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Case Report

HER2-positive, *RAS*-mutant, MSS colorectal cancer: a rare subtype report and novel insights into immunotherapy and ADC combinations

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Abstract: This study reports a case of HER2-positive, *RAS*-mutant, microsatellite-stable (MSS) metastatic colorectal cancer (mCRC) exhibiting intrinsic resistance to 5-fluorouracil (5-FU)-based chemotherapy and bevacizumab. Despite multiple lines of prior systemic therapy, the patient achieved significant tumor shrinkage and durable disease control with a combination of PD-1 blockade and a HER2-targeted antibody-drug conjugate (ADC), resulting in a progression-free survival (PFS) exceeding 10 months. Based on this case, we review the current landscape of immunotherapy and ADCs in mCRC, emphasizing the emerging potential of combination strategies for patients harboring rare molecular profiles such as HER2-positive, *RAS*-mutant MSS tumors. In addition, we discuss the importance of HER2 testing in CRC and the available diagnostic modalities, including immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), next-generation sequencing (NGS), and liquid biopsy. Given the heterogeneity of HER2 expression in CRC, optimizing testing strategies and treatment selection remains a critical research priority. This review underscores the need for large prospective studies and biomarker-driven trials to refine treatment approaches and improve outcomes in this challenging patient population.

Keywords: Metastatic colorectal cancer, HER2-positive, *RAS*-mutant, serplulimab, trastuzumab deruxtecan

Introduction

Colorectal cancer (CRC) ranks as the third most common malignancy worldwide, with the increasing incidence and mortality, representing a significant global health challenge [1]. Metastatic disease remains the leading cause of CRC-related deaths, with a 5-year survival rate of only about 13% [2]. Unfortunately, therapeutic progress in advanced CRC has been limited over the past few decades. First- and second-line treatments still rely heavily on fluoropyrimidine-based chemotherapy, while later-line options, such as regorafenib and trifluridine/tipiracil offer limited clinical benefit, with median progression-free survival (mPFS) of approximately 2-3 months and objective response rates (ORR) below 5% [3-7].

Moreover, CRC is a highly molecularly driven disease in which therapeutic strategies are strongly influenced by primary tumor location, pathological characteristics, and genetic alterations, thereby necessitating close multidisciplinary collaboration [8]. Within this paradigm, HER2 overexpression (3-5% of metastatic CRC [mCRC]) and *RAS* mutations (up to 45% of mCRC) exemplify key clinical challenges [9-11]. While HER2-targeted antibody-drug conjugates (ADCs), such as trastuzumab deruxtecan (T-DXd), have shown promising activity with an ORR of 45.3% in the DESTINY-CRC01 trial, their activity in the HER2-positive and *RAS*-mutant subset remains largely uncharted [12]. In addition, more than 80% of HER2-positive CRCs also exhibit microsatellite stability (MSS) and low PD-L1 expression, forming an "immune-

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cold" phenotype that is largely resistant to immunotherapy [13]. The failure of PD-1 inhibitors in MSS CRC, with an ORR of less than 1% in the KEYNOTE-016 study, reflects the profoundly "cold" tumor immune microenvironment characterized by limited T-cell infiltration and abundant immunosuppressive myeloid cells [14, 15]. Intriguingly, preclinical data suggest that HER2-targeted ADCs may help remodel tumor immunity through multiple mechanisms: (1) releasing tumor antigens via immunogenic cell death induced by T-DXd's topoisomerase I inhibitor payload; (2) influencing macrophage polarization through Fcγ receptor engagement, potentially shifting from an immunosuppressive M2 phenotype to a pro-inflammatory M1 phenotype, thereby enhancing antitumor immune responses; and (3) reducing dominant tumor clones, which may expose previously subdominant T-cell epitopes and promote a broader and more effective T-cell-mediated antitumor response [16-18].

