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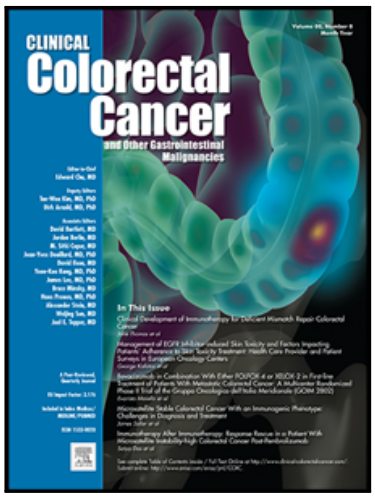


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# Single-Agent Neoadjuvant Immunotherapy with a PD-1 Antibody in Locally advanced Mismatch Repair-Deficient or Microsatellite Instability-High Colorectal Cancer

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**Abstract**

***Background:*** PD-1 blockade has been recommended as first-line therapy for nonresectable or metastatic mismatch repair-deficient/microsatellite instability-high (dMMR/MSI-H) colorectal cancer (CRC). However, the safety and efficacy of neoadjuvant PD-1 blockade immunotherapy for locally advanced dMMR/MSI-H CRC remain unclear.

***Patients and methods:*** From June 2020 to June 2022, eleven locally advanced dMMR/MSI-H CRC patients treated at the Sixth Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) were enrolled. All patients received 6 sintilimab (Innovent, LTD) injections (200 mg/injection, every 3 weeks) before radical laparoscopic resection. The patient clinical and pathological data were analyzed retrospectively.

***Results:*** dMMR was confirmed by immunohistochemistry (IHC) for all patients. However, polymerase chain reaction (PCR) or next-generation sequencing (NGS) confirmed MSI-H for only 90.9% (10/11) of the patients, while one patient had microsatellite stable (MSS) disease. After 6 injections of neoadjuvant anti-PD-1 therapy, 90.9% (10/11) of the patients (those confirmed to have dMMR and MSI-H disease) achieved pathological complete response (pCR). The other patient, who achieved major pathological response (mPR) with residual tumor <1%, had dMMR but MSS disease. No grade 3 or above immunotherapy-related adverse events (irAEs) occurred [Common Terminology Criteria for Adverse Events (CTCAE); version 5.0]. Overall, 72.7% (8/11) of the patients had grade 1-2 irAEs. No operational mortality or complications occurred within 30 days after surgery.

***Conclusions:*** Single-agent neoadjuvant PD-1 antibody immunotherapy was safe and effective in locally advanced dMMR/MSI-H CRC. Dual confirmation of MMR and MSI status by IHC and NGS or PCR is necessary for dMMR/MSI-H CRC patients before immunotherapy. The immunotherapy regimen used in this study deserves further validation in phase II and III clinical studies.

***Keywords:*** Colorectal cancer, dMMR/MSI-H, neoadjuvant immunotherapy, PD-1 blockade

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**Ethical Compliance:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Data Access Statement:** The data used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Conflict of Interest declaration:** The authors declare that they have NO affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

**Author Contributions** Data curation, Fengyun Pei; Formal analysis, Jingjing Wu and Qijun Yao; Investigation, Yandong Zhao and Qijun Yao; Methodology, Wan He; Project administration, Jun Huang; Resources, Jun Huang;

Validation, Wan He and Jun Huang; Visualization, Meijin Huang; Writing ï original draft, Fengyun Pei, Jingjing Wu and Yandong Zhao; Writing ï review & editing, Meijin Huang and Jun Huang.

