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Review Article

PD-1 blockade as a future treatment for colorectal cancer with microsatellite instability

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ABSTRACT

Introduction: Colorectal cancer is the third most common cancer worldwide, with about 15% of these tumours related with microsatellite instability, which confers distinct characteristics to these tumours, both clinicopathological and in the response to treatments. In fact, the poor response to chemotherapy in these tumours has led to the investigation for new treatments, with immunotherapy being the most successful one to date. The focus of this review is to assess the response of microsatellite unstable colorectal cancer to PD-1 blockade, and the mechanisms behind that response.

Methods: A PubMed research was conducted, resulting in the inclusion of 47 articles in this review.

Results: Microsatellite instability results in a high neoantigen load, leading to a highly active immune microenvironment of the tumour, mainly due to T-cells. To counteract this, there is an upregulation of PD-1, acting as a "brake" for immune cells, facilitating tumour growth and metastasis. This upregulation makes these tumours great candidates for treatment with PD-1 blockade, as seen in many clinical trials, where the overall responses and progression free survival rates were higher than those observed in microsatellite stable tumours.

Conclusion: With the importance of colorectal cancer with microsatellite instability new treatments are necessary. Therefore, PD-1 blockade is a promising treatment for colorectal cancer with microsatellite instability, with improvement in survival rates and a better prognosis for these patients.

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Bloqueio do PD-1 como futuro tratamento do cancro colorretal com instabilidade de microssatélites

Palavras-chave:
Câncer colorretal
Instabilidade de microssatélites
Imunoterapia
Receptor PD-1
Tratamento

R E S U M O

Introdução: O câncer colorretal é o terceiro mais comum em todo o mundo, com cerca de 15% desses tumores relacionados com instabilidade dos microssatélites, o que confere características distintas a esses tumores, tanto clínico patológicas quanto na resposta aos tratamentos. De fato, a fraca resposta à quimioterapia nesses tumores levou à investigação de novos tratamentos, sendo a imunoterapia a mais bem sucedida até o momento. O foco desta revisão é avaliar a resposta do câncer colorretal com microssatélites instáveis ao bloqueio do PD-1 e os mecanismos por trás dessa resposta.

Métodos: Foi realizada uma pesquisa na base de dados PubMed, resultando na inclusão de 47 artigos nesta revisão.

Resultados: A instabilidade de microssatélites resulta em uma alta carga de neoantígenos, levando a um microambiente imunológico altamente ativo do tumor, principalmente devido às células T. Para neutralizar isso, há uma maior expressão do PD-1, atuando como um "freio" para as células imunes, facilitando o crescimento do tumor e suas metástases. Essa expressão faz desses tumores grandes candidatos ao tratamento com bloqueio PD-1, como demonstrado em vários ensaios clínicos, onde as respostas globais e as taxas de sobrevivência livres de progressão foram maiores do que as observadas em tumores com microssatélites estáveis.

Conclusão: Com a importância do câncer colorretal com instabilidade de microssatélites, novos tratamentos são necessários. Portanto, o bloqueio do PD-1 é um tratamento promissor para o câncer colorretal com instabilidade de microssatélites, com melhora nas taxas de sobrevivência e melhor prognóstico para esses pacientes.

Introduction

Colorectal Cancer (CRC) is currently the third most common cancer in men worldwide, and the second in women, with circa 55% occurring in more developed regions, with surgery and chemotherapy remaining the standard of care at the moment.[1]

In the USA, the 5-year survival rates after surgical removal of CRC for localized (Stage I), regional (Stages II and III) and distant (Stage IV) tumours are 91%, 71% and 13%, respectively.[2]

Acquired Somatic Microsatellite Instability (MSI) is seen in 15% of sporadic CRCs, and hereditary MSI is associated with 5% of all CRCs[3,4] but with only 5% of metastatic cancers.[5]

MSI is associated with dMMR (deficient Mismatch Repair), commonly associated with a better prognosis and improved Overall Survival (OS), except in head and neck cancer and pancreatic cancer.[6,7] Loss of function in one of the mismatch repair proteins leads to accumulation of mistakes in microsatellite regions in the DNA, therefore resulting in MSI and accumulation of replication errors, responsible for about 15% of all CRCs.[7] MSI can be determined by immunohistochemistry, PCR and next-generation sequencing.[5]