In this study, we present an illustrative case of a 68-year-old female diagnosed with MSS, low PD-L1 expression (combined positive score [CPS] 2) CRC, characterized by HER2 overexpression (immunohistochemistry [IHC] 3+) and *HER2* gene amplification confirmed by next-generation sequencing (NGS). The patient's tumor was located in the right colon, with concurrent liver and lung metastases, and harbored a *KRAS* p.G12V mutation (variant allele frequency 15.55%). Following multiple failed lines of prior therapy, the patient achieved a sustained deep response and remained progression-free for more than 10 months on a combination regimen of serplulimab (an anti-PD-1 monoclonal antibody [mAb]) and T-DXd. Based on this case, we further review the current landscape of immunotherapy and ADC therapies in advanced CRC, focusing on their potential to overcome treatment resistance and improve outcomes.

Case presentation

A 68-year-old female with right-sided colon adenocarcinoma and known liver and lung metastases for more than six months, who had undergone primary tumor resection five months earlier, presented to Nanjing Tongren Hospital for further evaluation and treatment (**Table 1**; **Figure 1**). NGS of both tumor tissue and peripheral blood identified a *KRAS* mutation and *ERBB2* (*HER2*) amplification, with wild-type *NRAS* and *BRAF*. Pathologic examination confirmed HER2 overexpression (IHC 3+), MSS based on intact expression of MLH1, MSH2, MSH6, and PMS2, and a high proliferative index with Ki-67 staining of 70% (**Table 1**; **Figure 2**). Based on these findings, the patient was diagnosed with *KRAS*-mutant, HER2-positive, MSS stage IV right-sided colon adenocarcinoma (post-surgery), with a TNM stage of ypT3N1b cM1b and an Eastern Cooperative Oncology Group (ECOG) performance status of 1.

Prior to presenting to our institution, the patient had undergone multiple lines of systemic therapy, including chemotherapy and bevacizumab, with limited antitumor activity (best response: stable disease [SD]; **Table 1**; **Figures 1** and **2**). According to the patient's account and the available medical records, she initially presented over 6 months earlier to a local hospital with paroxysmal abdominal pain. Laboratory testing revealed elevated serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels (**Figure 2A**). Computed tomography (CT) demonstrated bowel wall thickening of the ascending colon near the ileocecal valve, retroperitoneal lymphadenopathy, and multiple metastatic lesions in the liver and lungs (**Figure 1**). The patient was clinically diagnosed with stage IV colon cancer. She initially received one cycle of FOLFOX (5-fluorouracil [5-FU], leucovorin, and oxaliplatin); however, the serum CEA and CA19-9 levels continued to increase (**Figure 2B**), indicating treatment failure. The regimen was subsequently switched to FOLFIRI (5-FU, leucovorin, and irinotecan). After one cycle, the patient developed malignant bowel obstruction and underwent palliative right hemicolectomy, ileocolic anastomosis, and lymphadenectomy. Postoperatively, the patient continued treatment with FOLFIRI plus bevacizumab for four additional cycles. During this period, the patient's general condition remained stable, but follow-up imaging showed increased size and number of liver and lung metastases, indicating progressive disease (PD). Third-line treatment with XELOX (capecitabine plus oxaliplatin) plus bevacizumab was initiated, but after two cycles, the CEA and CA19-9 levels increased again (**Figure 2B**), prompting referral to our institution for further management.

