion, which can be reversed by anti-PD-1 or anti-PD-L1 antibody (62,63). Based on the ligand-receptor inhibitory checkpoint signaling in TME, multiple antibodies have been developed to prevent this interaction and to activate T-cells to kill tumors, which lead to the development of ICI therapy. However, the development of T cell exhaustion during treatment also results in resistance of cellular immunotherapy (57,58). How this can be prevented requires thorough investigations.

Immunotherapy was initially demonstrated to benefit cancer treatments of melanoma, as ICIs ipilimumab (targeting CTLA4), pembrolizumab and nivolumab (targeting PD-1) were shown to improve the survival of metastatic melanoma patients for $\geq$10 years (64–67). Subsequently, these ICIs were also found to be effective for non-small-cell lung cancer (NSCLC) (68–70). Efforts to identify the biomarkers responsible for the ICI sensitivity have revealed that the difference between the ICI-responsive and non-responsive melanoma and NSCLC is the degree of tumor mutation burdens (TMB). In comparison to tumors that do not respond to immunotherapy, the ICI-responsive tumors display high levels of mutations (71,72), suggesting that high tumor mutation burdens (TMB-H) benefit immunotherapy. This is likely because these mutations cause protein truncations, leading to the production of short peptides, which can function as neoantigens to stimulate the immune response. Indeed, it has been shown that neoantigens are mainly tumor-specific and are derived from mutations in tumor cells (73). They are ideal targets for T cells to recognize cancer cells to trigger anti-tumor response. Therefore, neoantigens are key components in cancer immunotherapy.

MMR-deficiency benefits checkpoint blockade immunotherapy

As discussed above, dMMR tumors exhibit a mutator phenotype, characterized with MSI and elevated frame-shift mutations (4). When insertion/deletion mutations occur in coding regions of a gene, they induce frame-shift mutations and truncated polypeptides or mutated proteins (74–76). These mutated proteins are not stable and can be easily degraded into peptides, which act as tumor neoantigens to form neoantigen-MHC I complex. The latter is then specifically recognized by cytotoxic $CD8^+$ T cells. The increased mutations in dMMR tumor cells provide a reservoir of neoantigens, thereby increasing the infiltration of immune cells and triggering host immune response. Other single-nucleotide substitution-induced somatic mutations in dMMR tumors also increase the non-self mutant peptides and enable the generation of neoantigen to stimulate immune response (74–78). These properties make dMMR tumors excellent candidates for the ICI therapy.

Le et al. (19) conducted a phase 2 clinical trial by treating 41 patients with progressive metastatic carcinoma with anti-PD-1 antibody pembrolizumab and found that 40% (4 of 10) and 78% (7 of 9) of dMMR colorectal cancer (CRC) patients obtained immune-related objective response and progression-free survival (PFS), respectively, but none (0 of 18 patients) objective response and 11% (2 of 18 patients) PFS were observed in microsatellite-stable (MSS) CRC patients. Similar results were also obtained in dMMR non-CRC patients. These observations clearly indicate that patients with dMMR tumors acquired clinical benefit from ICI therapy (19). Their continued clinical trial involving larger cohorts of patients with dMMR tumors ($n = 86$) across 12 different tumor types showed essentially the same result, with 53% (46 in 86 patients) objective response and 21% (18 in 86 patients) PFS (20), further confirming that the dMMR status benefits ICI therapy. This conclusion has been supported by other clinical trials (79–85), as well as a number of animal studies using various models with depleted *MLH1* or *MSH2*, including those

of murine syngeneic CRC, breast cancer, pancreatic cancer and melanoma (74,76). In addition, Le *et al.* also observed rapid expansion of neoantigen-specific T cell clones, which can react with mutant neopeptides in the tumor (20), suggesting that the mutator phenotype-generated neoantigens in dMMR tumors render them sensitive to ICI therapy. Consequently, FDA approved the ICI treatment for dMMR tumors in 2017. However, although all dMMR tumors express a high-level of neoantigens, not all of them respond to the ICI therapy, suggesting that additional mechanisms regulate the ICI therapy in dMMR tumors.

dMMR triggers activation of the cGAS-STING pathway

The cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS), a critical factor in the innate immune response pathway, has been shown to be essential for anti-PD-L1 therapy in animal models (86). Upon binding cytosolic DNA, cGAS synthesizes the second messenger molecule cyclic GMP-AMP (cGAMP), which, in turn, binds and activates the adaptor stimulator of interferon genes (STING). The activated STING then triggers a signaling cascade that induces type I interferons (IFNs) and other immune molecules (87,88). Since cGAS recognizes endogenously derived tumor DNA to induce innate immune response to enhance anti-tumor immunity (86,89–92), it is possible that cGAS is a required component for dMMR-mediated immunotherapy.

We tested this hypothesis using *MLH1*-deficient (dMLH1) murine syngeneic CRC, breast cancer and melanoma as models (13,93). As expected, tumors derived from dMLH1 cancer cells are highly responsive to ICI treatment (93). Interestingly, we found that the dMLH1 tumor cells are associated with large quantity of cytosolic DNA, activated cGAS-STING pathway, increased production of type-I interferon, enhanced dendritic cell-mediated T cell priming and enriched tumor-infiltrating lymphocytes (TILs) (93), suggesting that the cGAS-STING-type I IFN signaling pathway is involved in dMMR-mediated immunotherapy (Figure 3). Consistently, our clinical data showed that dMLH1 tumor patients who express normal levels of cGAS and STING responded very well to anti-PD-1 treatment, but those who express no or low levels of cGAS or STING failed to respond to the treatment (93). These observations also indicate that cGAS and STING, as well as their downstream factors, are independent ICI-therapy predictor for dMMR (at least dMLH1) tumors.

The question is how dMLH1 activates the cGAS-STING innate immune sensing pathway. It is known that activation of the cGAS-STING pathway is associated with DNA damage-induced genome instability, which leads to the formation of micronuclei and cytosolic DNA release (94,95). MLH1, a subunit of the MutLα heterodimer (96), functions together with its partner PMS2 to initiate the MMR reaction by interacting with MutSα (11) and to negatively regulate Exo1′s nuclease activity upon mismatch removal (7,11). In addition to participating in MMR, MLH1 is also involved in repairing double strand DNA breaks (DSBs) (97), which may explain why dMLH1 cancer cells exhibit DSBs (98). Similarly, Exo1 is also a DSB repair factor by conducting the DNA end resection to facilitate homologous recombination-mediated DSB repair (99–101). Because DNA hyper-resection is mutagenic and toxic to the genome (102), Exo1-catalyzed DNA end resection must be properly regulated, and MutLα could be the factor that regulates the Exo1-catalyzed end resection, as it is in MMR (7). Therefore, loss of MLH1 may cause Exo1-catalyzed hyper-excision, which leads to genome instability and activation of the cGAS-STING pathway.

Indeed, Guan et al. (13) showed that in dMLH1 tumor cells, Exo1 undergoes uncontrolled DNA end resection. In vitro end resection assay using a purified system revealed that MutLα regulates Exo1′s nuclease activity via its physical interaction with Exo1, as disrupting the MLH1-Exo1 interaction leads to uncontrolled DNA end resection. The hyper-excision by Exo1 causes the formation of large quantity of ssDNA and RPA exhaustion, DNA breaks, and eventually chromosome abnormalities. This results in the release of nuclear DNA into the cytoplasm to activate the cGAS-STING pathway, thereby leading to increased chromosome aberrations and accumulation of cytosolic DNA (13).

