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Mini-review

Immunotherapy holds the key to cancer treatment and prevention in constitutional mismatch repair deficiency (CMMRD) syndrome

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ABSTRACT

Monoallelic germline mutations in one of the DNA mismatch repair (MMR) genes cause Lynch syndrome, with a high lifetime risks of colorectal and endometrial cancer at adult age. Less well known, is the constitutional mismatch repair deficiency (CMMRD) syndrome caused by biallelic germline mutations in MMR genes. This syndrome is characterized by the development of childhood cancer. Patients with CMMRD are at extremely high risk of developing multiple cancers including hematological, brain and intestinal tumors. Mutations in MMR genes impair DNA repair and therefore most tumors of patients with CMMRD are hypermutated. These mutations lead to changes in the translational reading frame, which consequently result in neoantigen formation. Neoantigens are recognized as foreign by the immune system and can induce specific immune responses. The growing evidence on the clinical efficacy of immunotherapies, such as immune checkpoint inhibitors, offers the prospect for treatment of patients with CMMRD. Combining neoantigen-based vaccination strategies and immune checkpoint inhibitors could be an effective way to conquer CMMRD-related tumors. Neoantigen-based vaccines might also be a preventive treatment option in healthy biallelic MMR mutation carriers. Future studies need to reveal the safety and efficacy of immunotherapies for patients with CMMRD.

Introduction

Lynch syndrome (LS) is an autosomal dominant tumor syndrome predisposing to predominantly colorectal cancer (CRC) and endometrial carcinoma at an early age of onset, with a mean age of 45 years [1]. Monoallelic germline mutations in one of the DNA mismatch repair (MMR) genes cause malignancies in patients with LS when a second hit inactivates the wildtype allele (Fig. 1) [2–5]. The MMR genes involved in LS are *MLH1, MSH2, MSH6,* and *PMS2*. Biallelic mutations in one of these MMR genes cause constitutional mismatch repair-deficiency (CMMRD). CMMRD is a rare recessively inherited syndrome mainly characterized by café-au-lait spots and a broad spectrum of childhood malignancies, primarily hematological, brain and intestinal tract tumors [6–9]. The prognosis for patients with CMMRD is much worse than for patients with LS due to the types of cancer and the high risk of multiple primary malignancies [10]. In children with CMMRD, no somatic mutations have to arise in the MMR genes to start tumorigenesis, since affected individuals inherit a germline MMR mutation from each parent. These biallelic MMR mutations, either homozygous or compound heterozygous, cause loss of genomic integrity by the inability of cells to repair DNA damage. This results in high numbers of mutations, mainly consisting of insertion and deletion mutations at repetitive DNA sequences known as microsatellites. Repeated DNA structures are prone to DNA polymerase slippage during DNA replication [11]. Due to these insertions and deletions, the length of the repeating sequences increases or decreases leading to

Abbreviations: CMMRD, constitutional mismatch repair deficiency; COX, cyclooxygenase; CRC, colorectal cancer; CTLs, cytotoxic T lymphocytes; GBM, glioblastoma multiforme; LS, Lynch syndrome; MMR, mismatch repair; MSI, microsatellite instability; Tregs, regulatory T cells.

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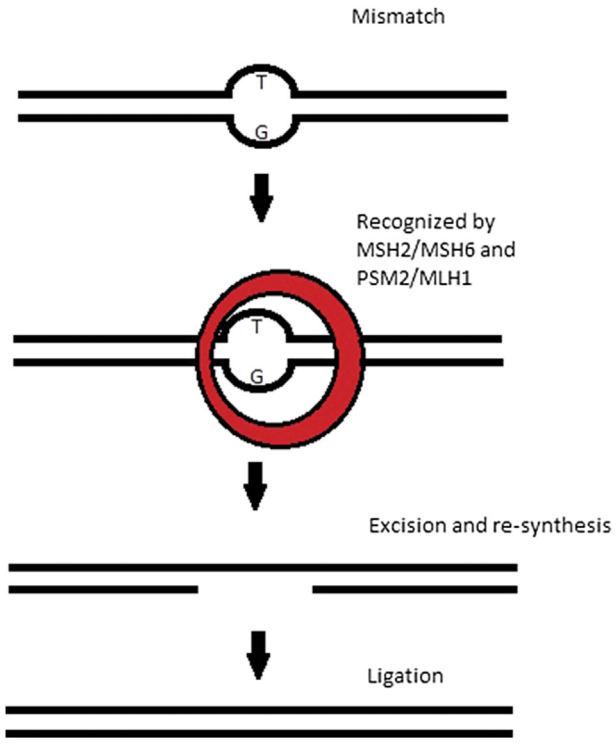