Table 1. Patient's clinical characteristics and treatment history

	Details
General Information	Age/Sex: 68 years/Female
	BMI: 26.83 kg/m^2
	Comorbidities: Hypertension (well-controlled with nifedipine for over ten years)
	Medical History: Never smoked, no history of alcohol abuse, no family history of cancer
Physical Examination	Unremarkable, blood pressure: 128/65 mmHg
Imaging Findings	Right colon: Postoperative changes
	Liver: Multiple round low-density lesions, largest: 3.6 cm × 2.7 cm
	Lung: Bilateral multifocal pulmonary masses, some with cavitation, largest: 2.0 cm × 1.6 cm
Pathological & Molecular Findings	HER2 status: Positive (IHC 3+)
	PD-L1 (22C3): CPS 2, TPS 0
	MMR proteins: Intact (MLH1, MSH2, MSH6, PMS2) → MSS
	Ki-67: ~70%
	Genetic testing (tumor): *KRAS* mutation (p.G12V, NM_033360.4, Exon 2, c.35G>T, frequency: 15.55%), *APC* mutation (NM_000038.6, Exon 16, c.4666dupA, p.T1556Nfs*3, frequency: 11.22%), *PIK3CA* mutation (NM_006218.4, Exon 2, c.263G>A, p.R88Q, frequency: 16.61%), *TP53* mutation (NM_000546.6, Exon 6, c.637C>T, p.R213*, frequency: 18.58%), increased *ERBB2* copy number (n=7.62), wild-type *BRAF*, *NRAS, NTRK1/2/3* Genetic testing (blood): *KRAS* mutation (p.G12V, NM_033360.4, Exon 2, c.35G>T, frequency: 2.28%), *PIK3CA* mutation (NM_006218.4, Exon 2, c.263G>A, p.R88Q, frequency: 2.64%), *TP53* mutation (NM_000546.6, Exon 6, c.637C>T, p.R213*, frequency: 1.96%), increased *ERBB2* copy number (n=2.85), wild-type *BRAF*, *NRAS*, *NTRK1/2/3*
Clinical Staging & Performance Status	TNM staging: ypT3N1b cM1b (post-surgery)
	ECOG PS score: 1
Diagnosis	RAS-mutant, HER2-positive, MSS stage IV right-sided colon cancer (post-surgery) with liver and lung metastases
Treatment History	First-line therapy: FOLFOX (1 cycle) → Increased CEA and CA19-9, suggesting PD → Best Response: NE
	Second-line therapy: FOLFIRI (1 cycle) → Bowel obstruction → Underwent palliative right hemicolectomy, ileocolic anastomosis, lymphadenectomy → FOLFIRI + bevacizumab (4 additional cycles) → Increased size and number of liver/lung metastases, suggesting systemic PD → Best Response: SD
	Third-line therapy: XELOX + bevacizumab (2 cycles) → Increased CEA and CA19-9, suggesting PD → Best Response: NE

BMI, body mass index; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; CPS, combined positive score; TPS, tumor proportion score; MMR, mismatch repair; MSS, microsatellite stable; KRAS, Kirsten rat sarcoma viral oncogene homolog; APC, adenomatous polyposis coli; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; TP53, tumor protein p53; ERBB2, Erb-B2 receptor tyrosine kinase 2 (HER2 gene); BRAF, B-Raf proto-oncogene serine/threonine kinase; NRAS, neuroblastoma RAS viral oncogene homolog; NTRK, neurotrophic receptor tyrosine kinase; TNM, tumor-node-metastasis; ECOG PS, Eastern Cooperative Oncology Group performance status; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; XELOX, capecitabine plus oxaliplatin; PD, progressive disease; SD, stable disease; NE, not evaluable; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