However, our studies have also raised many questions. First, because *MLH1* and *EXO1* double-knockout cells display reduced cytosolic DNA and interferon production in comparison to cells with *MLH1* knockout alone (13), does loss of Exo1 function abolish ICI therapy? If this is true, the status of Exo1 can be used as a prognostic predictor for dMMR tumors. Second, dMMR is known to cause base-base substitution and small insertion-deletion mutations, but we observed chromosomal abnormalities in dMLH1cells (13). This appears to be consistent with a previous study showing that *Mlh1*-knockout mice, which are infertile because of prematurely separated chromosomes and unsuccessful completion of recombination, display abnormal crossover in meiosis and severely damaged chromosomes (103). How does chromosomal instability (CIN) occur in dMLH1 cells? Jardim *et al.* reported that partial knockdown of ATR or Chk1 in multiple dMLH1 CRC lines resulted in cells displaying a CIN phenotype, including chromosomal breaks and gaps, chromosome bridge, and micronuclei formation (98). It is possible that the observed CIN phenotype in these dMLH1 cells and animals are caused by Exo1-generated ssDNA, which can be degraded by nucleases, particularly when RPA is exhausted. What are these nucleases? Are they essential for dMMR-mediated immunotherapy? Third, does loss of other key MMR components, such as MSH2 and MSH6, induce cytosolic DNA and cGAS-STING activation? Finally, irradiation strongly increases *MLH1* knockout-associated accumulation of cytosolic DNA and cGAS-STING mediated immune response (13), can irradiation enhance the response of ICI therapy in dMLH1 and other dMMR tumors? These interesting questions await future investigations.

dMMR reshapes tumor microenvironment

The most evaluable feature for an effective ICI therapy is the density of TILs in TME. Indeed, dMMR tumors across various tumor types are associated with high infiltration of cytotoxic T cells and other lymphocytes, which may contribute to better immunotherapy response. For instance, in

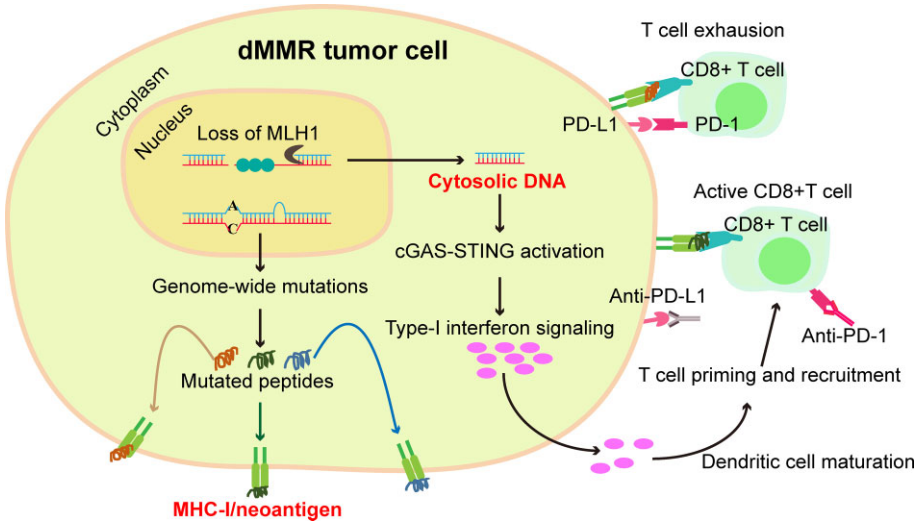


Figure 3. Mechanisms by which dMMR tumors respond to anti-PD-1 therapy. Defects in MMR cause genome-wide mutations, and some of these mutations produce mutated proteins, which can be packaged into proteasomes for degradation to generate mutated peptides. The mutated peptides are then loaded to MHC-I complex and processed as neoantigens for antigen presentation, resulting in high immunogenicity of dMMR tumors. In addition, dysfunction of MLH1, a subunit of MutLα, also leads to Exo1-mediated hyperexcision and accumulation of cytosolic DNA fragments, which activates the cGAS-STING pathway and the type-I interferon signaling. The type-I interferons then promote maturation of dendritic cells and T cell priming. With increased neoantigens, dMMR tumors are infiltrated with high densities of T lymphocytes to promote anti-PD-1/anti-PD-L1 therapy.

a phase III trial, 561 stage III CRC patients consisting of 278 dMMR cases and 283 MMR-proficient (pMMR) cases were treated with adjuvant FOLFOX-based chemotherapy, the densities of $CD3^+$ and $CD8^+$ TILs in dMMR tumors are much higher than those in pMMR tumors (104). These higher densities of TILs in dMMR tumors were found to be associated with the number of frame-shift mutations (76,84,85), supporting the notion that dMMR status reshapes TME. In addition, the degree of tumor infiltrating natural killer (NK) cells in TME can also predict prognostic outcome of ICI therapy. NK cells are a subtype of cytotoxic lymphocytes that can produce robust cytokines to initiate their tumor cell killing activity upon activation, which is modulated by their activating receptors, inhibitory receptors and co-stimulatory receptors (105). Accordingly, cGAS-STING activation-mediated IFNβ has been reported to prime and activate NK cells for cytotoxicity (106). Since dMMR tumors have increased interferon production, it would be interesting to investigate whether or not NK cells are also enriched in dMMR tumors to benefit ICI therapy.

The expression of immune checkpoints is another key factor in regulating immune response in TME, which limits the activation of cytotoxic T cells and helps tumors to escape from immune surveillance. Previous studies have reported that defects in DNA damage response pathways or treatment with DNA damage agents can upregulate the expression of immune checkpoints on tumor cells. Similarly, dMMR tumors are associated with a large amount of neoantigens, increased cGAS-STING activation-mediated type-I interferon signaling and high density of TILs, which also triggers upregulation of IFNγ, PD-L1 and other inhibitory signals, leading to immune-suppressive TME (107,108). In 2015, Llosa and coworkers first described a link between cancer features with its corresponding expression of immune checkpoints in TME. They reported that dMMR tumors displayed high densities of activated $CD8^+$ cytotoxic T cells and $CD4^+$ Helper T Cells Type 1 cells with increased IFNγ expression, which is counterbalanced by highly upregulated PD-1, PD-L1, CTLA-4, LAG-3, and IDO (108). Consistently, in a cohort of 63 patients with endometrial cancer, Howitt *et al.* revealed that MSI-H tumors exhibited higher tumor-infiltrating $CD3^+$ and $CD8^+$ lymphocytes, associating with PD-1 overexpression in TILs and peritumoral lymphocytes compared with MSS tumors (107). However, they found that PD-L1 expression was not frequent in dMMR tumor cells but occurred in intraepithelial immune cells, suggesting that PD-L1 expression is heterogenous between tumor cells and TILs (107). Similarly, the majority of PD-L1 expression was found in myeloid cells, rather than in tumor cells in MSI CRC, which is much different from melanoma, renal, or lung cancer (108). Vanderwalde and coworkers analyzed 11348 cases across 26 cancer types, and showed that only 26% of MSI-H tumors were PD-L1 positive (109). The lack of PD-L1 expression in dMMR tumor cells, even with high level of IFNγ, is highly intriguing and requires further investigating.

Tumor resistance to ICI therapy in dMMR cancers

Although dMMR status acts as a prognostic predictor for ICI therapy, ∼50% of dMMR tumors exhibit primary and acquired resistance with lack of clear mechanisms. To date, loss of cGAS-STING pathway, disruption of antigen presenting machinery, alterations in JAK-STAT Pathway, and deregulation of suppressive TME have been reported to affect efficacy of ICI therapy in dMMR tumors. Since the density of TILs directly determines the efficacy of ICI therapy, resistance mechanisms have been focused on why some patients with dMMR tumors lack TILs.

Although high levels of neoantigens are associated with dMMR tumors, not all of them are highly immunogenic, which results in reduced activation of cytotoxic T cells and resistance to the ICI therapy (74,76). Our recent study in mice model have revealed that loss of cGAS or STING expression in dMLH1 tumor cells attenuates the tumor-infiltrating $CD8^+$ T lymphocytes and inhibits the ICI therapy efficacy by attenuating type-I interferon signaling (Figure 4A). In addition, clinical data from 7 patients with dMLH1 tumors who had received anti-PD-1 therapy showed that low expression of cGAS or STING is associated with poor survival, suggesting that loss of the cGAS-STING pathway confers resistance to ICI therapy in dMMR tumors (93).

In 2018, Grasso et al. analyzed a cohort of CRC tumors ($n = 1211$ in total, with 79 dMMR cases) and found that MSI-H tumors had a high rate of the disrupted antigen presentation machinery, including losses of Beta 2 microglobulin (B2M) and human leukocyte antigen (HLA) (110). B2M plays a pivotal role in the antigen presentation process by dimerizing with MHC-I molecule to assist loading mutant peptides. Thus, mutation or loss of B2M and HLA in dMMR tumors impairs antigen process and presentation to $CD8^+$ T cell, which may contribute to ICI therapy resistance. Notably, in a cohort of 1751 CRC patients, 24% (44 in 182) of MSI-H CRCs harbored B2M mutations, among which 73% (32 in 44) had complete loss of B2M expression, but B2M mutation status was not associated with tumor infiltration of lymphocytes; in addition, 85% (11 in 13 for patients with B2M mutations who had received anti-PD-1 or anti-PD-L1 ICI therapy) still achieved clinical benefit. Consistently, Germano *et al.* reported that B2M loss did not affect response to ICI therapy in dMMR CRC tumors, which is dependent on increased tumor-infiltration of $CD4^+$ T cells but not $CD8^+$ T cells (111). These results suggest that B2M mutation is not the main resistance mechanism and cannot be as a good predictor for most dMMR tumors.

