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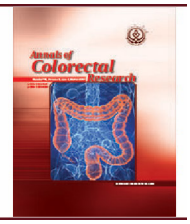

CTLA-4 Blockade in the Treatment of Colorectal Cancer with Microsatellite Instability

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Abstract

Context: Colorectal cancer is one of the most common tumors worldwide, with around 10-15% of cases being related to microsatellite instability, which is in turn responsible for a high neoantigen load and tumor mutational burden. These characteristics are responsible for the poor response of these tumors to chemotherapy, highlighting the need for a different approach in the treatment of patients with microsatellite-unstable colorectal cancer. Immunotherapy was proven important in the treatment of these patients, with immune checkpoint inhibition such as CTLA-4 blockade being one of the most promising targets so far.
Evidence Acquisition: A PubMed search was done on February 2021 where the used query obtained a total of 33 articles. After implementing the inclusion and exclusion criteria, a total of 21 articles were filtered and used in this narrative review.
Results: Several studies with microsatellite-unstable colorectal tumors have been done in order to evaluate the advantages and adverse events of CTLA-4 blockade in these patients. Studies show a benefit regarding the progression-free survival, overall survival, and overall response rates in patients receiving ipilimumab (anti-CTLA-4) when compared to those who weren't. Besides, the main adverse events were manageable and were more tolerable than those observed with chemotherapy. Nonetheless, unlike PD-1 blockade, anti-CTLA-4 drugs are currently only approved for use as part of combination therapy in microsatellite-unstable colorectal cancer, still awaiting approval as monotherapy.
Conclusion: Microsatellite-unstable colorectal tumors deserve a different treatment path as their characteristics make them poor responders to chemotherapy. At the same time, these tumors are excellent candidates for immunotherapy, particularly with CTLA-4 inhibitors.

Keywords: Colorectal cancer, CTLA-4, Immune checkpoint, Immunotherapy, Ipilimumab, Microsatellite instability

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Context

Colorectal cancer (CRC) is the third most common cancer worldwide, possessing a high mortality rate when in an advanced stage (1-4). Microsatellite instability (MSI) is responsible for 10-15% of these tumours (5), with about 3-5% of microsatellite-unstable colorectal tumors being related to germline mutations, a famous example of which is Lynch Syndrome (3).

It is known, however, that MSI is related to a poorer response to fluoropyrimidine-based therapy (such as the FOLFOX and FOLFIRI treatment schemes) and a poor response to other therapies such as oxaliplatin, irinotecan, or even targeted therapies such as bevacizumab (2, 6, 7).

MSI is responsible for a high load of neoantigens produced by the tumor cells resulting in a high tumor mutation burden (TMB), making them more easily recognizable by the immune system of the host, which in turn results in a highly active tumor microenvironment (TME) (1, 2). This microenvironment has a high infiltration of T cells, inducing a strong anti-tumor immunological response while also being a biomarker of response to immune checkpoint inhibitors (ICI), such as programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockers (6).

Several studies have shown a good response to ICI in the treatment of CRC with MSI, as seen in the KEYNOTE and CHECKMATE trials (1, 2, 4, 6).

In this review, we aim to show the rationale for the treatment of microsatellite-unstable CRC with immunotherapy, focusing on the role of CTLA-4 blockade in the treatment of patients with these tumors.

Evidence Acquisition

This narrative review is based on a web search on the PubMed database on February 2021, with the query "((Anti-CTLA4 OR ipilimumab OR tremelimumab OR ticilimumab) AND (microsatellite instability OR MSI)) AND (colorectal cancer OR CRC OR colorectal neoplasm)", having obtained 33 articles with this query.

Upon selection of articles published in the last five years in English and Portuguese only, a total of 31 articles were obtained. No exclusion criteria were used (Figure 1).

Based on the title alone, one article was excluded, as it was referring to microsatellite stable (MSS) tumors. With this exclusion, 30 article abstracts were examined, resulting in the exclusion of 3 articles, one referring to HIV-positive patients, one looking at other biomarkers, and one with only two MSI patients. With this selection, 27 remaining articles were analyzed by full-text analysis, resulting in the exclusion of six more articles, mainly due to not being in direct link with the subject (referring only to PD-L1 blockade, for example). With this process, 21 articles were selected and included in this review.