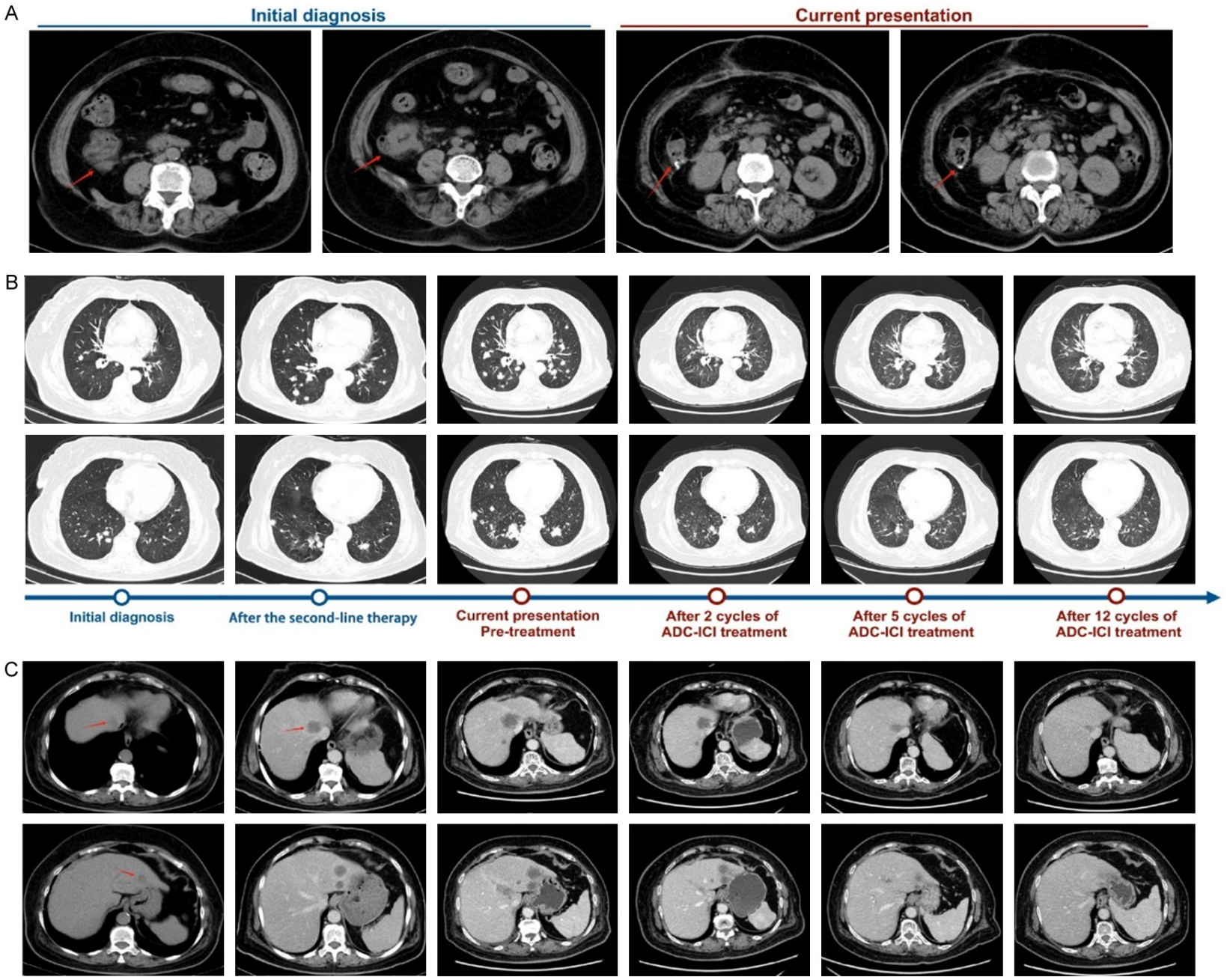
A
Initial diagnosis
Current presentation
B
Initial diagnosis
After the second-line therapy
Current presentation
Pre-treatment
After 2 cycles of
ADC-ICI treatment
After 5 cycles of
ADC-ICI treatment
After 12 cycles of
ADC-ICI treatment
C

Figure 1. Tumor changes during treatment. A. Abdominal computed tomography (CT) scans at initial and current diagnosis show the primary right-sided colon tumor and postoperative changes after surgical resection (arrow). B. Chest CT scan show changes in pulmonary metastases during treatment. At the current presentation, bilateral multifocal pulmonary masses, some with cavitation, were evident. After five cycles of serplulimab in combination with T-DXd, significant shrinkage and partial disappearance of the pulmonary lesions were observed, correlating with a partial response (PR). C. Abdominal CT scan show changes in liver metastases. After treatment with serplulimab and T-DXd, the liver lesions showed significant regression, achieving PR. Abbreviations: ADC, antibody-drug conjugate; ICI, immune checkpoint inhibitor; ADC-ICI, combination treatment with serplulimab and T-DXd; CT, computed tomography; PR, partial response.