The existing therapies for CRC include surgery for early staged tumours and surgery, chemotherapy and/or radiotherapy in more advanced stages.[8] However, MSI status can be considered a predictive factor of non-response to chemotherapy based in 5-Fluorouracil (5-FU).[9] It is also known that stage II-III MSI CRCs achieve similar Progression-Free Survival (PFS) and OS, with or without 5-FU-based neoadjuvant chemotherapy,[4,6] making them good candidates for a different therapeutic approach, namely immunotherapy.[3,10–12] In fact, after failure of therapy with FOLFOX and FOLFIRI, prolonged survival isn't common.[13]

Indeed, tumours with dMMR/MSI are commonly resistant to therapies with methylating agents, platinum compounds and fluoropyrimidines.[7,14,15]

On the other hand, the improved prognosis of MSI CRCs may be a result of the high anti-tumour immune response of the host.[4] In fact, tumours with MSI have an important lymphocytic infiltration, making them good candidates for therapies based in the activation of the immune system.[7]

Microsatellite unstable tumours are characteristically tumours with a highly active tumour microenvironment, with dense T-cell infiltrations and upregulation on checkpoint regulators, assuring they are great candidates to Immune Checkpoint Inhibitors (ICIs).[16] Besides, MSI is accompanied by a high tumour mutational burden, yet another marker related to the response to ICIs.[17]

Tumours can evade immune surveillance by expressing multiple molecules responsible for the immune response against the tumour, such as Il-10, Transforming Growth Factor-β (TGF-β), immune co-inhibitory signalling proteins such as Programmed cell Death protein 1 (PD-1), Programmed cell Death 1 ligand 1 (PD-L1) and Cytotoxic T Lymphocyte-Associated antigen 4 (CTLA4).[18] PD-1 acts as a "brake" for

immune cells inhibiting T-cells, and therefore the immune response to tumours.[16,19] Thus, the PD-1/PD-L1 axis may be responsible for inducing immune inhibition or exhaustion, resulting in a decrease in activated T-cells. Therefore, the anti-tumour immune response is impaired, making this the rationale for the treatment with blockade of this axis, which allows the recovering of native anti-tumour function of T-cells, facilitating tumour regression.[20] Inhibitors of the PD1/PD-L1 axis interrupt the immunoediting incited by this axis, resulting in stimulation of T cells and antitumor activity.[15]

With the increase in OS of patients with CRC, there is also an increase in the cost of treatment,[21] making the choice of treatment even more important.

With the favourable outcomes of these immunotherapies, the Food and Drug Administration (FDA) approved pembrolizumab for the treatment of adult and paediatric patients with advanced MSI/dMMR tumours in the 23rd of May 2017. Furthermore, on the 1st of July 2017, an accelerated approval was granted by the FDA for the use of nivolumab in patients with metastatic CRC with a MSI/dMMR phenotype.[22] Besides, FDA has also approved dMMR/MSI as indicators of response to ICIs for metastatic cancers irrespective of cancer type.[6]

This review is aimed at understanding the characteristics of CRC with MSI and the ways in which MSI influences tumour response to immunotherapy, focusing on the role of PD-1 blockade in the treatment of CRC with MSI.

Methods

In this narrative review, a web search was done on the PubMed database on September 2019, using the following query: "(((Immunotherapy OR pembrolizumab OR nivolumab)) AND Colorectal Cancer) AND (Microsatellite instability OR MSI)". Using this query, a total of 245 articles were obtained.

When refining the search for articles published only in the last five years and filtering for the languages of Portuguese and English, a total of 226 articles were obtained. No exclusion criteria were used (Fig. 1). Based on the title, 97 articles were excluded mainly due to the referral of other tumour types such as melanoma, endometrium and urological. This exclusion was also based on studies based only on specific populations, genetic alterations and on genomic sequencing. With this exclusion, 129 articles were analysed based on their abstract. This analysis resulted in the exclusion of 34 articles, with articles focusing on tumour diagnosis methods, explanation of tumour pathophysiology, studies with the main focus on microsatellite stable tumours and only specific populations studied.

This resulted in a total of 95 articles remaining for the full text analysis, with the exclusion of 48 articles, mainly due to the unavailability of the full text and the language of the article (some articles in Czech and French). Besides, some articles were also excluded because of their outdated information (mainly the articles from 2015). With this process, 47 articles were selected and included in this review.

MeSH terms

"immunotherapy"; "microsatellite instability"; "nivolumab"; "colorectal neoplasms"

Supplementary Concept: "pembrolizumab".