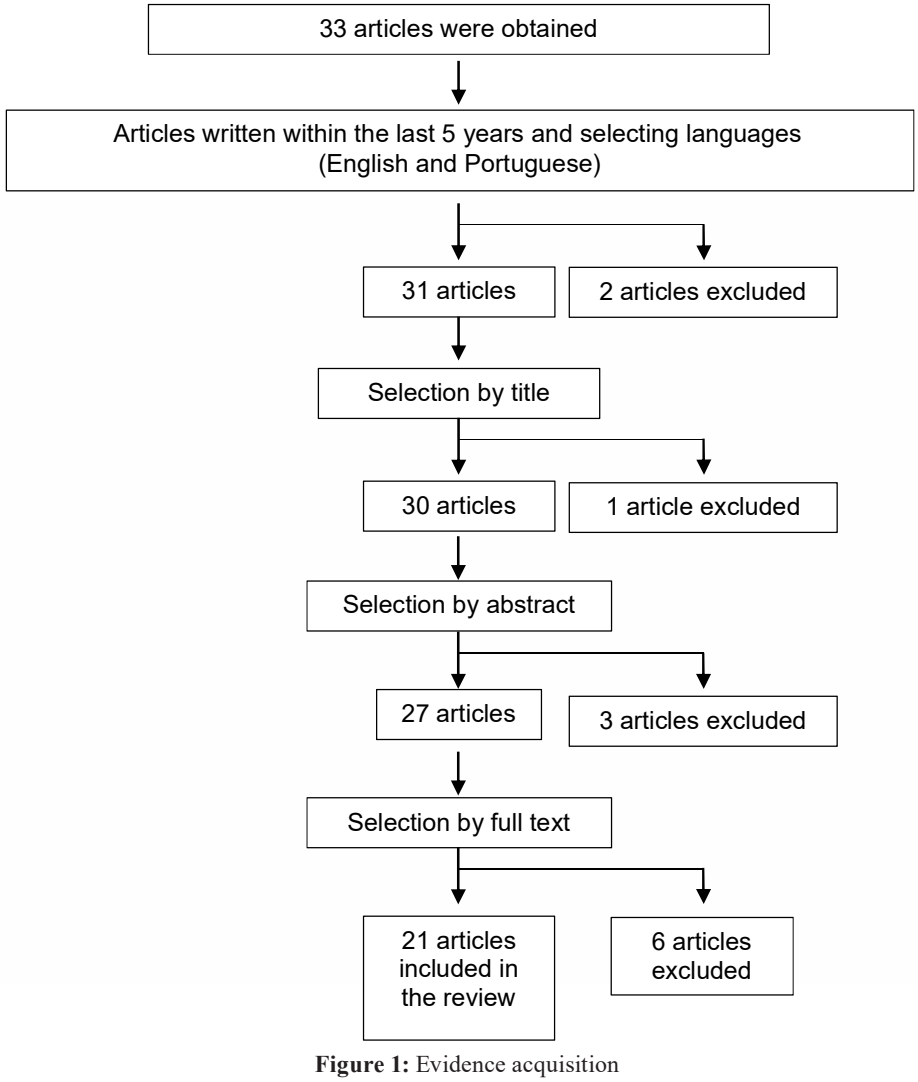

Figure 1: Evidence acquisition

Results

Colorectal Cancer with Microsatellite Instability

Microsatellite-unstable colorectal tumors are more frequently found on the right side of the colon and in younger patients, with a strong mucinous component and inflamed stroma and a high rate of tumor-infiltrating lymphocytes (TILs) (2, 6).

Furthermore, MSI is responsible for a high neoantigen load, making the tumor more easily recognized by the host's immune system and resulting in a highly active TME and the upregulation of immune checkpoint expression (5, 7, 8).