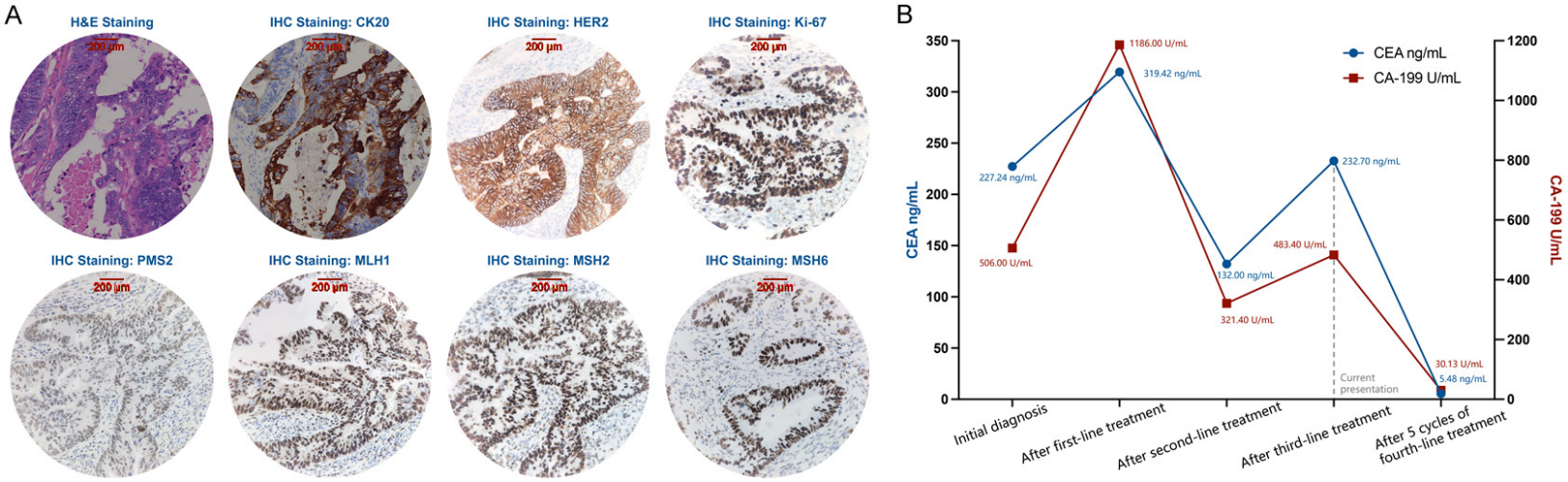

Figure 2. Pathological analysis and blood tumor marker changes. A. Representative pathological images of the patient's resected right-sided colon tumor show hematoxylin and eosin (H&E) staining, HER2 overexpression (IHC 3+), Ki-67 proliferation index (~70%), intact mismatch repair (MMR) protein expression (MLH1, MSH2, MSH6, and PMS2), and CK20 positivity, confirming HER2-positive, MSS, and right-sided colon adenocarcinoma. B. Changes in carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels during treatment. Both serum tumor markers dynamics showed significant increases after first- and second-line treatment, indicating disease progression. After five cycles of fourth-line treatment with serplulimab plus T-DXd, both markers decreased significantly and reached normal levels, which correlated with tumor regression and durable response. H&E, hematoxylin and eosin; IHC, immunohistochemistry; HER2, human epidermal growth factor receptor 2; CEA, carcinoembryonic antigen; CA-199, carbohydrate antigen 19-9; MMR, mismatch repair; MSS, microsatellite stable.

Given the limited treatment options in the later-line setting for mCRC and the failure of prior targeted therapy plus chemotherapy, a multidisciplinary team recommended an innovative combination regimen comprising the HER2-targeted ADC, T-DXd, and the anti-PD-1 mAb, serplulimab. The regimen consisted of 300 mg of serplulimab on day 1 and 300 mg of T-DXd on day 2, repeated every 21 days until disease progression or unacceptable toxicity. After two treatment cycles, radiographic imaging demonstrated significant regression of several pulmonary lesions, with some lesions completely resolving, and a modest reduction in liver metastases, achieving a partial response (PR) (**Figure 1**). Following the fifth cycle, the target lesions continued to shrink, including marked regression of liver metastases, maintaining a PR with a depth of response (DpR) of 68% (**Figure 1**). Concurrently, serum CEA and CA19-9 levels progressively decreased, normalizing after five cycles (**Figure 2B**). Following 12 cycles of combination therapy, the patient maintained a sustained deep response of target lesions, with no evidence of PD, achieving a fourth-line PFS (PFS4) exceeding 10 months.

The combination therapy was well tolerated overall. According to the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0), most adverse events (AEs) were grade 1-2 and were attributed to T-DXd. The patient experienced mild diarrhea and neutropenia, both of which resolved with symptomatic treatment. Additionally, the patient reported mild fatigue lasting approximately 10 days, which resolved spontaneously without medical intervention.