Characteristics of CRC with MSI

MSI manifests with an increased variability in the size of microsatellites throughout the patientsǵenome, leading to further DNA mutations, which increases neoantigen production, making the tumour more recognizable to the immune system.[4,6,23] Associated with the increase in dendritic cells, this facilitates the presentation of tumour cells to cytotoxic T lymphocytes, resulting in an increase in tumour infiltrating lymphocytes, mainly $CD8^+$. This is, in part, related to the better prognosis usually associated with CRC with MSI.[4–24]

MSI CRC is clinicopathologically different than other subtypes of CRC, namely with a younger age of onset, proximal location, lymphocytic reaction, mucinous/signet ring differentiation and a medullary growth pattern. Besides, MSI is usually associated with a reduced risk for nodal and distant metastases, with an increased Disease Free Survival (DFS) in early stages.[4,6]

The majority of MSI CRCs are included in the Molecular Subtype 1 of the Consensus Molecular Subtype Classification, a group of hypermutated tumours with strong immune activation.[2,3,25] This subgroup is usually associated with a better prognosis, with intratumoral heterogeneity (mixed conventional, mucinous, and poorly differentiated carcinoma), and a prominent inflammatory reaction at the advancing edge of the tumour (Crohn-like reaction). Tumour infiltrating lymphocytes are mainly $CD8^+$ cytotoxic T-cells.[3]

MSI is associated with a high mutation load, with MSI tumours presenting with a mutational rate 20 times higher than tumours with Microsatellite Stability (MSS).[22] This high tumour mutational load is also typically seen in right-sided CRC.[26] MSI CRC are associated with a higher tumour mutational load, especially with high Microsatellite Instability (MSI-H), and a high tumour mutational load is in turn associated with MSI-H ($p < 0.0001$).[23]

In fact, MSI is essentially a marker of dMMR, which in turn results in an inhability to correct possible DNA mistakes that arise during replication, therefore increasing the tumour mutational load.[6,22] The higher mutational burden results in neoantigens, triggering the host immune system and increasing T-cell infiltration[27,28] and, therefore, the tumour immunogenicity. These tumours have a higher activation of $CD8^+$ T-cells, as well as an upregulation of checkpoints such as CTLA-4 and PD-1/PD-L1, amongst others.[1,26] However, tumour cells are not eliminated by the immune system because of the upregulation of inhibitory checkpoints (such as PD-1, CTLA4, LAG3, and Indoleamine 2,3-Dioxygenase)(IDO),[26] which in turn favour an immune-suppressive microenvironment.[28]

Microsatellite unstable CRCs are associated with a high expression of PD-1/PD-L1. This signalling axis may be associated with immune inhibitory/exhaustion signalling to activated T-cells resulting in a significant impairment of anti-

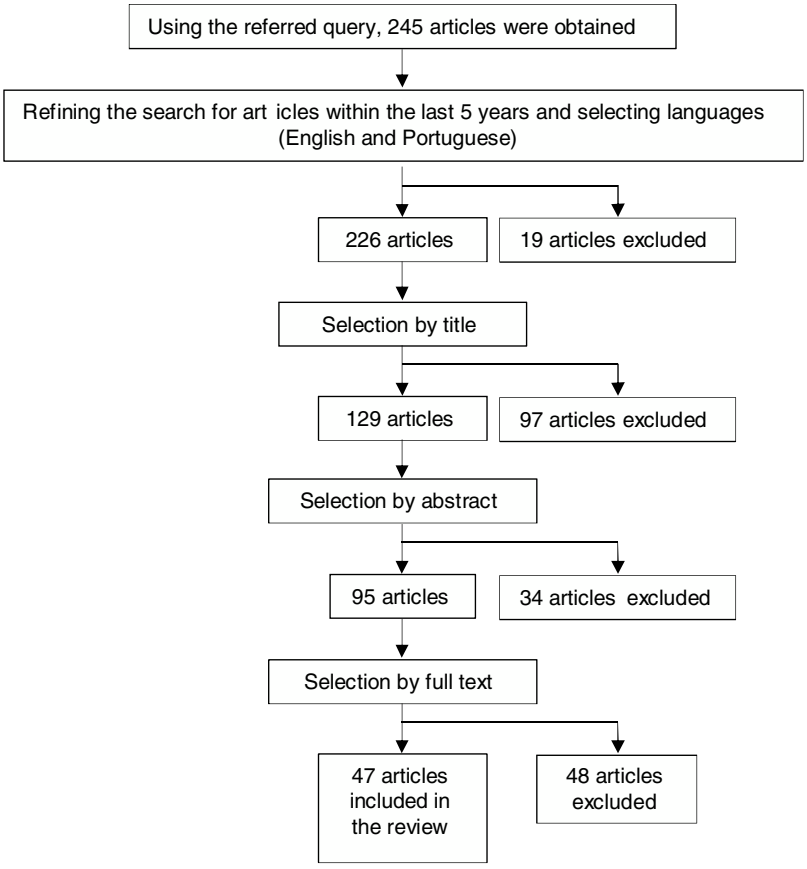

Fig. 1 – Methods.