This upregulation of immune checkpoints like PD-1 and CTLA-4 is responsible for an effective T-cell response to immune checkpoint inhibition with ICIs, proving the rationale for the treatment of MSI CRC with CTLA-4 and PD-1 blockers (7). In addition, right-sided colorectal adenocarcinomas, such as MSI CRC, have the highest prevalence of TMB-high tumors, with TMB being a marker of response to ICIs (7).

MSI is also responsible for a better prognosis in CRC patients, with a lower likelihood of deficient mismatch repair to be found in more advanced stage tumors (6).

On the other hand, MSI is a marker of poor response to conventional therapies such as chemotherapy (7), favoring the importance of microsatellite testing before treating CRC patients (2, 9).

Indeed, given the inferior response of microsatellite-proficient CRC to immunotherapy (10) and the better response of MSI CRC to immunotherapy when compared to chemotherapy (2), it is now advised to test patients with these tumors for MSI before treatment.

With the good response to immunotherapy seen in MSI CRC when compared to MSS tumors, microsatellite testing should be done upon diagnosis and before treatment with ICIs (2, 6, 8).

The Immune Microenvironment in Colorectal Cancer

MSI is responsible for unique biological features of colorectal tumors, resulting in a high TMB, which in turn is responsible for an increased amount of neoantigens (2, 7, 11). These antigens are recognized by the immune system as foreign antigens and elicit a robust immune response, associated with high infiltration of T cells in the TME, particularly T CD4+ cells, with an important role in the robust anti-tumor response that is usually associated with MSI CRC (1-3, 5, 7, 12).

On the other hand, tumor cells have a high expression of cell surface inhibitory molecules, such as PD-1 and CTLA-4, which allow them to downregulate the host's immune reaction, thereby protecting MSI cancer cells from the hostile TME as they continue to thrive (2, 5, 10).

The highly infiltrated TME seen in MSI CRC is associated with a favorable response to ICIs (1), as blockade of immune checkpoints allows the activation of peritumoral lymphoid cells and enables the immune system to react against tumor cells (5, 13). Contrarily, MSS tumors are associated with limited T cell infiltration in the TME, which is related to the poor antigenicity owing to their low TMB; this is one of the main reasons for the poor response of MSS CRC to ICIs (1, 5). MSI is, in fact, one of the most important biomarkers of response to immunotherapy with ICIs (6, 14).

Immune Checkpoints and Immune Checkpoint Inhibitors

Tumor cells elicit a host immune response characterized by releasing pro-inflammatory cytokines and immune cells' infiltration into the TME. At the same time, this is also responsible for a feedback mechanism where activated T cells upregulate inhibitory receptors such as PD1 and CTLA-4, while tumor cells upregulate counter-receptors such as the PD-1 ligand (PD-L1), which are also known as immune checkpoints (6). Tumor cells use these checkpoints as a mechanism to suppress the anti-tumor response of these cancers, enabling them to avoid immunosurveillance (6, 10).

CTLA-4 is a coinhibitory molecule that regulates signal transduction pathways and regulates T-cells' activation and function. This molecule binds to B7-1 and B7-2 on antigen-presenting cells, downregulating tumor-reactive T-cells' activation and thereby suppressing the immune response to tumor-associated antigens (5, 15).

With this rationale in mind, several drugs have been developed targeting immune checkpoints, such as the monoclonal antibodies for PD1 (nivolumab, pembrolizumab), PDL1 (atezolizumab, durvalumab, avelumab), and CTLA-4 (ipilimumab, tremelimumab) (6).

Pembrolizumab is a PD1 inhibitor that is currently part of the standard of care for metastatic CRC (6, 7).

Ipilimumab, a CTLA-4 inhibitor, prevents the binding of B7-1 and B7-2 in antigen-presenting cells to T cells, which allows for T-cell activation, proliferation, and amplification, enabling an immune response against tumor cells (15).