Another possible resistance mechanism is due to alteration of JAK-STAT signaling, which play an important role in regulating immune response. The signal transducers and activators of transcription (STAT) function downstream of JAK regulate the production of chemokines and IFN-stimulated genes (ISG), most of which can promote anti-tumor immunity (112,113). In 2016, Zaretsky *et al.* analyzed biopsy samples from 4 patients with melanoma who had acquired resistance to anti-PD-1 therapy and found that 2 of them had loss-of-function mutations in JAK1/JAK2 that lead to a lack of response to IFNγ (114). Subsequently, Shin et al. revealed that JAK1/2-inactivating mutations occurred in one patient with melanoma (1 in 23) and one patient with dMMR CRC (1 in 16), both of whom were bearing high TMB but lack of response to anti-PD-1 therapy, suggesting that loss-of-function mutations in JAK1/2 caused inactivation of IFNγ and resulted in primary resistance to anti-PD-1 therapy in dMMR tumors (115). Accordingly, Albacker et al. (116) analyzed a database of solid tumors (n = 61704) and found that recurrent frameshift mutations of JAK1 are associated with MSI-H and TMB-H in multiple tumor types, including endometrial cancer, CRC, stomach, and prostate carcinomas. And the tumors with frameshift mutations of JAK1 exhibited reduced expression of IFN response signatures and other anti-tumor immunity signatures, which contributed to tumor escape from the immune system and may cause resistance to ICI therapy (116). Thus, loss-of-function mutations in the JAK-STAT signaling pathway can result in impaired interferon signaling and lead to resistance to ICI therapy (Figure 4B).

The WNT-β-catenin pathway has been reported to affect anti-tumor immunity and efficacy of ICI therapy by modulating cancer immune evasion (117). Spranger et al. showed that activation of the WNT-β-catenin pathway is associated with loss of the T cell gene expression signature in metastatic melanoma, leading to the lack of T cell infiltration. Further studies using autochthonous mouse melanoma models revealed that activated WNT-β-catenin causes T cell exclusion and resistance to anti-PD-L1/anti-CTLA-4 therapy (117). Accordingly, Trabucco *et al.* analyzed 67000 patient tumor samples with next-generation sequencing and found activation of the WNT pathway is enriched in many dMMR tumors, which can induce T cell exclusion from tumors and reduced TILs (118). Grasso *et al.* investigated a CRC cohort ($n = 1211$, with 179 dMMR tumors), and reported that the WNT pathway is commonly mutated, which associates with an activated WNT-β-catenin pathway, but low level of TILs (110). Furthermore, analysis of the whole-exome data of 6747 human tumors identified several somatic microsatellite insertion/deletion mutations in WNT-regulating genes, including DOCK3 and RNF43, loss of which could induce activation of WNT signaling. For instance, RNF43 functions as a negative regulator of WNT, and the insertion/deletion mutation p.G654fs in *RNF43* has been found in 40% (16/40) MSI CRC, 35% (24/69) MSI stomach cancer and 23% (36/155) MSI endometrial cancer, respectively (119). Taken together, these observations indicate that a significant portion of dMMR tumors are associated with high frameshift insertion/deletion mutations-induced activation of WNT signaling, which can lead to reduced TILs and resistance to ICI therapy (Figure 4C).

Whereas inhibitory immune signals, such as PD-1, PD-L1 and CTLA-4, are upregulated to counterbalance the high density of TILs in TME, which contributes to the resistance of ICI therapy in dMMR tumors (Figure 4D). In addition, increased suppressive immune cells in certain dMMR tumors, which restrict the activity of cytotoxic T cells and affect anti-tumor immune response, have been observed (120–122). In a cohort study of 69 CRC patients (36 MSS, 33 MSI-H), Bauer et al. found that infiltrations of dendritic cells and macrophages are elevated in dMMR CRCs compared to those of MSS CRCs, but a subset of MSI-H tumors display high infiltration of $Foxp3^+$ regulatory T cells associated with low proportion of $CD208^+$ mature dendritic cells (121,123). This suggests that regulatory T cells may hamper maturation of dendritic cells and confer resistance to ICI in dMMR tumors (Figure 4E). In addition, Hu et al. reported that macrophages of the suppressive M2 phenotype are enriched in a subgroup of MSI-H tumors with poor prognosis (122).

Taken together, the resistance mechanisms of ICI therapy in dMMR tumors are summarized as follow: first, the loss-of-function in the cGAS-STING pathway (due to frameshift mutations or hypermethylation) can induce im-

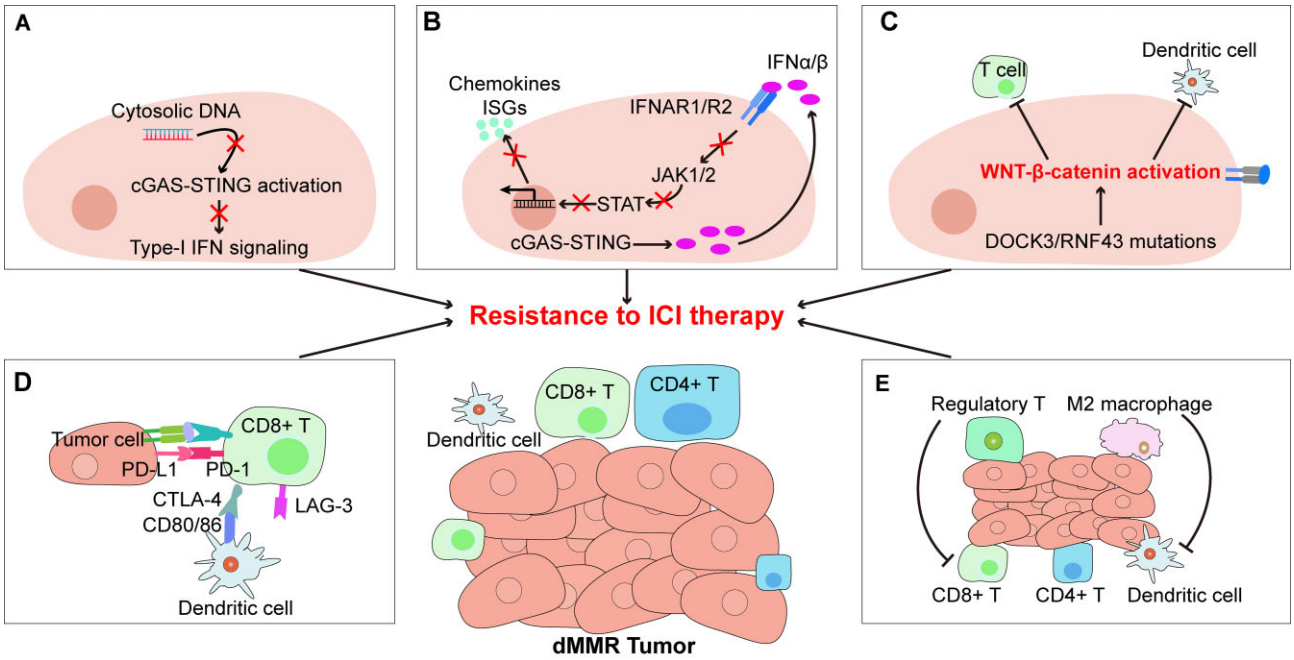


Figure 4. Mechanisms of tumor resistance in dMMR-mediated immunotherapy. dMMR tumors exhibit primary and acquired resistance from ICI therapy, which is frequently associated with low density of tumor-infiltration of T lymphocytes. The resistance mechanisms include the followings. (**A**) Loss-of-function in cGAS or STING, which inactivates the type-I interferon signaling, and leads to low infiltration of T lymphocytes to impair ICI therapy in dMMR tumors. (**B**) Loss function of the JAK-STAT pathway, which blocks production of chemokines and interferon stimulated genes (ISG), leading to impaired anti-tumor immune response. (**C**) Frame-shift mutations in coding regions of DOCK3 or RNF43, which induces hyper-activation of WNT-β-catenin and upregulates WNT-targeted genes, resulting in exclusion of T cells and dendritic cells from tumors. (**D**) Upregulation of immune checkpoints including PD-1, PD-L1, CTLA-4, LAG-3 and IDO, which confers immuno-suppressive TME in dMMR tumors. (**E**) Increase of regulatory T cells and M2 macrophages, which impairs the activity of cytotoxic T cells and presents immuno-suppressive TME, thereby inhibiting ICI therapy.

paired interferon signaling or antigen presentation process, which hampers priming, activates T lymphocytes and reduces TILs; second, activation of the WNT signaling pathway may lead to T cells exclusion from tumors and result in low density of TILs; third, hyper-upregulation of inhibitory immune signals and suppressive immune cells cause impaired maturation of DC and reduced activity of cytotoxic T cells in TME.