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**Introduction**

Colorectal cancer (CRC) is one of the most common malignant tumors worldwide, and the incidence of CRC in China is constantly rising.[1] According to the expression status of mismatch repair (MMR) proteins, CRC is divided into two types: mismatch repair-deficient (dMMR) and mismatch repair-proficient (pMMR) disease.[2-4] Human MMR genes can express corresponding MMR proteins after transcription and translation. Any lack of MMR protein expression can lead to MMR defects, and defects in MMR function during DNA replication will lead to the accumulation of microsatellite instability (MSI).[2, 5] dMMR/MSI-H CRC patients have been identified as potential beneficiaries of immunotherapy.[6] The results of the KEYNOTE-016 (NCT01876511) study established a milestone in immunotherapy of CRC.[7] Previous studies showed that neoadjuvant immunotherapy was highly effective in most dMMR/MSI-H CRC patients.[8-10] The phase III clinical study KEYNOTE-177 (NCT02563002) showed that pembrolizumab had better safety and efficacy than the standard first-line treatment regimen (chemotherapy ± bevacizumab or cetuximab) in advanced dMMR or MSI-H CRC.[11, 12] The Food and Drug Administration (FDA) thus officially approved pembrolizumab for the first-line treatment for metastatic dMMR/MSI-H CRC. The 2022 edition of the Chinese Society of Clinical Oncology (CSCO) CRC diagnosis and treatment guidelines also specifically note that patients with metastatic dMMR/MSI-H CRC should receive immunotherapy for all first-line, second-line and third-line therapies, further supporting the role of immunotherapy in dMMR/MSI-H CRC treatment.

Patients with metastatic dMMR/MSI-H CRC account for only approximately 4% of CRC patients, while the proportion of patients with locally advanced dMMR or MSI-H CRC is approximately 12%-20%.[13, 14] Currently, major clinical guidelines recommend immunotherapy for nonresectable or metastatic dMMR/MSI-H CRC, while the safety and efficacy of neoadjuvant immunotherapy in early or locally advanced dMMR/MSI-H CRC remain unknown. The PICC study (NCT03926338), a phase II clinical study of neoadjuvant toripalimab (3 mg/kg administered intravenously on day 1, 14 days/cycle, 6 cycles) treatment in locally advanced dMMR/MSI-H CRC, showed a pathological complete response (pCR) rate of 64.7% (11/17).[15] The PICC study largely enriched the evidence supporting neoadjuvant immunotherapy for locally advanced dMMR/MSI-H CRC. However, the safety and efficacy of different neoadjuvant immunotherapy regimens for locally advanced dMMR/MSI-H CRC still need to be explored in more clinical studies.

Therefore, in this pilot study, we retrospectively analyzed the safety and efficacy of single-agent neoadjuvant immunotherapy with a PD-1 antibody using 6 injections (200 mg/injection, every 3 weeks) of sintilimab (Innovent, LTD) in locally advanced dMMR/MSI-H CRC.



## Materials and methods

### *Study design and patients*

Using a descriptive case series study approach, this study retrospectively analyzed eleven dMMR/MSI-H CRC patients with imaging stages $T_{3-4}N_{0-2}M_0$ who were treated in the Sixth

Affiliated Hospital of Sun Yat-sen University from June 2020 to June 2022. Among the eleven patients, seven were male, and four were female. The clinical stage was stage II in three patients and stage III in eight patients. The median age was 60 (range 41-81) years. The whole group had confirmed dMMR by immunohistochemistry (IHC). MSI status was detected by polymerase chain reaction (PCR) or next-generation sequencing (NGS), which showed that ten patients had MSI-H disease, and one patient had MSS disease.

The key enrollment criteria were as follows: (1) initial diagnosis with clinical stage $T_{3\sim4}N_{0\sim2}M_0$, (2) diagnosis of dMMR/MSI-H CRC by IHC, PCR or NGS, (3) Eastern American Oncology Group (ECOG) performance score of 0 or 1, (4) no previous colorectal surgery, (5) no previous chemotherapy or radiotherapy, and (6) no previous biological therapy or immunotherapy.

All patients received neoadjuvant immunotherapy in the form of 200 mg sintilimab injection intravenously on day 1 of each 3-week cycle, for a total of 6 injections. Radical laparoscopic resection for all patients was scheduled to be completed within 14 to 30 days after the end of the last neoadjuvant immunotherapy treatment.

***Efficacy and safety assessment***

The primary indicator for assessing efficacy in this study was the pCR rate of neoadjuvant immunotherapy. The main indicator for assessing safety was the incidence of irAEs. pCR was defined as lack of detection of any residual invasive tumor cells after radical surgical of primary colorectal lesions and in all sampled regional lymph nodes by pathological examination.[16] The

irAEs were graded according to the Common Terminology Criteria for Adverse Events (CTCAE; version 5.0).

