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The strategic treatment of advanced Colorectal Cancer with Microsatellite instability and mismatch repair using Neoadjuvant immunotherapy and treatment resistance

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There is a broad variety of current treatments for colorectal cancer; particularly metastatic colorectal cancer (mCRC) however, their outcomes remain unpredictable. In the last 10 years, advancements in comprehending the immune mechanism's role in cancer progression have led to the development of new treatments. Immunotherapy has been recently incorporated in the treatment of mCRC mainly the phenotype with Microsatellite Instability-High (MSI-H) and Deficient Mismatch Repair (dMMR). Those patients have received a little advantage from neoadjuvant chemotherapy (nCT). The treatment regimen passed through several therapeutic approaches over eight years, and immune check point inhibitors (ICIs) were the only adjuvant therapies that added significant prognostic values to mCRC patients. Anti-programmed cell death-1 (PD1) inhibitor (pembrolizumab and nivolumab) as single agent or combined with ipilimumab (anti-CTL4) have demonstrated long-term response in CRC patients. Nevertheless, many patients did not show a significant response after the treatment. Although treatment response is multifactorial, a better understanding of the molecular alterations associated with these drug outcomes is essential. Wide genomic mutations including B2M mutations were explored as mechanisms of treatment resistance to anti-PD-1/PD-L1. This review discusses the current treatment protocols of immunotherapy for patients with advanced CRC including mCRC (MSI-H/dMMR).

Keywords: Colorectal cancer, metastatic colorectal cancer, MSI, dMMR, Treatment, Resistance

INTRODUCTION

Worldwide, colorectal cancer (CRC) is ranked as the third most frequently diagnosed cancer among men and the second among women, associated with high mortality rate (Sung et al. 2021) (Oliveira et al. 2019) During the disease's follow-up, it has been estimated that 50% of patients with CRC are diagnosed with liver metastasis. (Manfredi et al. 2006). In the last two decades, there has been an improvement in clinical outcomes aimed at preventing metastatic stages of CRC. Nonetheless, patients with metastatic colorectal cancer (mCRC) exhibit an overall survival (OS) of 25 months when RAS-mutated and 30 months when RAS wild-type status. (Van custem et al. 2016) (Venook et al. 2017). Because most of CRC patients are diagnosed during the local progression stage of the disease, treating patients in this stage is challenging. Locally advanced colorectal cancer (LACRC) is a progressive cancer stage dominated by a pathological stage II (cT3-4, N0) or pathological stage III (any cT, +N) that possess a high risk of post-operative recurrence and remote metastasis. To prevent disease recurrence, chemotherapy is given to all patients with stage III cancers and some of stage II cancers. The choice of chemotherapeutic agents is based on various factors, including tumor size and location, as well as the grade of cancer cells. Additionally, age and co-morbidities of the patients are important factors to consider. Once the disease is metastasized outside the colon, mCRC stage IV is diagnosed. The approach to treat patients with locally advanced CRC (stage III) or mCRC (stage IV) underwent sequence of development in the last eight years. Microsatellite stability (MSS) status Mismatch Repair (MMR) is recently incorporated as an essential requirement for treating patients with CRC. The microsatellite status of cancer is classified into a stable (MSS), and high instability (MSI-H). An alteration caused by dMMR is the insertion or deletion of mutations during DNA replication, affecting the length or the base of microsatellite sequences (Cohen et al. 2019). Deficient-Mismatch repair (dMMR) frequently occurs from sporadic mutations of the dMMR associated with CpG island methylation (CIMP). It can also occur due to a hereditary germline mutation of MMR gene (Altonen et al. 1998). Patients who are diagnosed with MSI in the early stages of cancer generally have a more positive prognosis as compared to those who are diagnosed with more

advanced stages. Additionally, patients who suffer from advanced cancer stages (stage III) and are diagnosed with MSI-H/dMMR exhibit better outcomes when they receive postoperative adjuvant chemotherapy as compared to those who are diagnosed with MSS.

Determining the molecular genotype and MSS status has emerged as a valuable addition to the latest treatment options for patients suffering from CRC. It has been observed that around 15% of colorectal cancer patients present MSI-H/dMMR. in particular III/IV CRC than proficient -MMR (pMMR) (Altonen et al. 1998) (Hause et al. 2016). However, this rate was found less in rectal cancer patients. Their prognosis is marked, variably based on disease stage, from 50%- 80% chance of 5-year survival rate after surgical resection and traditional chemotherapy (Hause et al. 2016).