New strategies for combination therapy in dMMR tumors

Given that ∼50% of patients with dMMR tumors are resistance to ICI therapy and that a subgroup of these patients exhibit acquired resistance, overcoming the ICI-therapy resistance is very important for effective ICI therapy. Here, we briefly discuss potential strategies to overcome tumor resistance (Figure 5).

First, since loss of cGAS or STING in dMMR tumors attenuates type-I IFN signaling and leads to few TILs and resistance to ICI therapy, developing strategies to activate type-I IFN signaling independent of cGAS-STING will benefit dMMR tumors with a defective cGAS-STING pathway. In addition, loss-of-function in JAK1/2 genes can also hamper IFN signaling and result in low density of TILs. Recovering IFN signaling using mRNA delivery technologies could be a promising strategy to conquer this kind of resistance. Lipid nanoparticles have been widely used to deliver mRNA for personalized therapy (124). In a recent study, Liu et al. delivered a constitutively active STING mRNA ($STING^{R284S}$) to STING-deficient cancer cells using lipid nanoparticles and achieved the production of IFNs and other anti-tumor cytokines to enhance the anti-tumor cell killing activity (125). Unlike traditional STING agonists, which can induce host T cell cytotoxicity and only work in STING-proficient tumors, $STING^{R284S}$ mRNA delivery with lipid nanoparticles can specifically target on tumor cells without activating the host innate immune response and causing systemic inflammation. This strategy can recover IFN signaling and induce antitumor response in STING-silenced tumors, which would help conquer the resistance of ICI therapy for dMMR tumors.

Second, since frame-shift mutation-induced upregulation of the WNT pathway in a subset of dMMR tumors causes T cell exclusion and low TILs, ICI-based combination therapy with WNT inhibitor should improve the survival (Figure 5A). Recently, WRN helicase has been found to be a synthetic lethal target in dMMR tumors (126,127), combination of ICI agents with WRN inhibitors would also be a promising strategy.

Third, inhibitory checkpoints including PD-1, PD-L1, CTLA-4, LAG3 and IDO have been found in dMMR tumors. Thus, targeting PD-1/PD-L1 together with CTLA-4, LAG3 or IDO would enhance anti-immunity activity than monotherapy (Figure 5B). In fact, this has been demonstrated nicely in a recent clinical trial of dMMR metastatic CRCs, where nivolumab plus low-dose ipilimumab was used (128). In addition, pembrolizumab plus epacadostat (IDO-inhibitor) has been trialed for advanced

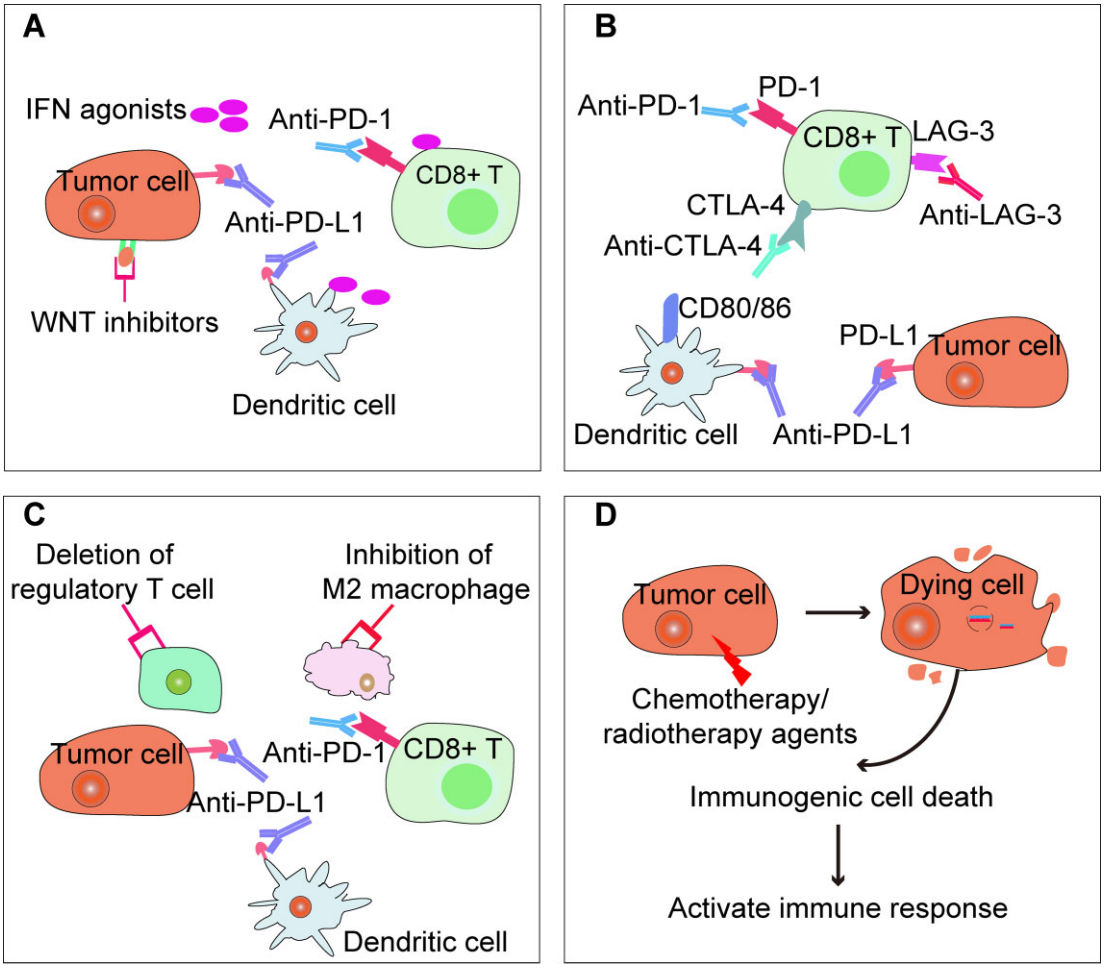


Figure 5. Potential strategies to overcome tumor resistance to ICI therapy. Although ICI therapy represents a remarkable efficacy in patients with dMMR tumors, primary and acquired resistance restricts the survival rate of the non-responders. New strategies can be developed for dMMR tumors to conquer the resistance. (**A**) Combination with targeted therapy such as STING/interferon agonists, cytokines or WNT inhibitors to increase tumor-infiltration of T lymphocytes. (**B**) Combination with inhibitors of other immune checkpoints such as anti-CTLA-4 or anti-LAG-3. (**C**) Combination with deletion or inhibition of regulatory T cells and M2 macrophage to block immune-suppressive TME. (**D**) Combination with chemotherapy or radiotherapy to induce damage-associated molecular pattern (DAMP), tumor-associated antigen presentation, and release of costimulatory molecules.

solid dMMR tumors including endometrial cancer, CRC and gastric cancer (NCT02178722). Furthermore, deletion of M2 macrophages or regulatory T cells together with ICI therapy could also be taken into consideration (Figure 5C).

Finally, several other strategies combining ICI with chemotherapy, radiotherapy or targeted therapy under clinical trials could also improve the survival of patients with dMMR tumors (Figure 5D). For instance, pembrolizumab combined with radiotherapy and nivolumab or ipilimumab combined with radiotherapy have been used for treating patients with dMMR CRC in clinical trials (NCT03104439 and NCT04001101).

In conclusion, new strategies for patients with dMMR tumors should take into consideration for the personalized resistant mechanisms based on predictor features and goals to enrich TILs and enhance anti-tumor immunity. However, preclinical and clinical data for resistant mechanisms are still lacking, and thus, thorough investigations should be conducted in the future to conquer tumor resistance.

Future perspectives of dMMR immunotherapy

Tumors with defective MMR genes are associated with MSI-H features and frequent frameshift mutations, thus leading to increased generation of neoantigens that can be loaded to the MHC-I complex and recognized by antigen presenting cells or cytotoxic T cells. In addition, cytosolic DNA is accumulated in dMMR tumor cells and activates the cGAS-STING pathway, which promotes type-I IFN signaling and enhances T cell priming, thus resulting in high densities of TILs. Both neoantigens and cGAS-STING pathway-mediated type-I IFN signaling contribute to anti-tumor immunity and benefit from anti-PD-1/PD-L1 based ICI therapy (Figure 3).

However, >50% of patients with dMMR tumors still exhibit resistance to ICI therapy. This portion of dMMR tumors is associated with low densities of TILs caused by various mechanisms, including low or no expression of cGAS or STING, mutations-induced loss-of-function in IFN regulatory genes like JAK1/2, activation of WNT-β-catenin path-

way, overexpression of immune checkpoints like PD-1/PD-L1, and immune-suppressive cells like regulatory T cells.