Fig. 1. Schematic figure of the mismatch repair pathway. A base mismatch is recognized by mismatch repair proteins (MSH2/MSH6 and PMS2/MLH1). The mismatch on the newly synthesized strand is excised and the correct nucleotides are synthesized. Finally, the DNA strands are ligated.

microsatellite instability (MSI). When microsatellites in gene-encoding regions are affected it can cause inactivation of the gene products through shifting of the translational reading frame leading to truncated or nonfunctional proteins (Fig. 2) [12–14]. Truncated proteins can be processed into peptides and presented on the surface of mutated cells. Eventually, so-called ultra-hypermutated tumor cells arise. All ultra-hypermutated tumors harbor mutations in polymerase genes *POLE* or *POLD1* and have an upper limit of exonic mutations which is tolerable [15,16]. Fortunately, endogenous ultra-hypermutated tumor cells can be recognized by the immune system as foreign as they can be identified by their private mutanome-derived epitopes called neoantigens. Most of the tumors that present in patients with CMMRD are ultra-hypermutated and thus express neoantigens, which can result in an immune response against these tumor-specific neoantigens [17]. Generally speaking, tumors that are recognized by the patients' own immune system have an improved prognosis [18,19]. Studies have shown that microsatellite instable CRCs of LS patients with a high density of tumor-infiltrating lymphocytes are associated with a better prognosis than microsatellite stable (MSS) CRCs [20,21]. Until now, very little is known about immune responses against neoantigens in CMMRD patients. In this review we discuss the potential of neoantigens to elicit immune responses in patients with CMMRD and probable immunotherapeutic treatment options.

Biallelic mutations in MMR genes and their risk of cancer

Individuals with CMMRD are at increased risk for developing malignant gliomas, hematologic malignancies, and gastrointestinal tract cancers at young age. It is a highly penetrating cancer predisposition syndrome, with most biallelic mutation carriers developing cancer in the first two decades of life [8,9]. Hematological malignancies usually arise in infancy or early childhood and are more frequent in patients with *MLH1* or *MSH2* mutations than in patients with mutations in *MSH6* or *PSM2* [6]. The latter group appears to have a higher prevalence of brain tumors which develop later during childhood. CRC in patients with CMMRD is most frequently found as a second or third primary malignancy and arises in adolescence or young adulthood. The prevalence of CRC is higher in patients with biallelic *PMS2* and *MSH6* [6,22–24].

Prophylactic cancer surveillance and preventive treatments

When a patient is diagnosed with CMMRD syndrome, family members can be screened for heterozygous and homozygous mutations by performing a mutation analysis of peripheral blood DNA. Especially, siblings need to be screened since the chance for a homozygous mutation is 25% resulting in CMMRD, and an additional