Treatment Options for Advanced CRC

Neoadjuvant chemotherapy (nCT) was considered as a treatment option for locally advanced CRC (LACRC) to reduce local tumour recurrence and improve patient prognosis (Sung et al. 2021). Traditional nCT typically involves a combination of two or more than chemotherapeutic agents such as 5-fluorouracil (5-FU), leucovorin, and oxaliplatin. This regimen was commonly refereed as FOLFOX therapy (Overman et al. 2017). There was a more than 70% a tumour regression with the FOLFOX therapy while 26% of patients with MSI/MMR showed no tumour regression (Saltz et al. 2000) (Foxtrot, 2012). Around 30% of patients with MSI-H/dMMR had a tumour progression versus regression. However, studies used FOLFOX therapy with MSI-H/ dMMR did not show any significant clinical outcome. For patients with locally advanced rectal cancers, the National Comprehensive Cancer Network (NCCN) recommended nCT with total excision as a standard treatment (Foxtrot, 2012) (Benson et al. 2018). About 20-25% of those patients were insensitive to nCT, after progression even after adjuvant chemotherapy. Other protocols used in the treatment of LACRC included CAPOX (Capecitabine and oxiplatin), and FOLFIRI (Folinic acid, fluorouracil, and irinotecan). Their response rate was 51% and 45%, respectively (Cassidy et al. 2008) (Douilard et al. 2000). When it comes to the metastatic stage, dMMR CRC is seen in only about 5% of cases and is linked to a poorer prognosis. (Zhang et al. 2022). Patients with mCRC accompanied with MSI-H/dMMR has 13 months OS (Zhang et al. 2022). This prognosis become more unfavorable when the patient had BRAFV600E mutation (Goldstein et al. 2014).

Patients with CRC who display these characteristics are a minority, and their tumors typically exhibit dMMR or MSI-H. Additionally, individuals with mCRC can potentially experience benefits from a combination of nCT and targeted therapies such as anti-epidermal growth factor receptor (anti-EGFR) and anti-angiogenic treatments. Bevacizumab has been approved for mCRC treatment due to its ability to prolong survival when administered alongside chemotherapy. According to a study conducted by Cainap et al., two commonly used chemotherapeutic regimens that were combined with bevacizumab included FOLFIRI/CAPIRI and FOLFOX4/CAPEOX. (Cainap et al. 2021). They found that interaction between an irinotecan-based regimen as a second-line treatment and double-dose bevacizumab after progression was associated with an improved OS.

Mechanism of Immune Microenvironment with Neoadjuvant Therapies

Additional adjuvant therapies became necessary to improve patient survival however, pre-clinical studies involved immune microenvironment of the tumour added more values in the last decade. Gelsomino et al found that CD8+ cytotoxic cells, T-helper cells, and T-cell markers were significantly expressed in patients with MSI-H/dMMR CRC than in those with MSS (Gelsomino et al. 2014). It was clear that this group of patients enrolled in their study showed many CD8+ cytotoxic T-cells and upregulated checkpoints (Le DT et al. 2015) (Liosa et al. 2015). It has been also reported that patients with MSI-H/dMMR CRC had a higher tumor mutation burden (TMB), with high lymphocyte infiltration count in tumour microenvironment. Those patients presented with an overexpression of PD-L1 in the tumor microenvironment, indicating that immune checkpoint inhibitors (ICIs) may have promising targeted therapeutic potential for this cohort. (Luchini et al. 2019). Anti PD-1, anti-PD-L1, or anti-CTLA-4, have emerged as effective therapies for many cancers (Rotte et al. 2019). The interaction between PD-L1 and PD-1 results in the suppression of T-cells, which inhibits the antitumor response. This is because the interaction between PD-1 and CD80 on T-cells in the microenvironment leads to a counter-inhibitory negative feedback loop that serves as a protective mechanism against attacks by the immune system. However, PD-1 and PD-L1 inhibitors disrupt immune checkpoint activity, which ultimately leads to heightened T-cell activation and improved immune function. (Ganesh et al. 2019) (Wu et al. 2019). Hence, this mechanism clarifies that ICIs induce T-cells activation in early cancer stage. The T-cells can kill tumor cells, abolish tiny metastatic niches, and promote disease remission (Topalian et al. 2020).