Nevertheless, the interplay between dMMR, TME and anti-tumor immunity is still not fully understood. More tumor intrinsic mechanisms and predictive biomarkers in dMMR tumors for efficacy of ICI therapy should be exploited and integrated for evaluation in future personalized therapy. In addition, since lacking preclinical and clinical studies for ICI-based combination therapy for dMMR tumors, investigators can take into consideration of the primary and acquired resistance mechanisms to develop new approaches.

DATA AVAILABILITY

No new data were generated or analysed in support of this research.

ACKNOWLEDGEMENTS

The work is supported in part by grants from the Talents Program of the Lanzhou University Second Hospital (yjrckyqdj-2022–02 to J.G.) and the Cancer Prevention & Research Institute of Texas (RR160101 to G.-M.L.). G.-M.L. is a CPRIT Scholar in Cancer Research and the Reece A. Overcash, Jr. Distinguished Chair for Research on Colon Cancer.

FUNDING

Lanzhou University Second Hospital (yjrckyqdj-2022–02 to J.G.) and the Cancer Prevention & Research Institute of Texas (RR160101 to G.-M.L.).
Conflict of interest statement. None declared.

REFERENCES

1. Kinzler,K.W. and Vogelstein,B. (1996) Lessons from hereditary colorectal cancer. *Cell*, **87**, 159–170.
2. Kolodner,R.D. and Marsischky,G.T. (1999) Eukaryotic DNA mismatch repair. *Curr. Opin. Genet. Dev.*, **9**, 89–96.
3. Kunkel,T.A. and Erie,D.A. (2005) DNA mismatch repair. *Annu. Rev. Biochem.*, **74**, 681–710.
4. Li,G.M. (2008) Mechanisms and functions of DNA mismatch repair. *Cell Res.*, **18**, 85–98.
5. Modrich,P. and Lahue,R. (1996) Mismatch repair in replication fidelity, genetic recombination, and cancer biology. *Annu. Rev. Biochem.*, **65**, 101–133.
6. Constantin,N., Dzantiev,L., Kadyrov,F.A. and Modrich,P. (2005) Human mismatch repair: reconstitution of a nick-directed bidirectional reaction. *J. Biol. Chem.*, **280**, 39752–39761.
7. Zhang,Y., Yuan,F., Presnell,S.R., Tian,K., Gao,Y., Tomkinson,A.E., Gu,L. and Li,G.M. (2005) Reconstitution of 5'-directed human mismatch repair in a purified system. *Cell*, **122**, 693–705.
8. Holmes,J. Jr, Clark,S. and Modrich,P. (1990) Strand-specific mismatch correction in nuclear extracts of human and Drosophila melanogaster cell lines. *Proc. Nat. Acad. Sci. U.S.A.*, **87**, 5837–5841.
9. Thomas,D.C., Roberts,J.D. and Kunkel,T.A. (1991) Heteroduplex repair in extracts of human HeLa cells. *J. Biol. Chem.*, **266**, 3744–3751.
10. Kadyrov,F.A., Dzantiev,L., Constantin,N. and Modrich,P. (2006) Endonucleolytic function of MutLalpha in human mismatch repair. *Cell*, **126**, 297–308.
11. Ortega,J., Lee,G.S., Gu,L., Yang,W. and Li,G.M. (2021) Mispair-bound human MutS-MutL complex triggers DNA incisions and activates mismatch repair. *Cell Res.*, **31**, 542–553.
12. Ramilo,C., Gu,L., Guo,S., Zhang,X., Patrick,S.M., Turchi,J.J. and Li,G.M. (2002) Partial reconstitution of human DNA mismatch repair in vitro: characterization of the role of human replication protein A. *Mol. Cell. Biol.*, **22**, 2037–2046.
13. Guan,J., Lu,C., Jin,Q., Lu,H., Chen,X., Tian,L., Zhang,Y., Ortega,J., Zhang,J., Siteni,S. *et al.* (2021) MLH1 Deficiency-triggered DNA hyperexcision by exonuclease 1 activates the cGAS-STING pathway. *Cancer Cell*, **39**, 109–121.
14. Kat,A., Thilly,W.G., Fang,W.H., Longley,M.J., Li,G.M. and Modrich,P. (1993) An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. *Proc. Nat. Acad. Sci. U.S.A.*, **90**, 6424–6428.
15. Fink,D., Nebel,S., Aebi,S., Zheng,H., Cenni,B., Nehme,A., Christen,R.D. and Howell,S.B. (1996) The role of DNA mismatch repair in platinum drug resistance. *Cancer Res.*, **56**, 4881–4886.
16. York,S.J. and Modrich,P. (2006) Mismatch repair-dependent iterative excision at irreparable O6-methylguanine lesions in human nuclear extracts. *J. Biol. Chem.*, **281**, 22674–22683.
17. Li,G.M. (1999) The role of mismatch repair in DNA damage-induced apoptosis. *Oncol. Res.*, **11**, 393–400.
18. Wu,J., Gu,L., Wang,H., Geacintov,N.E. and Li,G.-M. (1999) Mismatch repair processing of carcinogen-DNA adducts triggers apoptosis. *Mol. Cell. Biol.*, **19**, 8292–8301.
19. Le,D.T., Uram,J.N., Wang,H., Bartlett,B.R., Kemberling,H., Eyring,A.D., Skora,A.D., Luber,B.S., Azad,N.S., Laheru,D. *et al.* (2015) PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.*, **372**, 2509–2520.
20. Le,D.T., Durham,J.N., Smith,K.N., Wang,H., Bartlett,B.R., Aulakh,L.K., Lu,S., Kemberling,H., Wilt,C., Luber,B.S. *et al.* (2017) Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*, **357**, 409–413.
21. Aaltonen,L.A., Peltomaki,P., Leach,F.S., Sistonen,P., Pylkkanen,L., Mecklin,J.P., Jarvinen,H., Powell,S.M., Jen,J., Hamilton,S.R. *et al.* (1993) Clues to the pathogenesis of familial colorectal cancer. *Science*, **260**, 812–816.
22. Ionov,Y., Peinado,M.A., Malkhosyan,S., Shibata,D. and Perucho,M. (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature*, **363**, 558–561.
23. Thibodeau,S.N., Bren,G. and Schaid,D. (1993) Microsatellite instability in cancer of the proximal colon. *Science*, **260**, 816–819.
24. Parsons,R., Li,G.M., Longley,M.J., Fang,W.H., Papadopoulos,N., Jen,J., de la Chapelle,A., Kinzler,K.W., Vogelstein,B. and Modrich,P. (1993) Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell*, **75**, 1227–1236.
25. Boland,C.R., Thibodeau,S.N., Hamilton,S.R., Sidransky,D., Eshleman,J.R., Burt,R.W., Meltzer,S.J., Rodriguez-Bigas,M.A., Fodde,R., Ranzani,G.N. *et al.* (1998) A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.*, **58**, 5248–5257.
26. Hause,R.J., Pritchard,C.C., Shendure,J. and Salipante,S.J. (2016) Classification and characterization of microsatellite instability across 18 cancer types. *Nat. Med.*, **22**, 1342–1350.
27. Gu,L., Cline-Brown,B., Zhang,F., Qiu,L. and Li,G.M. (2002) Mismatch repair deficiency in hematological malignancies with microsatellite instability. *Oncogene*, **21**, 5758–5764.
28. Mao,G., Yuan,F., Absher,K., Jennings,C.D., Howard,D.S., Jordan,C.T. and Gu,L. (2008) Preferential loss of mismatch repair function in refractory and relapsed acute myeloid leukemia: potential contribution to AML progression. *Cell Res.*, **18**, 281–289.
29. Gu,L., Wu,J., Zhu,B.B. and Li,G.M. (2002) Deficiency of a novel mismatch repair activity in a bladder tumor cell line. *Nucleic Acids Res.*, **30**, 2758–2763.
30. Murata,H., Khattar,N.H., Kang,Y., Gu,L. and Li,G.M. (2002) Genetic and epigenetic modification of mismatch repair genes hMSH2 and hMLH1 in sporadic breast cancer with microsatellite instability. *Oncogene*, **21**, 5696–5703.
31. Parsons,R., Li,G.M., Longley,M., Modrich,P., Liu,B., Berk,T., Hamilton,S.R., Kinzler,K.W. and Vogelstein,B. (1995) Mismatch repair deficiency in phenotypically normal human cells. *Science*, **268**, 738–740.