***Follow-up***

All patients were scheduled to be followed up every 3 months during the first year after radical laparoscopic resection. During each follow-up, all patients underwent blood tests, tumor-related marker detection and computed tomography (CT) of the thorax, abdomen and pelvis. If the first-year follow-up results were normal, subsequent follow-up appointments were performed semiannually in the second and third years. The latest follow-up of all patients was conducted on July 1, 2022.

***Statistical analysis***

All data were processed using SPSS 24.0 statistical software. All continuous data are represented as M (range). Count information is expressed as absolute numbers. T tests and Wilcoxon tests were performed to explore the differences between the two groups. A P value $<0.05$ was set as statistical significance.

## Results

### *Characteristics of the Patients*

The whole group had confirmed dMMR by immunohistochemistry (IHC). MSI status was detected by polymerase chain reaction (PCR) or next-generation sequencing (NGS), which showed that ten patients had MSI-H disease, and one patient had MSS disease (Table 1). All patients received 6 injections of neoadjuvant immunotherapy with single-agent PD-1 monoclonal antibody before radical laparoscopic resection. The pathological examination results of surgically resected specimens showed that mPR was achieved in all patients in this study, and pCR was achieved in 90.9% (10/11) patients. In this cohort, all patients with dMMR and MSI-H CRC achieved pCR. The details of the patient clinical data are shown in Table 1.

### *Safety and Feasibility*

None of the patients in this study had any immune-related adverse events (irAEs) of grade 3 or above. Three (27.3%) patients did not have any irAEs. Eight (72.7%) patients had grade 1-2 irAEs, including decreased appetite, dizziness, dry mouth, dry eyes, arthralgia, aspartate aminotransferase increase, taste impairment, pruritus or rash, nausea, and somnipathy. All irAEs are shown in Table 2. All irAEs were relieved by symptomatic management during treatment. None of the patients had radical surgery delayed due to irAEs. None of the patients had surgery-related adverse events, such as intestinal obstruction, anastomotic leakage, stenosis, fistula, or incision infection. The latest follow-up (July 1, 2022) results suggested that none of

the patients had any signs of CRC recurrence. The median follow-up time and disease-free survival time (DFS) of all patients were 335 days.

***Clinical Activity***

Representative radiologic and pathologic responses after 6 preoperative doses of sintilimab are shown in Figure 1. Among the 11 patients in this cohort, significant pathological downstaging from the pretreatment clinical stage occurred in all patients (Table 3). In this cohort, 3 (27.3%) patients exhibited clinical complete response (cCR), 8 (72.7%) exhibited partial response (PR), and 3 (27.3%) had stable disease (SD) (Table 3). Notably, the pathologic response was significantly greater than the radiologic response ($P=7.03 ¦ 10^{-25}$) (Figure 2).

**Discussion**

In this pilot study, all patients with locally advanced dMMR/MSI-H CRC significantly benefited from single-agent neoadjuvant immunotherapy with a PD-1 monoclonal antibody. The pCR rate was 90.9%, which is better than those reported in other single-agent neoadjuvant immunotherapy studies, although the case number in this cohort was relatively small.[15, 17] Compared with traditional neoadjuvant therapies, the advantages of immunotherapy in dMMR/MSI-H CRC patients have been proven.[8-10] The neoadjuvant immunotherapy regimen in this study increased not only the preoperative downstaging effect but also the pCR rate in locally advanced dMMR/MSI-H CRC patients. Based on the satisfactory pCR rate of neoadjuvant immunotherapy in this cohort, neoadjuvant immunotherapy is especially worthy of further exploration and verification in locally advanced dMMR/MSI-H inferior rectal cancer patients whose organ, sexual and defecation function preservation are particularly required.