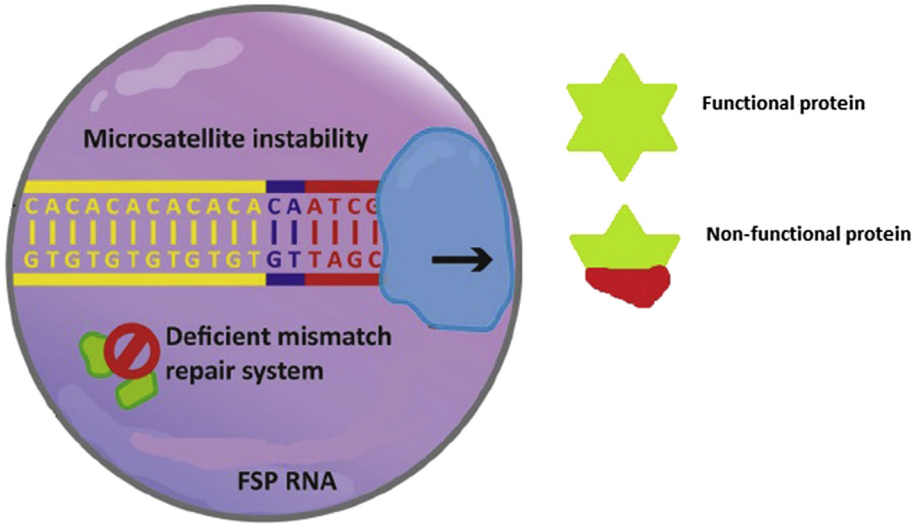

Fig. 2. Normally an insertion or deletion in a microsatellite sequence is repaired by the mismatch repair pathway to prevent that mutations become permanent and affect the protein. Mismatch repair deficient cells are unable to repair insertions or deletion, here indicated with an inserted CA sequence (purple), leading to a frameshift mutation. The coding sequence is altered due to shifting of the translational reading frame, which can result in a truncated or non-functional protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chance of 50% for heterozygous mutation resulting in LS. Patients with CMMRD may benefit from prophylactic cancer surveillance or preventive treatments through use of aspirin or ibuprofen. Surveillance may lead to earlier recognition of asymptomatic tumors which are better resectable and might be curable [25]. To improve the prognosis of CMMRD patients, surveillance should be offered for CRC and brain tumors [9]. The effectiveness of hematological screening is questionable since non-Hodgkin lymphomas and acute lymphoid leukemia are rapidly growing tumors and surveillance may not improve the outcome for patients with these malignancies [10]. In heterozygous MMR mutation carriers, daily intake of aspirin for approximately two years reduced the CRC incidence after a period of almost five years [26]. Another study reported that both the use of aspirin and ibuprofen might be effective in reducing the CRC risk for MMR gene mutation carriers [27]. Aspirin and ibuprofen, which belongs to the group of drugs called non-steroidal anti-inflammatory drugs, both inhibit cyclooxygenase (COX) enzymes resulting in decreased prostaglandin synthesis [28,29]. One isoform of COX, named COX-2 promotes inflammation and cell proliferation. In colorectal carcinogenesis the COX-2 enzyme is often overexpressed [28]. Prostaglandins are thought to regulate apoptosis, angiogenesis, and tumor-cell invasiveness [30]. Therefore, aspirin and non-steroidal anti-inflammatory drugs could reduce the risk of COX-2 expressing tumors. Whether there is overexpression of COX-2 in LS-associated CRC or CMMRD-related CRC is unknown. Regular aspirin use was also shown to lower the risk of CRC with low numbers of tumor-infiltrating lymphocytes. This was independent of MSI status [31]. It is postulated that aspirin, either related or unrelated to the inhibition of prostaglandins, may inhibit cancer development by overcoming suppression of T cell mediated antitumor immunity [32]. These results improve the understanding of the mechanisms through which aspirin exerts antineoplastic effects and provides support for the potential of exploiting immune mechanisms for cancer prevention [31]. Preventive treatments with aspirin and non-steroidal anti-inflammatory drugs might also reduce the incidence of cancer in patients with CMMRD.

Current treatment strategies

The treatment of childhood cancer is dependent of the specific location and the type of cancer. Since CMMRD is very rare, there is limited information on optimal therapeutic strategies. All described responses to chemotherapy are based on case report studies only. Chemotherapy treatment comes at the cost of toxicity. Therefore, careful selection of therapies is required for children with CMMRD. This should be based on the toxicity profile of specific agents as well as known tumor resistance to specific therapies [33].