32. Fishel,R., Lescoe,M.K., Rao,M.R., Copeland,N.G., Jenkins,N.A., Garber,J., Kane,M. and Kolodner,R. (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell*, **75**, 1027–1038.
33. Kolodner,R.D., Hall,N.R., Lipford,J., Kane,M.F., Morrison,P.T., Finan,P.J., Burn,J., Chapman,P., Earabino,C., Merchant,E. *et al.* (1995) Structure of the human MLH1 locus and analysis of a large hereditary nonpolyposis colorectal carcinoma kindred for mlh1 mutations. *Cancer Res.*, **55**, 242–248.
34. Kolodner,R.D., Hall,N.R., Lipford,J., Kane,M.F., Rao,M.R., Morrison,P., Wirth,L., Finan,P.J., Burn,J., Chapman,P. *et al.* (1994) Human mismatch repair genes and their association with hereditary non-polyposis colon cancer. *Cold Spring Harbor Symp. Quant. Biol.*, **59**, 331–338.
35. Kolodner,R.D., Tytell,J.D., Schmeits,J.L., Kane,M.F., Gupta,R.D., Weger,J., Wahlberg,S., Fox,E.A., Peel,D., Ziogas,A. *et al.* (1999) Germ-line msh6 mutations in colorectal cancer families. *Cancer Res.*, **59**, 5068–5074.
36. Leach,F.S., Nicolaides,N.C., Papadopoulos,N., Liu,B., Jen,J., Parsons,R., Peltomaki,P., Sistonen,P., Aaltonen,L.A., Nystrom-Lahti,M. *et al.* (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell*, **75**, 1215–1225.
37. Leach,F.S., Polyak,K., Burrell,M., Johnson,K.A., Hill,D., Dunlop,M.G., Wyllie,A.H., Peltomaki,P., de,l. *et al.* (1996) Expression of the human mismatch repair gene hMSH2 in normal and neoplastic tissues. *Cancer Res.*, **56**, 235–240.
38. Liu,B., Parsons,R.E., Hamilton,S.R., Peterson,G.M., Lynch,H.T., Watson,P., Markowitz,S., Willson,J.K.V., Green,J., de la Chapelle,A. *et al.* (1994) hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. *Cancer Res.*, **54**, 4590–4594.
39. Nicolaides,N.C., Papadopoulos,N., Liu,B., Wei,Y.-F., Carter,K.C., Ruben,S.M., Rosen,C.A., Haseltine,W.A., Fleischmann,R.D., Fraser,C.M. *et al.* (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature*, **371**, 75–80.
40. Papadopoulos,N., Nicolaides,N., Liu,B., Parsons,R., Lengauer,C., Palombo,F., D'Arrgo,A., Markowitz,S., Willson,J., Kinzler,K. *et al.* (1995) Mutations of GTBP in genetically unstable cells. *Science*, **268**, 1915–1917.
41. Veigl,M.L., Kasturi,L., Olechnowicz,J., Ma,A.H., Lutterbaugh,J.D., Periyasamy,S., Li,G.M., Drummond,J., Modrich,P.L., Sedwick,W.D. *et al.* (1998) Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. *Proc. Nat. Acad. Sci. U.S.A.*, **95**, 8698–8702.
42. Herman,J.G., Umar,A., Polyak,K., Graff,J.R., Ahuja,N., Issa,J.P., Markowitz,S., Willson,J.K., Hamilton,S.R., Kinzler,K.W. *et al.* (1998) Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc. Nat. Acad. Sci. U.S.A.*, **95**, 6870–6875.
43. Kane,M.F., Loda,M., Gaida,G.M., Lipman,J., Mishra,R., Goldman,H., Jessup,J.M. and Kolodner,R. (1997) 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.*, **57**, 808–811.
44. Markowitz,S., Wang,J., Myeroff,L., Parsons,R., Sun,L., Lutterbaugh,J., Fan,R.S., Zborowska,E., Kinzler,K.W., Vogelstein,B. *et al.* (1995) Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science*, **268**, 1336–1338.
45. Duval,A. and Hamelin,R. (2002) Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. *Cancer Res.*, **62**, 2447–2454.
46. Janssen,L.M.E., Ramsay,E.E., Logsdon,C.D. and Overwijk,W.W. (2017) The immune system in cancer metastasis: friend or foe?*J. Immunother. Cancer*, **5**, 79.
47. Khalil,D.N., Smith,E.L., Brentjens,R.J. and Wolchok,J.D. (2016) The future of cancer treatment: immunomodulation, cars and combination immunotherapy. *Nat. Rev. Clin. Oncol.*, **13**, 273–290.
48. Schreiber,R.D., Old,L.J. and Smyth,M.J. (2011) Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science*, **331**, 1565–1570.
49. Chaplin,D.D. (2010) Overview of the immune response. *J. Allergy Clin. Immunol.*, **125**, S3–S23.
50. Sanchez-Danes,A. and Blanpain,C. (2018) Deciphering the cells of origin of squamous cell carcinomas. *Nat. Rev. Cancer*, **18**, 549–561.
51. Sia,D., Villanueva,A., Friedman,S.L. and Llovet,J.M. (2017) Liver cancer cell of origin, molecular class, and effects on patient prognosis. *Gastroenterology*, **152**, 745–761.
52. Baumeister,S.H., Freeman,G.J., Dranoff,G. and Sharpe,A.H. (2016) Coinhibitory pathways in immunotherapy for cancer. *Annu. Rev. Immunol.*, **34**, 539–573.
53. Gonzalez,H., Hagerling,C. and Werb,Z. (2018) Roles of the immune system in cancer: from tumor initiation to metastatic progression. *Genes Dev.*, **32**, 1267–1284.
54. Waldman,A.D., Fritz,J.M. and Lenardo,M.J. (2020) A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat. Rev. Immunol.*, **20**, 651–668.
55. Grivennikov,S.I., Greten,F.R. and Karin,M. (2010) Immunity, inflammation, and cancer. *Cell*, **140**, 883–899.
56. Zou,W. and Chen,L. (2008) Inhibitory B7-family molecules in the tumour microenvironment. *Nat. Rev. Immunol.*, **8**, 467–477.
57. Chow,A., Perica,K., Klebanoff,C.A. and Wolchok,J.D. (2022) Clinical implications of T cell exhaustion for cancer immunotherapy. *Nat. Rev. Clin. Oncol.*, **19**, 775–790.
58. Wherry,E.J. and Kurachi,M. (2015) Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol.*, **15**, 486–499.
59. Gardner,D., Jeffery,L.E. and Sansom,D.M. (2014) Understanding the CD28/CTLA-4 (CD152) pathway and its implications for costimulatory blockade. *Am. J. Transplant.*, **14**, 1985–1991.
60. Lee,K.M., Chuang,E., Griffin,M., Khattri,R., Hong,D.K., Zhang,W., Straus,D., Samelson,L.E., Thompson,C.B. and Bluestone,J.A. (1998) Molecular basis of T cell inactivation by CTLA-4. *Science*, **282**, 2263–2266.
61. Walunas,T.L., Bakker,C.Y. and Bluestone,J.A. (1996) CTLA-4 ligation blocks CD28-dependent T cell activation. *J. Exp. Med.*, **183**, 2541–2550.
62. Dong,H., Strome,S.E., Salomao,D.R., Tamura,H., Hirano,F., Flies,D.B., Roche,P.C., Lu,J., Zhu,G., Tamada,K. *et al.* (2002) Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat. Med.*, **8**, 793–800.
63. Freeman,G.J., Long,A.J., Iwai,Y., Bourque,K., Chernova,T., Nishimura,H., Fitz,L.J., Malenkovich,N., Okazaki,T., Byrne,M.C. *et al.* (2000) Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.*, **192**, 1027–1034.
64. Hodi,F.S., O'Day,S.J., McDermott,D.F., Weber,R.W., Sosman,J.A., Haanen,J.B., Gonzalez,R., Robert,C., Schadendorf,D., Hassel,J.C. *et al.* (2010) Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.*, **363**, 711–723.
65. Robert,C., Schachter,J., Long,G.V., Arance,A., Grob,J.J., Mortier,L., Daud,A., Carlino,M.S., McNeil,C., Lotem,M. *et al.* (2015) Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N. Engl. J. Med.*, **372**, 2521–2532.
66. Robert,C., Thomas,L., Bondarenko,I., O'Day,S., Weber,J., Garbe,C., Lebbe,C., Baurain,J.F., Testori,A., Grob,J.J. *et al.* (2011) Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.*, **364**, 2517–2526.
67. Schadendorf,D., Hodi,F.S., Robert,C., Weber,J.S., Margolin,K., Hamid,O., Patt,D., Chen,T.T., Berman,D.M. and Wolchok,J.D. (2015) Pooled analysis of long-term survival data from Phase II and Phase III trials of Ipilimumab in unresectable or metastatic melanoma. *J. Clin. Oncol.*, **33**, 1889–1894.
68. Borghaei,H., Paz-Ares,L., Horn,L., Spigel,D.R., Steins,M., Ready,N.E., Chow,L.Q., Vokes,E.E., Felip,E., Holgado,E. *et al.* (2015) Nivolumab versus Docetaxel in advanced nonsquamous non-small-cell lung cancer. *N. Engl. J. Med.*, **373**, 1627–1639.
69. Brahmer,J., Reckamp,K.L., Baas,P., Crino,L., Eberhardt,W.E., Poddubskaya,E., Antonia,S., Pluzanski,A., Vokes,E.E., Holgado,E. *et al.* (2015) Nivolumab versus Docetaxel in advanced squamous-cell non-small-cell lung cancer. *N. Engl. J. Med.*, **373**, 123–135.
70. Garon,E.B., Rizvi,N.A., Hui,R., Leighl,N., Balmanoukian,A.S., Eder,J.P., Patnaik,A., Aggarwal,C., Gubens,M., Horn,L. *et al.* (2015) Pembrolizumab for the treatment of non-small-cell lung cancer. *N. Engl. J. Med.*, **372**, 2018–2028.
71. Alexandrov,L.B., Nik-Zainal,S., Wedge,D.C., Aparicio,S.A., Behjati,S., Biankin,A.V., Bignell,G.R., Bolli,N., Borg,A., Borresen-Dale,A.L. *et al.* (2013) Signatures of mutational processes in human cancer. *Nature*, **500**, 415–421.