tumour immune response.[20] PD-L1 positivity in MSI CRCs immune cells was significantly correlated with an early stage disease (81%), peritumoural lymphoid reaction (77%) and a high density of tumour infiltrating lymphocytes (77%), whereas PD-L1 positivity in tumour cells was associated with a more advanced disease stage (73%), lymphovascular invasion (46%), high density of tumour infiltrating lymphocytes (81%) and a poorer differentiation (65%).[20] It was observed that tumours with higher positivity for PD-L1 were associated with high tumour infiltrating lymphocytes and peritumoural infiltration, which in turn is associated with a better prognosis.[20]

MSI tumours are associated with tumour infiltrating immune cells that are PD-L1 positive, but not with tumour cells expressing this marker.[29] The expression of PD-L1 in stromal tumour infiltrating immune cells is associated with a good prognosis ($p < 0.001$), however the presence of tumour cells expressing PD-L1 is a marker of poor prognosis. MSI-H CRC is associated with the presence of stromal tumour infiltrating immune cells expressing PD-L1 but not tumour cells expressing PD-L1.[29] dMMR patients show higher tumour infiltrating lymphocytes PD-1 (54.8% vs. 43.2%, $p = 0.039$) and lower stroma PD-L1 (non-tumour infiltrating lymphocyte and non-tumour) expression (22.0% vs. 43.8%, $p < 0.001$), when compared to proficient Mismatch Repair (pMMR) patients.[18]

The higher density of $CD8^+$ T-cells is associated with CRC tumours that don't have early metastases and with prolonged OS. In fact, high risk early stage CRCs have a low $CD8^+$ T-cells density.[2] Besides, MSI is associated with a positive prognosis in the early stages of CRC, without adjuvant chemotherapy, having the opposite effect and a worse prognosis with FU-based chemotherapy.[6]

Tumour microenvironment in microsatellite unstable CRC

The tumour microenvironment is made of tumour-associated antigens, antigen-presenting cells, T lymphocytes (that recognize and destroy cells marked with tumour-associated antigens), B lymphocytes (producing antibodies to tumour antigens) and stromal cells.[26] In order to get an immune response to tumour cells, a reactivation of T-cells in the tumour microenvironment is required.[19] tumour microenvironment with higher density of $CD8^+$ tumour infiltrating lymphocytes are accompanied by high numbers of IFN and IFN-related cytokines, making it the most responsive to immunotherapy.[19]

Tumour infiltrating lymphocytes are a mixture of T and B lymphocytes, NK cells, macrophages and other innate cells. MSI is associated with a high number of tumour infiltrating lymphocytes and lymphoid reaction, which is a good prognosis factor.[2] Tumours with high levels of tumour infiltrating lymphocytes are tumours located to the proximal colon, with H-MSI and high neoantigen load ($p < 0.001$).[30]

MSI-H CRCs usually have a prominent inflammatory response in the edge of the tumour, with an increased number

of tumour infiltrating lymphocytes, mainly T cytotoxic cells ($CD8^+$).[3,31] This pro-inflammatory status in MSI is associated with less lymphovascular invasion and metastasis, related to the better prognosis.[2,30,32]

A lower density of $CD4^+$ and $CD8^+$ T-cells is, in turn, associated with primary CRC with higher metastatic potential. Furthermore, the higher density of myeloid-derived suppressor cells and Th17 cells is significantly associated with tumour progression and a poorer prognosis of CRC, related to the immune suppressive effects of these cells.[2]

The immunoscore quantifies the number, density, and distribution of $CD3^+$ T lymphocytes and $CD8^+$ cytotoxic T-cells in the tumour core and its invasive margins and may even provide a good staging tool given its prognostic value. Tumours with high immunoscore (3 and 4) are associated with a better prognosis.[3,31] In fact, in tumours with low immunoscore, 72% of these relapsed after 5 years, versus 4.7% of patients with a high immunoscore.[32] The immune infiltration in the tumour is inversely associated with early metastasis and death.[30,32]