MMR deficient cells are profoundly resistant to alkylating antineoplastic agents, such as temozolomide, cisplatin or busulfan. Temozolomide is frequently used in the standard treatment of glioblastomas multiforme (GBM) [10,34]. This is of clinical importance in patients with CMMRD, since it has become clear that these agents are less effective in MMR-deficient tumors and even may provide a growth advantage for the tumor cells [35]. Indeed, several studies have reported that treatment of GBM with temozolomide can promote further MMR deficiency due to loss of MSH2 or MSH6 expression, leading to temozolomide resistance [36,37]. *In vitro* studies also showed reduced sensitivity to the DNA damaging agents cisplatin and busulfan. Only one out of six patients with CMMRD-related GBM treated with temozolomide and radiotherapy showed a clinical response. The other patients' tumors were resistant to this therapy [10]. Another study showed that temozolomide treatment leads to accumulation of somatic mutations [38]. Moreover, temozolomide increases the risk of second primary tumors in patients with CMMRD because of their inability to repair the accumulated somatic mutations [9,10,39,40]. On the contrary, recent data supports that temozolomide treatment in glioma cells can boost the adaptive immune response [41].

Whether temozolomide, cisplatin and busulfan are safe in patients with CMMRD is still controversial and requires further clinical studies [10]. Until now it remains unclear which chemotherapeutic regimen is most effective and the least dangerous.

The role of the immune system in cancer

The immune system is able to protect the body against cancer development. Cancer cells are immunogenic through the generation of tumor-specific antigens that are the consequence of somatic mutations and epigenetic alterations in the DNA. Immune cells, such as killer T lymphocytes and natural killer cells, are capable of tumor cell killing by the release of cytolytic granules, such as perforin and granzymes. Tumor cells with a less immunogenic phenotype are able to escape the immune attack and progress further, leading to resistance for immune detection. Tumors can acquire several immune escape mechanisms which include defective antigen presentation, lack of tumor antigen recognition, loss of sensitivity by cancer cells through secretion of immunosuppressive cytokines, induction of inhibitory checkpoint receptors, and infiltrating immunosuppressive immune cells [42–44]. Immunosuppressive cells, such as Foxp3-positive regulatory T cells (Tregs) and myeloid-derived suppressor cells can suppress the immune response against tumor-specific antigens by suppression of cytotoxic T lymphocytes (CTLs) [22]. Although, several studies have shown that high Treg infiltration in MSI CRC is correlated with a poor outcome [45,46]. Others did not confirm this observation. Recently, it has been shown that the loss of HLA class I antigen expression as a result of beta-2 microglobulin mutations in LS tumors, leads to less Treg infiltration [47]. Therefore, more research is needed to elucidate the role of Tregs and the exact location of these cells within the tumor [45–51]. Knowledge about the presence of Tregs in tumors and blood of patients with CMMRD is not yet available. Studies on the numbers and localization of CTLs, Tregs, myeloid-derived suppressor cells, and specific dendritic cell subsets could be very informative. The presence of these immune cells and the beta-2 microglobulin status may provide key information about the immunogenicity of CMMRD-associated tumors and about a proposed immunosuppressive tumor microenvironment.

Opportunities for cancer immunotherapy

The immune system encompasses inhibitory mechanisms to prevent excessive and therefore damaging immune responses. These inhibitory mechanisms are necessary for balanced immunity in normal homeostasis. However, in the presence of a growing malignancy the balance is disrupted and skewed towards excessive inhibition of immune reactivity due to tumor-induced immune suppression and enhanced immunologic tolerance [52]. Currently, immunotherapies are directed against inhibiting receptors, such as programmed death 1 (PD-1) protein, a T cell co-inhibitory receptor, and one of its ligands programmed death-ligand 1 (PD-L1). PD-L1 is typically expressed on the surface of tumor cells and inhibits activation of T cells through its receptor PD-1. In many tumors the expression of PD-L1 is aberrantly up-regulated and can provide inhibitory signals to activated T cells. PD-L1 expressing tumor cells can evade immune detection and prevent effective host antitumor immunity [42,53,54]. Antibodies that interfere with this pathway by blocking PD-1 or its ligand, have led to significant clinical responses in patients with many different types of cancer [54–58]. Several studies have shown that tumors with a high mutation load