Microsatellite Instability and Single Immune Checkpoint Inhibitors (ICIs)

Since 2017, MSI status became an important era in clinical oncology practice. Hence, NCCN recommended that all newly diagnosed CRC patients should be tested for MSI and for MMR. This testing should not only involve patients with suspected metastasis but involve all CRC stages. Clinical studies have also revealed that colorectal cancer patients with MSI-H/dMMR showed better clinical outcome after receiving ICIs (Overman et al. 2017) (Le DT et al. 2015) (Marebelle et al. 2020). Anti-PD1 inhibitor (Nivolumab) and anti CTLA-4 inhibitor (Ipilimumab) have

been proven as effective ICIs in CRC, regardless cancer stage. However, NCCN has proposed that high concentration of these two neoadjuvant immunotherapies are recommended for patients with mCRC. Pembrolizumab was the initial study to demonstrate clinical effectiveness in CRC patients with MSI by blocking PD-1 (Oliveira et al. 2019). The clinical trial investigated the impact of administering pembrolizumab treatment on MMR deficiency after patients experienced a disease recurrence following standard treatments. In a study conducted by Le DT et al., pembrolizumab was employed in three distinct groups of patients: those with dMMR, mCRC, pMMR, and a third group consisting of patients with dMMR in other malignancies. (Le DT et al. 2015). Among patients with dMMR mCRC, the overall response rates were recorded at 40%, with a progression-free survival rate of 75% at 20 weeks. Conversely, the response among patients with pMMR was less significant. Subsequently, following this trial, the FDA approved the use of pembrolizumab for CRC patients with MSI-H/dMMR. Furthermore, the utilization of pembrolizumab as a first-line treatment recommendation for CRC patients with MSI-H/dMMR status has also been under consideration. (NCCN 2021). Additional studies included KEYNOTE028 and KEYNOTE0164 have used pembrolizumab to treat patients with advanced CRC (O'Neil et al. 2017) (Koustas et al. 2018). The two studies demonstrated the long-lasting antitumor efficacy of pembrolizumab in patients with MSI-H, who had experienced disease progression after initial treatment. The phase I trial assessing the effectiveness of nivolumab (an anti-PD-1 inhibitor) included 14 patients with mCRC, and it was conducted across various solid tumors. Only one patient reported a complete response (Oliveira 2019). A phase 2 trial, CheckMate 142, provided evidence for the use of Nivolumab in dMMR/MSI-H mCRC (Overman, 2017). PFS and OS at 12 months were 50% and 70%, respectively.

Combined Immune Checkpoint Inhibitors (ICIs) Strategy

Scientific research has revealed that the majority of CRC patients are insufficiently responsive to single immunotherapy. This suboptimal response may be attributable to several factors, including a lack of T-cell infiltration, limited T-helper cell activity, and low immune cytotoxicity in the tumor microenvironment (Galon et al. 2006). Combination regimens have been evaluated to be used more efficiently than single immunotherapy. Starting from 2018, the NCCN has incorporated nivolumab ± ipilimumab or pembrolizumab as an adjuvant treatment option for patients diagnosed with MSI-H/dMMR CRC (NCCN 2021). However, nivolumab ± ipilimumab or pembrolizumab has also been selected as an alternative option for preoperative neoadjuvant therapy for resectable MSI-H/dMMR mCRC (NCCN 2021). NICHE phase II study (2020) used nivolumab and ipilimumab in patients with stage 1-3 CRC with dMMR and pMMR status. Studies have suggested that the combined nivolumab + ipilimumab regimen represents a safe and well-tolerated immunotherapy option for patients diagnosed with MSI-H dMMR (Chalabie et al. 2020). Another study (NICOLE) study (NCT04123925) done in 2021 in which nivolumab has been used for two cycles in patients with MSI-H versus MSS. The outcomes of the study demonstrated that over 50% of patients experienced substantial tumor regression, which was associated with high levels of CD8+ T-cell expression. In 2020, a Phase II study called VOLTAGE-A was conducted in Japan, examining the effects of nCT followed by neoadjuvant immunotherapy (nIT) and surgical resection on rectal cancer patients with both MSS and MSI-H. (Yuki et al. 2020). As of January 2020, the median follow-up duration for the MSS group was 22 months, whereas for the MSI-H group, it was only six months. According to the findings, two patients in the MSS group experienced local recurrence, and two patients had remote metastases. On the other hand, no patients belonging to the MSI-H group were subject to recurrence (Yuki et al. 2020). Numerous studies have investigated the effects of neoadjuvant immunotherapy (nIT) on CRC patients with MSI-H/dMMR. A study conducted by Zhang et al. found that two MSI-H/dMMR CRC patients received nivolumab treatment, resulting in no recurrence after surgery for one patient and successful TME surgery for the other patient. Another study by Zhang et al. explored the effects of nIT, either alone or in combination with targeted therapy, on two LACRC patients with MSI-H (Zhang et al. 2020). The response was similar to the previous studies. A study performed by a Liu DX et al on 8 patients with MSI-H LACRC had a complete remission after single or combined immunotherapy (Liu et al. 2020).