Although relevant clinical studies have confirmed the effectiveness of immunotherapy for dMMR/MSI-H CRC, immunotherapy regimens for dMMR/MSI-H CRC, such as single-agent immunotherapy combined with CTLA-4 blockade double immunotherapy or immunotherapy combined with COX-2 inhibitors, are still in the exploratory stage. The NICHE study (NCT03026140) adopted the neoadjuvant immunotherapy regimen of nivolumab combined with ipilimumab [nivolumab 3 mg/kg (days 1 and 15) + ipilimumab 1 mg/kg (day 1)], and the pCR rate of the treatment group was 60.0% (12/20).[17] In the PICC study (NCT03926338), a neoadjuvant immunotherapy regimen of toripalimab (3 mg/kg, 2 weeks/cycle, 6 cycles) with or

without celecoxib was used, and the results showed that the pCR rate of the combination group was 88.2% (15/17), while that of the monotherapy group was 64.7% (11/17).[15] Meanwhile, the remarkable efficacy of immunotherapy in dMMR/MSI-H CRC indicated which might be a potential therapeutic option for locally advanced rectal cancer (LARC) patients with dMMR/MSI-H status. A recent study has showed that single-agent PD-1 blockade could even avoid surgery in dMMR LARC patients, and the watch and wait strategy after PD-1 blockade was also safe in these patients.[10] In our study, the pCR rate of patients who received single-agent neoadjuvant immunotherapy with sintilimab was 90.9% (10/11), and the only patient who did not achieve pCR had dMMR and MSS CRC. However, the patient who did not achieve pCR still achieved mPR with residual tumor cells less than 1%. Notably, only 30% of the 10 pCR patients were accessed cCR. The radiological regression in this cohort was significantly lower than that of the pathological regression ($P<0.0001$), which means there might be a high probability of no residual cancer cells even the original lesions were still radiological visible. Compared with the reported neoadjuvant immunotherapy regimen for locally advanced dMMR/MSI-H CRC, the single-agent neoadjuvant immunotherapy in this study achieved a better pCR rate, which might be due to the increased number of preoperative immunotherapy cycles and treatment interval of the regimen, as well as the associated effects of immunotherapy. Furthermore, no patients in this study had grade 3 or above irAEs, and the proportion of patients with grade 1-2 irAEs was 72.7% (8/11). These incidences of irAEs demonstrate the safety of the

neoadjuvant immunotherapy regimen used in this study. All irAEs documented in this study have been reported in other relevant immunotherapy studies.[15, 18, 19]

In this cohort, the MMR protein expression status of all patients was confirmed by IHC, and the MSI status of all patients was confirmed by PCR or NGS. The status of MMR proteins in all patients was dMMR, and only one patient (9.1%) showed inconsistency in the MSI status results derived from different methods, whose status was actually dMMR and MSS. Notably, this dMMR and MSS patient with colon cancer was the only one who failed to achieve pCR after 6 complete doses of neoadjuvant immunotherapy. All ten CRC patients with both dMMR and MSI-H in this cohort achieved pCR after neoadjuvant monotherapy with PD-1 blockade. Previous studies showed that the results of IHC and PCR/NGS are not always consistent.[20-22] The efficacy of neoadjuvant immunotherapy for dMMR and MSS CRC may be different from that for dMMR and MSI-H CRC. Therefore, for dMMR/MSI-H CRC patients who are recommended to receive neoadjuvant immunotherapy, both IHC to detect the expression status of MMR protein expression and PCR or NGS to detect MSI status are essential before performing neoadjuvant immunotherapy.

However, this study has some limitations. This pilot cohort had a relatively small case number, and the postoperative follow-up time was relatively short. Long-term follow-up of patients will be necessary to define the role of neoadjuvant monotherapy with PD-1 blockade in reducing recurrence and curing early-stage cancer. Furthermore, according to the results of this study, as well as those of the Checkmate142 study (NCT02060188) of immunotherapy in metastatic

dMMR/MSI-H CRC,[23] we designed and registered a prospective phase II clinical study (NCT04643041): Watch and Wait in PD-1 Monoclonal Antibody Treated dMMR/MSI-H Distal Rectal Cancer (BASKET). The study has started recruiting.