and therefore a higher chance of expressing neoantigens were most likely to respond to immune checkpoint blockers [59,60]. A more diverse repertoire of neoantigens increases the chance of a tumor-specific T cell response [61]. MMR deficient tumors are more responsive to PD-1 blockades than MMR proficient tumors [54]. MMR deficient tumors harbor 10–100 times more somatic mutations and therefore more neoantigens are present which are able to elicit an immune response. Tumors of MMR deficient patients also showed a high number of tumor-infiltrating lymphocytes which is correlated with a better prognosis [54,62]. This data strongly suggest that treatment with immune checkpoint blockade can be very attractive for MMR deficient tumors independent of the underlying tumor type [61]. Likewise, immunotherapy with checkpoint inhibitors could hold the key to an effective cancer treatment and prevention strategy of CMMRD syndrome.

Children with GBM have a poor prognosis and sometimes have underlying germline mutations in *TP53* (Li-Fraumeni syndrome) or the MMR genes [9]. Primary management consists of surgical resection followed by radiation therapy and chemotherapy. Pediatric CMMRD-related GBM have similar outcomes to sporadic childhood GBMs [63]. The mean time from relapse to death is less than three months in CMMRD GBM. In a recent study, two siblings with CMMRD and relapsed GBM were treated with anti-PD-1-directed immune checkpoint inhibitor. Clinically significant responses and a profound radiologic response were observed. No severe treatment-related side effects were observed, except the presentation of seizures, hyponatremia or apparent disease flaring. However, these symptoms are possibly GBM-related and not associated with anti-PD-1 treatment. The results of this study may have implications for treatment of GBM in general and other ultra-hypermutated cancers [59]. Other recent data have demonstrated that checkpoint inhibitors are effective in the treatment of Hodgkin lymphomas and also appear efficient against some non-Hodgkin lymphomas [64,65]. Non-Hodgkin lymphomas are over-represented among hematological tumors in CMMRD patients, and treatment of Non-Hodgkin lymphoma patients is improving through the development of targeted therapies [9]. In a subset of Hodgkin lymphomas and in genetically related non-Hodgkin lymphomas, genetic amplification of the loci encoding the PD-1 ligands have been discovered. The overexpression of PD-1 on lymphoma cells may play a critical role in immune evasion by these cancers and makes it therefore an attractive target for checkpoint inhibitor therapy [66,67]. Further studies are required to investigate whether other tumors in patients with CMMRD will benefit from checkpoint inhibitor therapy.

Cellular immunotherapies, such as vaccines can also be used as a strategy to elicit a potent immune response against cancer antigens. However, the overall clinical efficacy of therapeutic cancer vaccines is disappointing until now [68,69]. This is probably due to the difficulty of identifying tumor-specific target antigens and overcoming the immunosuppressive tumor microenvironment. Tumor-specific antigens should be uniquely expressed by the tumor or overexpressed on the tumors as compared to normal cells. In the past years, multiple tumor vaccine strategies have been developed, such as tumor cell vaccines, tumor-associated antigen vaccines, and dendritic cell vaccines [68,70,71]. Anti-cancer vaccines, especially dendritic cell vaccination, might be effective when combined with immune-checkpoint inhibitors [72].