Immunoediting is a process that occurs when the immune system attempts to eliminate tumour cells, following the designated elimination, equilibrium, and escape sequence. This process is regulated by checkpoint receptors mainly CTLA-4, PD-1/PD-L1 and LAG3.[26] PD-1/PD-L1 checkpoint blocks the interaction of tumour-expressed PD-L1 and PD-1 expressed in T-cells, inactivating T-cells and allowing the tumour to grow.[26]

The presence of Treg cells in tumour infiltrating lymphocytes produce immunosuppressive substrates (like IL-10) and express several immune checkpoints namely PD-L1 and CTLA-4, contributing to the inhibition of effector T-cells. A tumour microenvironment enriched with $CD8^+$ is associated with a reduced recurrence and metastasis, whereas a tumour microenvironment enriched with immunosuppressive cancer associated fibroblasts is associated with a poorer prognosis.[2]

MSI CRC is associated with PD-L1 expression in tumour infiltrating immune cells, both stromal ($p = 0.042$) and intraepithelial ($p < 0.001$).[29] The presence of these stromal cells was a marker of good prognosis ($p < 0.001$), whereas the presence of tumour cells expressing PD-L1 was a marker of poor prognosis.[29] Expression of PD-L1 in tumour infiltrating lymphocytes of patients with microsatellite unstable CRC is 68.6%, higher than the observed in MSS CRC.[6]

Large mucinous areas surrounding the tumour possibly result from immune destruction of tumour cells, making extracellular mucin a type of treatment response marker.[33] The CRC who presented clinical benefit from ICIs tend to present with high PD-L1 expression and a mucinous pattern.[33]

Microsatellite testing in patients with CRC

The current gold standard for assessing tumour Mismatch Repair (MMR) activity is molecular testing for MSI. It is also possible to assess MMR by immunohistochemistry (to identify the loss of one or more of the MMR proteins: MLH1, MSH2, MSH6 and PMS2), which is sensitive but not specific.[3,4]

In sporadic MSI CRCs, about 95% of the cases are associated with MLH1 promoter hypermethylation, with loss of function of MLH1 protein.[3,6] The reported sensitivity of DNA testing for MSI is 89% for MLH1/MSH2 and 77% for MSH6.[4]

If 30% or more of the repeats tested on PCR are unstable, the tumour is classified as MSI-H.[4,6] MSI testing with either method is found to be cost-effective, sensitive and specific, with benefits widely accepted.[4]

Furthermore, if the patient is shown to have MSI, they should be given the option of genetic counselling, to determine the germline mutation in the MMR gene.[3]

MSI is predictive of tumour response to conventional chemotherapy and immunotherapy, with consequences on the prognosis.[6] MSI is a response biomarker for PD-1 blockade, with PFS rates of as much as 78% in MSI-H CRC patients, versus only 11% of MSS patients.[13]

MSI is associated with favourable outcomes when compared with MSS tumours, a reason why some American guidelines recommend that MSI should be tested upon the CRC diagnosis.[34] With these results, MSI testing in patients with CRC can improve the prognosis is this subset of patients.

MSI as a biomarker of response to immunotherapy

MSI-H results in a high number of DNA replication errors that can be recognized as foreign by the host immune cells, eliciting an enhanced immune infiltration, making MSI a biomarker for the response to ICIs.[35] The high infiltration of $CD8^+$ T cells in MSI tumours ensures a more durable response to immunotherapy.[6] This was shown in the KEYNOTE-016 trial, where a much higher Overall Response Rate (ORR) was observed in MSI tumours when compared to MSS tumours (40% vs. 0%, respectively).[35]

MSI is a marker of dysfunction in MMR proteins, giving rise to an array of DNA mutations and neoantigens, with this higher tumour mutational burden as a marker of response to ICIs.[22] Cases with ORR to immunotherapies tend to have a higher tumour mutational burden (54 mutations/Mb) when compared to non-responders (29 mutations/Mb) ($p < 0.001$).[10] Therefore dMMR genotype is considered a probable marker of response to PD-1 inhibitors.[6,8]

Besides MSI, PD-L1 protein expression is also associated with benefit from immunotherapy.[14] MSI is associated with a higher expression of PD-1/PD-L1 when compared to MSS (20.6% vs. 7.8%, $p < 0.0001$),[23] associated with a good response to anti-PD1/PD-L1 therapies.[20] However, assessment of PD-L1 expression and tumour mutational burden is costly and not as easily accessible as MSI testing, making MSI a more feasible biomarker of response to immunotherapy.[14]

In summary, to balance the active tumour microenvironment in MSI CRC, tumour cells express immune checkpoint ligands, promoting an immune-suppressive environment. This is the rationale for why MSI is a good predictive biomarker of response to checkpoint inhibitors.[14,18,23,28,32] Furthermore, it is known that MSI, tumour mutational load and PD-1/PD-L1 expression are all markers associated with response to ICIs.[23]

Response of microsatellite unstable CRC to immunotherapy

Several studies have been conducted in order to sustain the benefits of immunotherapy in MSI CRC (Table 1). Nivolumab is a human IgG4 monoclonal antibody against PD-1, while pembrolizumab is a humanized IgG4 monoclonal antibody that

Table 1 – Main studies and results regarding PD-1 blockade in CRC.