In conclusion, single-agent neoadjuvant immunotherapy with a PD-1 monoclonal antibody was safe and significantly effective in locally advanced dMMR/MSI-H CRC. Dual confirmation of MMR and MSI status by IHC and NGS or PCR is necessary for dMMR/MSI-H CRC patients before immunotherapy. The immunotherapy regimen used in this study deserves further validation in phase II and III clinical studies.

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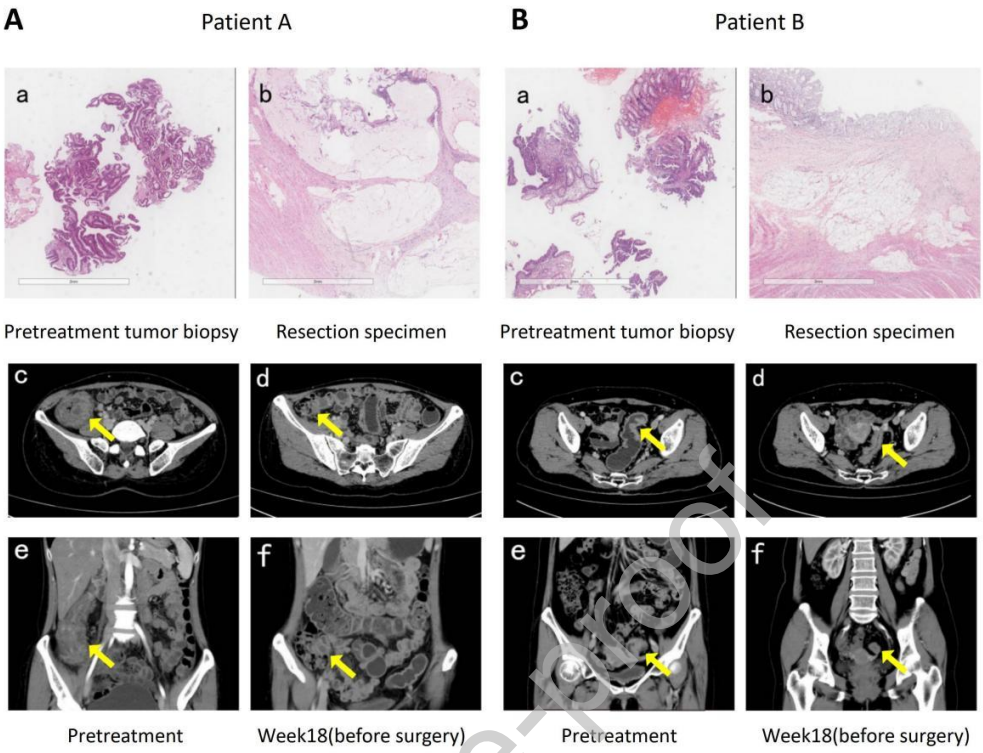
**Figure 1. Patterns of Pathologic and Radiologic Responses to Neoadjuvant Therapy with PD-1 Antibody.**




A. The upper row shows representative sections of tumor specimens obtained from an adult patient A with $cT_{3\sim4a}N_1M_0$ ascending colon cancer before (a) after (b) the neoadjuvant immunotherapy (hematoxylin and eosin staining). This patient had over 99% pathological regression of the tumor in the resection specimen. The lower row shows computed tomography (CT) scans of the abdomen of this patient before (c, e) and 18 weeks after (d, f) the neoadjuvant immunotherapy. A scan performed before surgery showed 55.6% shrinkage.

B. The upper row shows representative sections of tumor specimens obtained from another adult patient B with $cT_3N_2M_0$ sigmoid colon cancer before (a) and after (b) the neoadjuvant immunotherapy (hematoxylin and eosin staining). This patient had 100% pathological regression of the tumor in the resection specimen. The lower row shows CT scans of the abdomen of this patient before (c, e) and 18 weeks after (d, f) the neoadjuvant immunotherapy. A scan performed before surgery showed 28.3% shrinkage.