72. Rizvi,N.A., Hellmann,M.D., Snyder,A., Kvistborg,P., Makarov,V., Havel,J.J., Lee,W., Yuan,J., Wong,P., Ho,T.S. *et al.* (2015) Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*, **348**, 124–128.
73. Zamora,A.E., Crawford,J.C. and Thomas,P.G. (2018) Hitting the target: how T cells detect and eliminate tumors. *J. Immunol.*, **200**, 392–399.
74. Germano,G., Lamba,S., Rospo,G., Barault,L., Magri,A., Maione,F., Russo,M., Crisafulli,G., Bartolini,A., Lerda,G. *et al.* (2017) Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. *Nature*, **552**, 116–120.
75. Roudko,V., Bozkus,C.C., Orfanelli,T., McClain,C.B., Carr,C., O'Donnell,T., Chakraborty,L., Samstein,R., Huang,K.L., Blank,S.V. *et al.* (2020) Shared immunogenic poly-epitope frameshift mutations in microsatellite unstable tumors. *Cell*, **183**, 1634–1649.
76. Mandal,R., Samstein,R.M., Lee,K.W., Havel,J.J., Wang,H., Krishna,C., Sabio,E.Y., Makarov,V., Kuo,F., Blecua,P. *et al.* (2019) Genetic diversity of tumors with mismatch repair deficiency influences anti-PD-1 immunotherapy response. *Science*, **364**, 485–491.
77. Verdon,D.J. and Jenkins,M.R. (2021) Identification and targeting of mutant peptide neoantigens in cancer immunotherapy. *Cancers (Basel)*, **13**, 4245.
78. Marty,R., Kaabinejadian,S., Rossell,D., Slifker,M.J., van de Haar,J., Engin,H.B., de Prisco,N., Ideker,T., Hildebrand,W.H., Font-Burgada,J. *et al.* (2017) MHC-I genotype restricts the oncogenic mutational landscape. *Cell*, **171**, 1272–1283.
79. Le,D.T., Kim,T.W., Van Cutsem,E., Geva,R., Jager,D., Hara,H., Burge,M., O'Neil,B., Kavan,P., Yoshino,T. *et al.* (2020) Phase II open-label study of Pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J. Clin. Oncol.*, **38**, 11–19.
80. Overman,M.J., McDermott,R., Leach,J.L., Lonardi,S., Lenz,H.J., Morse,M.A., Desai,J., Hill,A., Axelson,M., Moss,R.A. *et al.* (2017) Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol.*, **18**, 1182–1191.
81. Overman,M.J., Lonardi,S., Wong,K.Y.M., Lenz,H.J., Gelsomino,F., Aglietta,M., Morse,M.A., Van Cutsem,E., McDermott,R., Hill,A. *et al.* (2018) Durable clinical benefit with Nivolumab plus Ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J. Clin. Oncol.*, **36**, 773–779.
82. Lenz,H.J., Van Cutsem,E., Luisa Limon,M., Wong,K.Y.M., Hendlisz,A., Aglietta,M., Garcia-Alfonso,P., Neyns,B., Luppi,G., Cardin,D.B. *et al.* (2022) 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.*, **40**, 161–170.
83. Thomas,J., Leal,A. and Overman,M.J. (2020) Clinical development of immunotherapy for deficient mismatch repair colorectal cancer. *Clin. Colorectal Cancer*, **19**, 73–81.
84. Maby,P., Tougeron,D., Hamieh,M., Mlecnik,B., Kora,H., Bindea,G., Angell,H.K., Fredriksen,T., Elie,N., Fauquembergue,E. *et al.* (2015) Correlation between density of CD8+ T-cell infiltrate in microsatellite unstable colorectal cancers and frameshift mutations: a rationale for personalized immunotherapy. *Cancer Res.*, **75**, 3446–3455.
85. Sena,L.A., Fountain,J., Isaacsson Velho,P., Lim,S.J., Wang,H., Nizialek,E., Rathi,N., Nussenzveig,R., Maughan,B.L., Velez,M.G. *et al.* (2021) Tumor frameshift mutation proportion predicts response to immunotherapy in mismatch repair-deficient prostate cancer. *Oncologist*, **26**, e270–e278.
86. Wang,H., Hu,S., Chen,X., Shi,H., Chen,C., Sun,L. and Chen,Z.J. (2017) cGAS is essential for the antitumor effect of immune checkpoint blockade. *Proc. Nat. Acad. Sci. USA*, **114**, 1637–1642.
87. Ablasser,A. and Chen,Z.J. (2019) cGAS in action: expanding roles in immunity and inflammation. *Science*, **363**, eaat8657.
88. Li,T. and Chen,Z.J. (2018) The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. *J. Exp. Med.*, **215**, 1287–1299.
89. Klarquist,J., Hennies,C.M., Lehn,M.A., Reboulet,R.A., Feau,S. and Janssen,E.M. (2014) STING-mediated DNA sensing promotes antitumor and autoimmune responses to dying cells. *J. Immunol.*, **193**, 6124–6134.
90. Woo,S.R., Fuertes,M.B., Corrales,L., Spranger,S., Furdyna,M.J., Leung,M.Y., Duggan,R., Wang,Y., Barber,G.N., Fitzgerald,K.A. *et al.* (2014) STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity*, **41**, 830–842.
91. Deng,L., Liang,H., Xu,M., Yang,X., Burnette,B., Arina,A., Li,X.D., Mauceri,H., Beckett,M., Darga,T. *et al.* (2014) STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity*, **41**, 843–852.
92. Ahn,J., Xia,T., Konno,H., Konno,K., Ruiz,P. and Barber,G.N. (2014) Inflammation-driven carcinogenesis is mediated through STING. *Nat. Commun.*, **5**, 5166.
93. Lu,C., Guan,J., Lu,S., Jin,Q., Rousseau,B., Lu,T., Stephens,D., Zhang,H., Zhu,J., Yang,M. *et al.* (2021) DNA sensing in mismatch repair-deficient tumor cells is essential for anti-tumor immunity. *Cancer Cell*, **39**, 96–108.
94. Harding,S.M., Benci,J.L., Irianto,J., Discher,D.E., Minn,A.J. and Greenberg,R.A. (2017) Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature*, **548**, 466–470.
95. Mackenzie,K.J., Carroll,P., Martin,C.A., Murina,O., Fluteau,A., Simpson,D.J., Olova,N., Sutcliffe,H., Rainger,J.K., Leitch,A. *et al.* (2017) cGAS surveillance of micronuclei links genome instability to innate immunity. *Nature*, **548**, 461–465.
96. Li,G.M. and Modrich,P. (1995) Restoration of mismatch repair to nuclear extracts of H6 colorectal tumor cells by a heterodimer of human MutL homologs. *Proc. Nat. Acad. Sci. U.S.A.*, **92**, 1950–1954.
97. Hong,Z., Jiang,J., Hashiguchi,K., Hoshi,M., Lan,L. and Yasui,A. (2008) Recruitment of mismatch repair proteins to the site of DNA damage in human cells. *J. Cell Sci.*, **121**, 3146–3154.
98. Jardim,M.J., Wang,Q., Furumai,R., Wakeman,T., Goodman,B.K. and Wang,X.F. (2009) Reduced ATR or Chk1 expression leads to chromosome instability and chemosensitization of mismatch repair-deficient colorectal cancer cells. *Mol. Biol. Cell*, **20**, 3801–3809.
99. Tsubouchi,H. and Ogawa,H. (2000) Exo1 roles for repair of DNA double-strand breaks and meiotic crossing over in Saccharomyces cerevisiae. *Mol. Biol. Cell*, **11**, 2221–2233.
100. Niu,H., Raynard,S. and Sung,P. (2009) Multiplicity of DNA end resection machineries in chromosome break repair. *Genes Dev.*, **23**, 1481–1486.
101. Cejka,P. (2015) DNA end resection: nucleases team up with the right partners to initiate homologous recombination. *J. Biol. Chem.*, **290**, 22931–22938.
102. Ochs,F., Somyajit,K., Altmeyer,M., Rask,M.B., Lukas,J. and Lukas,C. (2016) 53BP1 fosters fidelity of homology-directed DNA repair. *Nat. Struct. Mol. Biol.*, **23**, 714–721.
103. Baker,S.M., Plug,A.W., Prolla,T.A., Bronner,C.E., Harris,A.C., Yao,X., Christie,D.M., Monell,C., Arnheim,N., Bradley,A. *et al.* (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nat. Genet.*, **13**, 336–342.
104. Yoon,H.H., Shi,Q., Heying,E.N., Muranyi,A., Bredno,J., Ough,F., Djalilvand,A., Clements,J., Bowermaster,R., Liu,W.W. *et al.* (2019) Intertumoral heterogeneity of CD3(+) and CD8(+) T-cell densities in the microenvironment of DNA mismatch-repair-deficient colon cancers: implications for prognosis. *Clin. Cancer Res.*, **25**, 125–133.
105. Shimasaki,N., Jain,A. and Campana,D. (2020) NK cells for cancer immunotherapy. *Nat. Rev. Drug Discov.*, **19**, 200–218.
106. Marcus,A., Mao,A.J., Lensink-Vasan,M., Wang,L., Vance,R.E. and Raulet,D.H. (2018) Tumor-derived cGAMP triggers a STING-mediated interferon response in non-tumor cells to activate the NK cell response. *Immunity*, **49**, 754–763.
107. Howitt,B.E., Shukla,S.A., Sholl,L.M., Ritterhouse,L.L., Watkins,J.C., Rodig,S., Stover,E., Strickland,K.C., D'Andrea,A.D., Wu,C.J. *et al.* (2015) Association of polymerase e-mutated and microsatellite-instable endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1. *JAMA Oncol.*, **1**, 1319–1323.
108. Llosa,N.J., Cruise,M., Tam,A., Wicks,E.C., Hechenbleikner,E.M., Taube,J.M., Blosser,R.L., Fan,H., Wang,H., Luber,B.S. *et al.* (2015)