Study	Study characteristics	Patients/ tumour characteristics	Main results
Overman, M.J., et al.[38] (CheckMate-142)	Multicentre, open-label, phase 2 trial	Metastatic or recurrent CRC with dMMR or MSI-H tumours	Objective response: 31.1% Disease control for ≥ 2 weeks: 69% OS at 12 months: 73% PFS at 12 months: 50%
Overman, MJ, et al.[39] (ChekMate-142)	Multicentre, open-label, phase 2 trial	Histologically confirmed recurrent or metastatic CRC assessed as dMMR and/or MSI-H	Objective response: 54.6% Disease control for ≥ 12 weeks: 80% ORR: 49% PFS at 12 months: 71%
Ciardiello, D., et al.[40] Keynote-016	Phase 2 clinical trial	pMMR and dMMR chemo-refractory metastatic CRC or non-CRCs with dMMR	In dMMR-CRC: ORR of 40% and PFS rate of 78% (in the initial results) ORR of 52% and PFS rate at 2 years of 59% (in the updated results)
Franke, A.J., et al.[34] Keynote-164	Phase 2 clinical trial	MSI/dMMR previously treated metastatic CRC	Cohort A: ORR of 28%, 12 month-PFS of 34% and OS of 72% Cohort B: ORR of 32%, 12 month-PFS of 41% and OS of 76%
O'Neil, B.H., et al.[41] Keynote-028	Multicentre, open-label, nonrandomized, single-arm phase Ib trial	Histologically or cytologically confirmed, locally advanced, or metastatic colon or rectal adenocarcinoma	Partial Response in 4% (patient with MSI) 17% with stable disease ORR of 13%

binds to PD-1, preventing the interactions in the PD-1/PD-L1 axis. Therefore, both antibodies result in immune recognition of the tumour and subsequent response.[36] The first-line therapy is key as it can determine the successful systemic treatment in metastatic CRC (since it has the longest duration). However, pembrolizumab and nivolumab are currently only second-line and beyond for patients with MSI/Dmmr.[6,37]

The Checkmate-142 study was done in order to investigate the activity and safety of nivolumab in patients with locally determined dMMR/MSI metastatic or recurrent CRCs. Seventy-four patients were recruited and enrolled in this study, receiving 3 mg/kg intravenous nivolumab monotherapy every 2 weeks until disease progression, death, unacceptable toxic effects, withdrawal of consent or ending of the study occurred.[38] Besides tumour assessment and MSI/dMMR testing, the level of expression of PD-1 was also measured. The median follow-up was 12 months.[38] Twenty-three (31.1%) patients achieved an objective response and disease control for 12 weeks or longer occurred in 51 patients (69%). These responses seemed durable as only three responders experienced progression.[38] The median PFS was 14.3 months (with 50% PFS at 12 months).[38] Twelve-month OS was 73% with clinically important improvements in function, symptoms and global quality of life manifesting as early as week 13 of treatment. These outcomes were maintained for more than 37 weeks.[38]

Posteriorly, a second phase of the Checkmate-142 study was conducted in order to assess the benefit of adding anti-CTLA4 therapy (ipilimumab) to anti-PD1 therapy (nivolumab). One-hundred and nineteen patients with histologically confirmed recurrent or metastatic CRC assessed as dMMR and/or MSI-H received nivolumab 3 mg/kg + ipilimumab 1 mg/kg once every 3 weeks for four doses followed by nivolumab 3 mg/kg IV once every 2 weeks.[39] Fourty-four patients discontinued therapy due to disease progression (19%), adverse events related to the treatment (13%) and adverse events unrelated to the drug in study (2%). An objective response was achieved in 54.6% of patients (3.4% Complete Response [CR] and 51.3% partial response). In 80% of patients, disease control for ≥ 12 weeks was achieved. The ORR was 49% (4% CR and 45% partial response). Furthermore, the response was maintained, with 94% of responders having ongoing responses at data cut off and 83% lasting longer than 6 months.[39] Furthermore, improvements in functioning, global health status and symptoms were clinically important and statistically significant. These results favour a hypothesis that combination therapy with ICIs is beneficial.[39]