**Figure 2. Correlation of Pathological and Radiological Tumor Regression after Neoadjuvant Immunotherapy.**

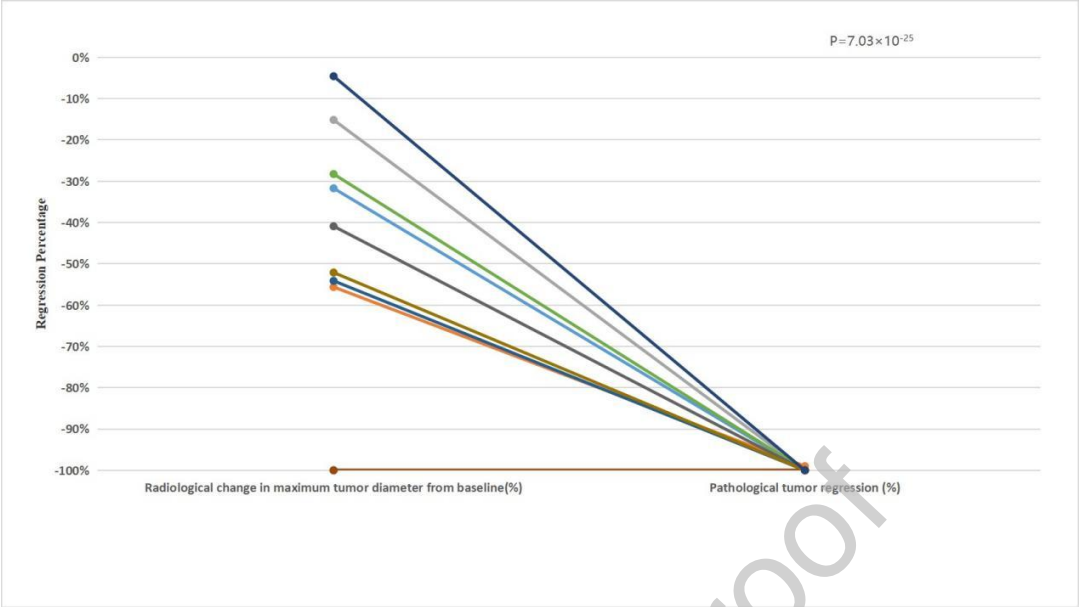



Table 1. Clinical information of the eleven patients

| No. | Age | Sex | Tumor location | Clinical TNM stage before immunotherapy | Clinical TNM stage after immunotherapy | Mismatch repair status | Loss of expression of mismatch repair proteins | Microsatellite instability test by PCR/NGS | Tumor response | TRG (NCCN) | Pathological type | Disease-free survival (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 69 | Female | Transverse colon | T3N1bM0 ( ) | T3N1bM0 | dMMR | MLH1, PMS2 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 644 |
| 2 | 41 | Female | Ascending colon | T3~4aN1M0 ( ) | T3N1M0 | dMMR | MSH2, MSH6 | MSS | mPR (<1%) | 1 | Moderately differentiated adenocarcinoma | 552 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 48 | Male | Transverse colon | T3N1bM0 ( ) | T3N1M0 | dMMR | PMS2 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 522 |
| 4 | 53 | Male | Rectum | T3N0M0 (II) | T0N0M0 | dMMR | MSH2, MSH6 | MSI-H | pCR | 0 | Poorly differentiated adenocarcinoma | 442 |
| 5 | 65 | Male | Ascending colon | T3N0M0 (II) | T0N0M0 | dMMR | MLH1, PMS2 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 410 |
| 6 | 55 | Female | Sigmoid colon | T3N2M0 ( ) | T3N0M0 | dMMR | MSH2 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 335 |
| 7 | 61 | Male | Hepatic flexure | T4aN2bM0 ( ) | T3bN0M0 | dMMR | MLH1, PMS2 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 324 |
| 8 | 60 | Male | Transverse colon | T3N0M0 (II) | T0N0M0 | dMMR | MLH1, PMS2 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 256 |
| 9 | 66 | Female | Sigmoid colon | T3-4aN2M0 ( ) | T2-3N1M0 | dMMR | MLH1, PMS2 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 53 |
| 10 | 54 | Male | Transverse colon | T4aN1M0 ( ) | T4aN1bM0 | dMMR | PMS2 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 29 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 81 | Male | Transverse colon | T4N1M0 ( ) | T3N0M0 | dMMR | MSH2, MSH6 | MSI-H | pCR | 0 | Moderately differentiated adenocarcinoma | 21 | |