MSI in LS is associated with lymphocyte infiltration and a comparatively favorable prognosis. Tumors with MSI give rise to the generation of potentially immunogenic frameshift-derived neoantigens. For example, the *OGT* gene is commonly mutated in MSI colorectal tumors, and neoantigens derived from this protein stimulate CTLs that recognize this neoantigen [21]. Also TGFβRII derived neoantigens are highly immunogenic and are applicable as target for tumor infiltrating $CD4^+$ T cells in MSI tumors [62]. Vaccination with neoantigens is a promising approach for the treatment of LS patients, but also a promising approach for tumor prevention in healthy LS mutation carriers [22,73]. Neoantigen-loaded vaccination strategies are, in contrast to checkpoint inhibitors, antigen specific and should therefore elicit a specific effector- and memory cell response [70]. Several clinical studies are investigating the response of anti-cancer vaccines in LS patients. Currently, in a clinical phase I/II trial neoantigen-loaded dendritic cells vaccinations are studied in CRC patients with MSI or healthy germline MMR-gene mutation carriers. The primary objective is safety and feasibility, and secondary endpoints cover induction or enhancement of an immune response (NCT01885702). In another ongoing clinical phase I/IIa trial frameshift-derived neoantigen vaccination against *AIM2, HT001,* and *TAF1B* is studied (NCT01461148). Preliminary data suggest that neoantigen vaccination is safe and well tolerated [74]. Strong immune responses against neoantigens in all vaccinated patients were observed so far. LS mutation carriers already show neoantigens-specific immune responses [75]. Therefore, immune surveillance mechanisms with specific T cell responses may play an important role in preventing MSI tumor development in LS mutation carriers. Neoantigen vaccination strategies may be applied in future standard of care as an adjuvant therapy in MSI CRC patients or even as a preventive vaccine in MMR germline mutation carriers. Moreover, in patients with CMMRD adjuvant or preventive neoantigens-based vaccinations could be the basis for an effective treatment regimen for the induction of anti-cancer immune responses [74]. The number of CMMRD patients is limited, which makes it difficult to identify commonly mutated coding microsatellites. However, there might be overlap in the genes that are mutated early in tumorigenesis or neoantigens that are shared among tumors of a particular organ [21]. One must be cautious for selecting neoantigens for vaccination because every cell in CMMRD patients is MMR deficient, and when the neoantigen is present in healthy cells it might result in immune-related adverse events and might trigger autoimmune diseases [76,77]. Therefore, selection of cell growth- or apoptosis-related neoantigens might be the safest way to go.

Conclusion and future perspectives

CMMRD is a cancer predisposition syndrome with a high mortality rate. Currently, no curative treatment options are available for patients with CMMRD. CMMRD-derived tumors are ultra-hypermutated, leading to a high neoantigen load. This makes these tumors attractive for treatment with checkpoint inhibitors or neoantigen-based vaccination strategies. However, as described, several immunosuppressive hurdles need to be overcome. It has been hypothesized that the potential of immunotherapies might increase in case of combination therapy. (Neo)antigen-specific vaccination and checkpoint inhibition could act complementary, as a vaccine activates the immune system in a (neo)antigen-specific manner and concomitant or subsequent treatment with immune checkpoint inhibitors could boost the induced response to overcome immunosuppression. In addition, it has been shown in mice that antigen cross-presentation by DCs is even necessary for immune checkpoint inhibitor-induced immune responses to tumor-associated (neo)antigens [78]. A number of studies have analyzed the effect of anti-CTLA4 treatment after vaccination with DCs loaded with shared antigens. Pierret and colleagues suggested that anti-CTLA-4 treatment of advanced melanoma may be more effective after prior treatment with DC vaccination [79]. Another study suggested that anti-CTLA-4 treatment enhanced the T cell responses induced by prior DNA or protein vaccination [80]. In addition, in small clinical trials in melanoma patients and prostate

cancer patients, the combination of anti-CTLA-4 antibodies and DC-based immunotherapy [81,82] or other forms of antigen-specific immunotherapy [83,84] seemed more effective than treatment with either agent alone. Future research is needed to investigate the clinical effects and potential risks of these therapies in metastatic, adjuvant, and even preventive settings in patients with CMMRD.

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Conflict of interest

All authors declare that they have no competing financial interests.

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