Another clinical trial (KEYNOTE-016) aimed to assess the response of pMMR and dMMR chemo-refractory metastatic CRC or non-CRCs with dMMR to the anti-PD1 monoclonal antibody pembrolizumab. In the beginning of this trial 41 patients were included and treated with 10 mg/kg of pembrolizumab every 3 weeks. Ten participants were dMMR CRC, with an ORR of 40% and PFS rate of 78%. In the updated trial, 86 patients with dMMR cancers were included, with an ORR of 52% (12% CR in the CRC cohort).[35,40] The PFS rate was 59% at two years, and the OS rate at two years was 72%.[40]

The Keynote-164 trial evaluated the response of MSI/dMMR CRC previously treated with at least two therapies including fluoropyrimidine, oxaliplatin, and irinotecan (Cohort A, with 61 patients) and in patients previously treated with at least one prior therapy including fluoropyrimidine, oxaliplatin, irinotecan, or anti- VEGF/EGFR (Cohort B, with 63 patients).[34] In Cohort A the ORR was 28% with a PFS at 12 months of 34%, and an OS of 72% at twelve months. On the other hand, in Cohort B, the ORR was 32%, with a 12-month PFS of 41% and an OS of 76% at twelve months. These results favour the utilization of pembrolizumab given its efficacy.[34] In this trial, the CR was 3%, higher than the usual 1% using combination chemotherapy, similar to the one observed in the CheckMate-142 trial, where the CR rose to 9% when the follow-up was prolonged to 21 months, meaning the response to treatment increases with time.[34]

Keynote-028 was a multicentre, open-label, nonrandomized, single-arm phase Ib trial with 23 patients with metastatic or locally advanced colon or rectal adenocarcinoma. Patients

received pembrolizumab intravenously at a dose of 10 mg/kg once every 2 weeks for 24 months (or until confirmed disease progression).[41] In this trial 1 patient experienced partial response and 17% of patients remained with stable disease, with a median duration of 5.1 months. Among the remaining patients, 65% had progressive disease. The ORR was 13%. The one patient that responded to pembrolizumab was the only one in this cohort of patients with MSI CRC, stating the importance of this biomarker to the response to PD-1 blockade.[41]

Keynote-177 is a future clinical trial proposed to study the comparison between standard chemotherapy and pembrolizumab in 270 previously untreated dMRR/MSI-H metastatic CRC, unlike the other existing trials, where the patients have previously been treated.[42]

Notably, primary resistance to ICIs vary from 12% to 40% of patients, with several mechanisms possibly responsible such as JAK loss-of-function mutations, truncating mutations of beta-2-microglobulin or even loss of Major Histocompatibility Complex (MHC) molecules.[12,43] Besides that, weak responses to ICIs are related to low expression of genes associated with infiltration of immune cells, mainly Th1 and cytotoxic T-cells.[8] It is known, however, that tumour response to ICIs was independent of KRAS and BRAF status,[42] known to have implications in the response to other treatments.

Possible adverse effects of treatment with immunotherapy

The most common side effect of ICIs is skin toxicity (rash and pruritus), in about 20% of patients. Less than 10% of side effects include cardiac arrhythmias, infusion-related reactions, iridocyclitis and peripheral and sensory neuropathy. Laboratory findings include disturbances in liver enzymes, hyponatremia, hyperkalaemia, disturbances in thyroid function and in cortisol levels.[22] Nevertheless, in 71%–96% of patients drug-related adverse events resolved, with the exception of endocrine events, resolving in 40% of patients.[39]

In fact, about 70% of the patients in the CheckMate-142 trial had drug-related adverse effects. However, serious drug-related adverse events were only observed in 12% of patients, including adrenal insufficiency, ALT levels increase, colitis, gastritis, stomatitis, acute kidney injury and arthritis. In fact, no deaths related to the study drug were reported.[38]

Combination therapy with nivolumab + ipilimumab results in an increase in adverse effects, with high levels of AST and/or ALT (11%), elevated lipase (4%), anaemia (3%) and colitis (3%) being the most common.[12,39] A case report showed a 61-year old woman who received nivolumab + ipilimumab developing rapidly extending proximal muscles weakness with significant muscular oedema, enhancing the possibility of synergy in adverse events when combining ICIs.[44]