As so, nivolumab, pembrolizumab, and nivolumab plus ipilimumab are all authorized by the United States Food and Drug Administration as options for treating refractory MSI CRC (16)

Effectiveness of Immune Checkpoint Inhibition with Anti-CTLA4

Ipilimumab is authorized in combination with nivolumab in patients with MSI CRC. This approval relies on the beneficial add-on provided by CTLA-4 blockade in patients already under PD-1 blockade therapy. CTLA-4 is involved in the suppression of dendritic cells' activity in lymphoid tissue, whereas PD-1 associates with T-cell inhibition and NK cell activation in peripheral tissues, as well as enabling

regulatory T cell (Treg) differentiation (17). These effects are the basis for the rationale of many studies that have evaluated the benefits of associating the PD-1 and CTLA-4 blockades.

The combination of nivolumab with ipilimumab results in an enduring clinical response with manageable adverse effects. In an open-label study, nivolumab was associated with an objective response rate (ORR) of 31% and a 12-month overall survival (OS) of 73% (17).

Checkmate-142 was a multicenter study with 119 patients, where patients received either nivolumab alone or a combination of nivolumab and ipilimumab. The ORR was 55% and the OS at 12 months was 85% (18, 19), demonstrating the benefit of ipilimumab addition in previously treated MSI CRC patients.

In the continuation of the study, Checkmate-142 subsequently added another cohort in order to evaluate the nivolumab plus ipilimumab combination in the first-line treatment of MSI metastatic CRC patients (8). This showed an ORR of 60% at a median follow-up of 13.8 months, with a 12-month progression-free survival (PFS) rate of 84% and 83% OS (8).

Furthermore, other CTLA-4 blockers are being studied, such as tremelimumab, a monoclonal antibody against CTLA-4. In a randomized controlled trial, tremelimumab was combined with durvalumab (a PD-1 blocker), where the control group was the best supportive care. The durvalumab plus tremelimumab association had a significatively higher OS, despite not having a significantly higher PFS (14).

Nonetheless, some resistance mechanisms to ICIs have been identified.

For example, regarding primary ICI resistance, tumors remain unrecognized by the infiltrating T-cells despite ICI therapy (16), which might be observed in 12-40% of patients (10). In acquired ICI resistance, tumors escape immune surveillance mechanisms with intrinsic and extrinsic pathways (16). Several mechanisms could be responsible for ICI resistance, such as JAK-loss of function mutations, beta-2-microglobulin truncating mutations, or loss of major histocompatibility complex molecules (10).

On the other hand, CTLA-4 blockade can be useful in patients with acquired resistance to anti-PD1 drugs. A case report of a woman with MSI CRC showed a response to nivolumab plus ipilimumab after progression with pembrolizumab (16), indicating the promising nature of the double blockade in patients with acquired resistance to PD-1 inhibitors. However, in deficient mismatch repair tumors, primary or adaptive resistance to ICI therapy (already present upon disease presentation) are much more common than acquired resistance which, in turn, is rare (8).

Most importantly, in order to identify patients for whom this dual blockade is useful, testing for deficient mismatch repair should be a part of the routine diagnostic workup in patients with CRC (9). Another marker of response to ICIs regardless of the MSI status is the TMB (14).

Response to therapy is usually evaluated with radiological criteria such as the RECIST criteria. However, in patients under ICI, the tumor diameter may initially grow due to immune cell infiltration in a phenomenon described as pseudoprogression (13). This called for the development of the iRECIST criteria. In a multicenter, phase II study, the nivolumab plus ipilimumab combination was associated with low pseudoprogression, showing that in patients having progressive disease criteria in iRECIST, confirmed progressive disease was much more probable than pseudoprogression (13). Nivolumab plus ipilimumab combination showed a 12-month PFS of 72.9% and an OS of 84.0% (13).

However, in addition to having an increase in adverse events with double checkpoint blockade therapy, the monetary cost might also be an issue. Nivolumab plus ipilimumab is associated with an annual cost of around $300,000/year (20). In a cost-effectiveness analysis, nivolumab plus ipilimumab was the most effective strategy, either as a first- or third-line therapy (20). This is mainly due to increased survival and less degree of adverse events than chemotherapy regimens. On the other hand, the double blockade is less cost-effective than mFOLFOX6 and cetuximab therapy. It is, however, important to define the duration of maintenance therapy, as ipilimumab and nivolumab would be the most cost-effective strategy if the duration of treatment was reduced to two years (20). ICI therapy discontinuation might not influence the outcome due to the reactivation of memory T cells with long-term tumor cell surveillance (2). In fact, Checkmate-142 patients who discontinued ICI therapy because of adverse events had an efficacy similar to the overall population of the study (2).