AJCC: American Joint Committee on Cancer, pCR: pathological complete response, mPR: major pathological response, dMMR: mismatch repair-deficient, MSI-H: microsatellite instability-high, MSS: microsatellite stable, TRG: tumor regression grade, NCCN: National Comprehensive Cancer Network

Table 2. Treatment-related adverse events during neoadjuvant immunotherapy

| Treatment-related adverse events | No. of patients | | |
|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 |
| Any | 8 | 2 | 0 |
| Decreased appetite | 3 | 0 | 0 |
| Dizziness | 2 | 0 | 0 |
| Dry mouth | 2 | 1 | 0 |
| Arthralgia | 2 | 1 | 0 |
| Pruritus or rash | 2 | 0 | 0 |
| Aspartate aminotransferase increase | 1 | 0 | 0 |
| Taste impairment | 1 | 0 | 0 |
| Nausea | 1 | 0 | 0 |

| | | | |
|---|---|---|---|
| Somnipathy | 1 | 0 | 0 |
| Dry eyes | 1 | 0 | 0 |

Table 3. Pretreatment clinical stage and posttreatment pathological stage

| No. | Pretreatment clinical stage, TNM (stage group) | Preoperative clinical stage TNM (stage group) | Pathological stage at resection, TNM (stage group) | Radiological regression in maximum tumor diameter from baseline (%) | Pathological tumor regression (%) |
|---|---|---|---|---|---|
| 1 | $T_3N_{1b}M_0$ ( B) | $T_3N_{1b}M_0$ ( B) | $T_0N_0M_0$ (0) | 31.7 | 100 |
| 2 | $T_{3\sim4a}N_1M_0$ ( B) | $T_3N_1M_0$ ( B) | $T_1N_0M_0$ (I) | 55.6 | 99 |
| 3 | $T_3N_{1b}M_0$ ( B) | $T_3N_1M_0$ ( B) | $T_0N_0M_0$ (0) | 15.2 | 100 |
| 4 | $T_3N_0M_0$ (IIA) | $T_0N_0M_0$ (0) | $T_0N_0M_0$ (0) | 100 | 100 |
| 5 | $T_3N_0M_0$ (IIA) | $T_0N_0M_0$ (0) | $T_0N_0M_0$ (0) | 100 | 100 |
| 6 | $T_3N_2M_0$ ( B-C) | $T_3N_0M_0$ (IIA) | $T_0N_0M_0$ (0) | 28.3 | 100 |
| 7 | $T_{4a}N_{2b}M_0$ ( C) | $T_{3b}N_0M_0$ (IIA) | $T_0N_0M_0$ (0) | 54.1 | 100 |
| 8 | $T_3N_0M_0$ (IIA) | $T_0N_0M_0$ (0) | $T_0N_0M_0$ (0) | 100 | 100 |
| 9 | $T_{3\text{-}4a}N_2M_0$ ( B-C) | $T_{2\text{-}3}N_1M_0$ ( A-B) | $T_0N_0M_0$ (0) | 40.9 | 100 |
| 10 | $T_{4a}N_1M_0$ ( B) | $T_{4a}N_{1b}M_0$ ( B) | $T_0N_0M_0$ (0) | 52.1 | 100 |
| 11 | $T_4N_1M_0$ ( B-C) | $T_3N_0M_0$ (IIA) | $T_0N_0M_0$ (0) | 4.6 | 100 |

Clinical Practice Points

Neoadjuvant immunotherapy is necessary in patients with locally advanced dMMR/MSI-H CRC. The neoadjuvant immunotherapy regimen in this study increased not only the preoperative downstaging effect but also the pCR rate in locally advanced dMMR/MSI-H CRC patients. In order to better benefit patients from immunotherapy, this regimen deserves further validation in phase II and III clinical studies.