Additional studies have documented the combination of pembrolizumab with chemotherapy. For example, a phase 2 clinical trial assessed the anti-tumor effects of pembrolizumab plus azacytidine in patients with previously treated mCRC (Lee et al. 2017). The findings revealed that there was only a minor improvement in response for those with MSS mCRC. Furthermore, a phase 2 clinical trial evaluated the efficacy of pembrolizumab in combination with mFOLFOX6 in patients with untreated or unresectable CRC (Shahada et al. 2017). An individual patient with dMMR showed a full pathologic response after receiving therapy for two months. In late 2022, Zhang et al. conducted a study treating 18 patients with MSI-H/dMMR LACRC with Tislelizumab, and all 18 patients exhibited complete remission and tumor regression. Out of these, two LARC patients and five LACRC patients underwent surgery, and it was confirmed that all achieved a pCR. Furthermore, two low LACRC patients utilized a W&W approach after achieving a complete response (Zhang et al. 2022). W&W non-surgical preservation strategy was initiated in 2004 (Habr-Gama et al. 2004). This strategy was frequently utilized for rectal cancer

patients who achieved full clinical response. It is important to evaluate whether this approach is also suitable for other patients who attain complete clinical response following neoadjuvant therapy. The effectiveness of nIT in patients with MSI-H/dMMR is extremely encouraging. Nonetheless, rectal cancer patients undergoing surgery may face more severe post-operative risks, including anal dysfunction, loss of fecal control, and sexual dysfunction

Treatment Resistance for Immune Checkpoint Inhibitors (ICIs)

Approximately, more than 30% of MSI-H/dMMR mCRC did benefit from anti-PD-1/PD-L1 (Le DT et al 2015) (Oliveira et al. 2019) (Andre et al. 2020). Further investigation is necessary to explore the molecular changes associated with nIT outcomes. According to Elez et al., the mechanisms of resistance to immunotherapy are complex and involve multiple factors (Elez et al. 2022). Elez at al performed a wide genomic analysis that identified group of mutations in B2M and CTNNB1 genes in immunotherapeutic-resistant group (Elez et al. 2022). in patients with MSI-H/dMMR CRC resistant to anti-PD-1/PD-L1 treatment, Le DT observed the presence of biallelic and monoallelic mutations in B2M. High and low T-cell infiltrates were associated with biallelic ARID1A mutation and CTNNB1 mutation, respectively. Moreover, Elez et al. discovered that nIT-responders had higher densities of T-cells and PD-L1 expression (Elez et al. 2022). PTEN loss also was found to promotes an immunosuppressive effect in CRC through releasing cytokines that boost the proliferation of tumour associated macrophages (TAMs), (Piro et al. 2019) (Vidotto et al. 2020). The non-MSI-H phenotype in dMMR is associated with poor benefit to immunotherapy. TMB does not correlate with immunotherapy benefits based on PD-1/PD-L1 inhibitors in MSI-H/dMMR CRC.

CONCLUSION

To ensure optimal results, the selection of nIT for CRC patients should involve a risk degree classification and molecular subtyping. It is worth noting that MSI-H/dMMR CRC patients generally exhibit low sensitivity to conventional treatments. A practical and effective therapeutic option for these patients can involve utilizing a single ICI, a combination of multiple ICIs, or an ICI combined with nCRT/nCT. Molecular identification through broad genomic sequencing panels can aid clinicians in determining the most suitable nIT for individual patients. Detection of MSI and MMR to select best nIT in patients of stage III or mCRC may help prevent poor long-term outcome caused by treatment divergence. Wide genomic studies also can help to identify the mutant variants associated with treatment resistance in poor IT responders. Further studies of nIT in CRC patients should be conducted to incorporate the predictive biomarkers for treatment resistance with MSI/dMMR**.**

CONFLICT OF INTEREST

The author declared that present study was performed in absence of any conflict of interest.

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