Safety of CTLA-4 Blockade and Possible Adverse Effects

Studies have shown the safety of ICIs in the treatment of patients with CRC (1). However, adverse events still occur in the course of treatment with these drugs, affecting mainly the skin (rash) and gastrointestinal tract (diarrhea; colitis). Other systems may also be affected such as the lungs (pneumonitis), cardiovascular (myocarditis), endocrine (hypothyroidism), and nervous systems (myasthenia gravis) (1, 15, 21). Increased serum transaminases and lipase levels and fatigue are also possible adverse events (13).

With nivolumab monotherapy, the occurrence of grade 3 or higher adverse effects was 13-22%, while that of dual immune checkpoint inhibition with nivolumab plus ipilimumab was 22-64% (1, 8). A case report described a 61-year-old female who developed rapidly extending proximal muscle weakness after treatment with nivolumab plus

ipilimumab combination therapy (22).

Most treatment-related adverse events occurred in the first 12 weeks of treatment and also resolved within 12 weeks of the event onset (2, 21). The commonest causes for treatment cessation were autoimmune hepatitis and acute kidney injury (21). No deaths have been attributed to study drug-related toxicity (21).

On the other hand, adverse events are usually manageable with supportive measures such as anti-histaminic drugs and/or corticosteroids and tend to resolve with a rare need for immunosuppressive drugs such as infliximab (15). Besides, studies have shown patients are more likely to tolerate the adverse events related to ICI rather than the ones usually associated with chemotherapy (20).

There are no known absolute contraindications to ICIs, but they can cross the placenta, being transmitted to the fetus (15).

Nonetheless, patients with and without drug-related adverse events had comparable ORR and survival rates (21). The documented adverse events are manageable and should therefore not interfere with the choice of treatment with these drugs (17, 21)

Conclusion

CRC is one of the most common types of tumors worldwide, with MSI being seen in about 10-15% of cases. Despite the better prognosis usually associated with MSI, their poorer response to chemotherapy when compared to MSS tumors is well known, raising the need for other treatment strategies for these patients.

Additionally, MSI grants tumors with characteristics that make them great candidates for therapies targeting the immune system, with high immune cell infiltration and upregulation of immune checkpoints. In fact, MSI and its high TMB are great biomarkers of response to ICIs.

Several studies showed the benefit of blocking PD-1 blockade, including good PFS, ORR, and OS, granting the approval of these drugs in the treatment of MSI CRC. More recently, some studies have shown the advantages of dual checkpoint inhibition, namely targeting PD-1 and CTLA-4 simultaneously. These studies have shown greater PFS, OR, ORR, and OS when compared to monotherapy with only PD-1 or CTLA-4 blockade while being associated with only a minor rise in the adverse events rate.

Furthermore, the combination of nivolumab plus ipilimumab in MSI CRC has shown great amelioration of these patients' quality of life, with more manageable side-effects than those seen with chemotherapy. In fact, the main disadvantage of this combination is the monetary cost associated with these drugs, which could be diminished with a decrease in cost price.

There is, however, the need for more studies regarding the use of anti-CTLA-4 drugs in a monotherapy context, which might also be a way to lower the cost of treatment in these patients.

Nonetheless, MSI testing before treating patients with CRC is of utmost importance as MSS tumors don't tend to respond well to ICIs. Moreover, MSI is a marker of response to these drugs and is at the same time a forecaster of inferior response to chemotherapy.

To sum up, MSI CRC should be treated with ICIs such as ipilimumab considering the great response rates that studies have shown. Furthermore, these treatments are associated with tolerable adverse events and an improvement in the patients' quality of life.

Conflicts of interests: None declared.

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