The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov.*, **5**, 43–51.
109. Vanderwalde,A., Spetzler,D., Xiao,N., Gatalica,Z. and Marshall,J. (2018) Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Med.*, **7**, 746–756.
110. Grasso,C.S., Giannakis,M., Wells,D.K., Hamada,T., Mu,X.J., Quist,M., Nowak,J.A., Nishihara,R., Qian,Z.R., Inamura,K. *et al.* (2018) Genetic mechanisms of immune evasion in colorectal cancer. *Cancer Discov.*, **8**, 730–749.
111. Germano,G., Lu,S., Rospo,G., Lamba,S., Rousseau,B., Fanelli,S., Stenech,D., Le,D.T., Hays,J., Totaro,M.G. *et al.* (2021) CD4 T cell-dependent rejection of beta-2 microglobulin null mismatch repair-deficient tumors. *Cancer Discov.*, **11**, 1844–1859.
112. Heim,M.H., Kerr,I.M., Stark,G.R. and Darnell,J.E. Jr (1995) Contribution of STAT SH2 groups to specific interferon signaling by the Jak-STAT pathway. *Science*, **267**, 1347–1349.
113. Philips,R.L., Wang,Y., Cheon,H., Kanno,Y., Gadina,M., Sartorelli,V., Horvath,C.M., Darnell,J.E. Jr, Stark,G.R. and O'Shea,J.J. (2022) The JAK-STAT pathway at 30: much learned, much more to do. *Cell*, **185**, 3857–3876.
114. Zaretsky,J.M., Garcia-Diaz,A., Shin,D.S., Escuin-Ordinas,H., Hugo,W., Hu-Lieskovan,S., Torrejon,D.Y., Abril-Rodriguez,G., Sandoval,S., Barthly,L. *et al.* (2016) Mutations associated with acquired resistance to PD-1 blockade in Melanoma. *N. Engl. J. Med.*, **375**, 819–829.
115. Shin,D.S., Zaretsky,J.M., Escuin-Ordinas,H., Garcia-Diaz,A., Hu-Lieskovan,S., Kalbasi,A., Grasso,C.S., Hugo,W., Sandoval,S., Torrejon,D.Y. *et al.* (2017) Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. *Cancer Discov.*, **7**, 188–201.
116. Albacker,L.A., Wu,J., Smith,P., Warmuth,M., Stephens,P.J., Zhu,P., Yu,L. and Chmielecki,J. (2017) Loss of function JAK1 mutations occur at high frequency in cancers with microsatellite instability and are suggestive of immune evasion. *PLoS One*, **12**, e0176181.
117. Spranger,S., Bao,R. and Gajewski,T.F. (2015) Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature*, **523**, 231–235.
118. Trabucco,S.E., Gowen,K., Maund,S.L., Sanford,E., Fabrizio,D.A., Hall,M.J., Yakirevich,E., Gregg,J.P., Stephens,P.J., Frampton,G.M. *et al.* (2019) A novel next-generation sequencing approach to detecting microsatellite instability and pan-tumor characterization of 1000 microsatellite instability-high cases in 67,000 patient samples. *J. Mol. Diagn.*, **21**, 1053–1066.
119. Maruvka,Y.E., Mouw,K.W., Karlic,R., Parasuraman,P., Kamburov,A., Polak,P., Haradhvala,N.J., Hess,J.M., Rheinbay,E., Brody,Y. *et al.* (2017) Analysis of somatic microsatellite indels identifies driver events in human tumors. *Nat. Biotechnol.*, **35**, 951–959.
120. Michel,S., Benner,A., Tariverdian,M., Wentzensen,N., Hoefler,P., Pommerencke,T., Grabe,N., von Knebel Doeberitz,M. and Kloor,M. (2008) High density of FOXP3-positive T cells infiltrating colorectal cancers with microsatellite instability. *Br. J. Cancer*, **99**, 1867–1873.
121. Bauer,K., Michel,S., Reuschenbach,M., Nelius,N., von Knebel Doeberitz,M. and Kloor,M. (2011) Dendritic cell and macrophage infiltration in microsatellite-unstable and microsatellite-stable colorectal cancer. *Fam. Cancer*, **10**, 557–565.
122. Hu,W., Yang,Y., Qi,L., Chen,J., Ge,W. and Zheng,S. (2019) Subtyping of microsatellite instability-high colorectal cancer. *Cell Commun Signal*, **17**, 79.
123. Onishi,Y., Fehervari,Z., Yamaguchi,T. and Sakaguchi,S. (2008) Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 10113–10118.
124. Mitchell,M.J., Billingsley,M.M., Haley,R.M., Wechsler,M.E., Peppas,N.A. and Langer,R. (2021) Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov*, **20**, 101–124.
125. Liu,W., Alameh,M.G., Yang,J.F., Xu,J.R., Lin,P.J.C., Tam,Y.K., Weissman,D. and You,J. (2022) Lipid nanoparticles delivering constitutively active STING mRNA to stimulate antitumor immunity. *Int. J. Mol. Sci.*, **23**, 14504.
126. Chan,E.M., Shibue,T., McFarland,J.M., Gaeta,B., Ghandi,M., Dumont,N., Gonzalez,A., McPartlan,J.S., Li,T., Zhang,Y. *et al.* (2019) WRN helicase is a synthetic lethal target in microsatellite unstable cancers. *Nature*, **568**, 551–556.
127. van Wietmarschen,N., Sridharan,S., Nathan,W.J., Tubbs,A., Chan,E.M., Callen,E., Wu,W., Belinky,F., Tripathi,V., Wong,N. *et al.* (2020) Repeat expansions confer WRN dependence in microsatellite-unstable cancers. *Nature*, **586**, 292–298.
128. Andre,T., Lonardi,S., Wong,K.Y.M., Lenz,H.J., Gelsomino,F., Aglietta,M., Morse,M.A., Van Cutsem,E., McDermott,R., Hill,A. *et al.* (2022) 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.*, **33**, 1052–1060.