Most adverse events are noted within 3 – 12 weeks of treatment,[34,45] resolving within 12 weeks of onset.[34] Some of the most common adverse events (skin rash, pruritus) can respond to antihistamines, and topical steroids,[45] which do not appear to interfere with the therapeutic effect of immunotherapy.[19] If this fails, early addition of immunosuppressive drugs (such as infliximab) may be considered.[45] Despite having adverse events related to treatment, PD-1/PD-L1 inhibitors have a significantly lower risk of fatigue, neuropathy, diarrhoea, hematologic toxicity, anaemia, nausea and anorexia, when compared with chemotherapy.[19]

Incorporating immunotherapy onto the treatment of MSI CRC

PD-1 blockers have been associated with good outcomes in the treatment of MSI CRC with durable responses, good survival rates and an acceptable safety profile, making them favourable for the treatment of these patients, especially given their low response rates to chemotherapy.[39]

In fact, pembrolizumab was approved by the FDA for all solid tumours with dMMR, regardless of histology, after failure of treatment with first-line therapy. Furthermore, nivolumab was also approved by the FDA, but only in MSI CRCs, after disease progression with chemotherapy with fluoropyrimidine, oxalipatin and irinotecan.[27,45] Currently, pembrolizumab and nivolumab are considered second-line treatments for CRC, and should only be used after molecular profiling of the tumour.[37]

Nevertheless, therapy with ICIs is expensive (reaching as far as 300,000 $/year per patient regarding ipilimumab + nivolumab combination). It isn't the most cost-effective, mainly due to the drug pricing in nivolumab. When talking about first-line therapy, the combination therapy was the most effective, however still not cost-effective in comparison with mFOLFOX6 and cetuximab. Nonetheless, the OS with ipilimumab + nivolumab was longer, with patients more likely to avoid chemotherapy, making these therapies a good choice of treatment in CRC with MSI, especially if the pricing of the drugs is lowered.[46]

For the majority of patients, first-line treatment remains combination chemotherapy with targeted therapy. However, for patients with MSI/dMMR, it is recommended the use of nivolumab (with or without ipilimumab) or pembrolizumab as one of several first-line regimens, especially in patients where chemotherapy cannot be done.[34] Importantly, the MSI status should be assessed before treatment, given these ICIs have greater outcomes in this subset of patients.[34] Indeed, the incorporation of PD-1 blockers in the treatment of CRC is of the greatest interest, given their clinical benefits and safety profiles, especially after their approval by the NCCN.[27,45]

Alternative immunotherapies

Besides ICIs, there are other immunotherapies in study for the treatment of CRC. For example, cancer autologous vaccines use the patient's tumour cells containing tumour-associated antigens, with limited efficacy to date.[36] Another option is adoptive cell transfer, treating patients with cell populations previously expanded ex vivo, in an attempt to enable the patients' T-cells to overcome the immune suppression occurring in vivo.[27,35] Toll-like receptors agonists are also under investigation.[35,36] Other novel therapies under investigation include inhibitors of enzymes responsible for generating nutritional stress for T-cells, such as IDO and immunomodulatory molecules like CD73.[47]

Conclusion

CRC is still one of the most common tumours worldwide, with about 15% of these tumours related with MSI. MSI tumours have distinct characteristics, namely the high neoantigen load and the enhanced immune infiltration. These characteristics have implications not only in the prognosis, but also when it comes to the response to different treatments, consequently justifying MSI testing before the treatment of patients with CRC.

In fact, despite the better prognosis of MSI tumours, their worse response to chemotherapy is known, enhancing the need for other therapies when treating these patients. Moreover, the high T-cell infiltration in these tumours favours the upregulation of checkpoint regulators, where the PD-1/PD-L1 axis has a main role, resulting in an immune system inactivation, thus facilitating tumour expansion and growth.

Similarly, with these characteristics of MSI CRC, it is possible to conclude the important role of immune checkpoint blockade in the treatment of these tumours, namely with the use of anti-PD1 antibodies like pembrolizumab and nivolumab. In fact, studies exploring the effects of PD-1 blockade in the treatment of patients with MSI CRC have demonstrated the high efficacy of the aforementioned antibodies in this context, with an acceptable safety profile.

In conclusion, patients with MSI CRC have tumours that justify a different approach, with PD-1 blockade being a good treatment alternative in these cases. Nonetheless, patients should be tested for MSI before initiating these treatments and approval by the regulatory authorities is required. In short, PD-1 blockade can improve symptoms, ORR and OS in patients with MSI CRC, making these antibodies one of the future treatments of these patients.

Conflicts of interest

The authors declare to have no conflicts of interest.

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