Discussion

This case highlights a rare molecular subset of CRC characterized by concurrent HER2 overexpression/amplification, *KRAS* mutation, and MSS status in a right-sided primary tumor, features that are infrequently represented in prospective clinical trials. Although HER2 overexpression has been reported in approximately 4%-5% of mCRCs, it most commonly occurs in *RAS* wild-type tumors; in contrast, the coexistence of HER2 positivity and *KRAS* mutations is observed in only about 1% of cases [9]. Moreover, HER2 overexpression is more frequently associated with left-sided colon or rectal cancers, as demonstrated in trials such as HERACLES (84% left colon/rectum vs. 16% right colon) and MyPathway (75% left colon/rectum vs. 21% right colon) [19, 20]. Notably, our patient exhibited primary resistance to 5-FU-based chemotherapy and anti-angiogenic regimens, as evidenced by the failure of three different treatment protocols, which potentially attributable to her unique molecular profile. Collectively, these overlapping molecular alterations presented a formidable therapeutic challenge with limited guidance from existing clinical evidence. Nevertheless, the innovative use of immunotherapy combined with a HER2-targeted ADC achieved a sustained deep response and durable clinical benefit, underscoring the potential of immunotherapy-ADC combinations in this exceptionally difficult-to-treat subset of mCRC.

Immunotherapy in CRC

In 2017, the U.S. Food and Drug Administration (FDA) approved pembrolizumab and nivolumab for the second-line treatment of mismatch repair-deficient (dMMR) and microsatellite instability-high (MSI-H) mCRC, marking a pivotal breakthrough for immunotherapy in this disease [21]. Landmark studies, including KEYNOTE-016 and CheckMate-142, consistently demonstrated significant clinical benefit with PD-1 inhibitors in dMMR/MSI-H CRC patients [15, 22]. KEYNOTE-177 further validated the efficacy of pembrolizumab monotherapy as first-line treatment for dMMR/MSI-H CRC, showing improved PFS compared to chemotherapy, although OS benefit was not statistically significant [23]. In sharp contrast, these pivotal trials showed that immunotherapy had minimal efficacy in proficient MMR (pMMR)/MSS CRC patients, who constitute the majority (about 85%) of the CRC cases. For instance, KEYNOTE-016 reported no objective responses among pMMR/MSS patients treated with pembrolizumab monotherapy, with median PFS and OS limited to 2.2 months and 5.0 months, respectively [15]. Taken together, these studies suggest that immunotherapy is effective only in dMMR/MSI-H CRC patients, while pMMR/MSS CRC patients show limited benefit with mono-immunotherapy.

Further studies have shown that dMMR/MSI-H CRCs are commonly associated with a high

tumor mutation burden (TMB), usually exceeding 12 mutations per 10^6DNA bases. This high TMB results in the presentation of mutated peptides on major histocompatibility complex (MHC) class I molecules; these complexes of mutant peptides with MHC class I are recognized as foreign neoantigens [24]. As a result, the tumor microenvironment (TME) in dMMR/MSI-H CRC is heavily infiltrated by immune cells, particularly $CD8^+$ tumor-infiltrating lymphocytes (TILs), T helper 1 (TH1) $CD4^+$ TILs, and macrophages [25-27]. In response, tumor cells upregulate T cell inhibitory ligands, including CD80, CD86 (members of the B7 family), and PD-L1, which bind to co-inhibitory receptors such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and PD-1. Moreover, a high proportion of tumor-associated macrophages (TAMs) express PD-1, further facilitating immune escape and promoting rapid tumor proliferation [28]. These immunosuppressive characteristics of the TME explain the remarkable clinical efficacy of ICIs, such as anti-PD-(L)1 antibodies, which can counteract T-cell and macrophage-mediated immune suppression and enable cytotoxic immune responses against tumor cells. However, dMMR/MSI-H tumors account for only about 15% of all CRC cases; the remaining approximately 85% of pMMR/MSS CRCs usually exhibit a much lower TMB (<8.24 mutations per 10^6DNA bases), lack significant immune infiltration resulting in T cell exclusion, and express lower levels of immunoinhibitory ligands [13]. These may explain the poor response of pMMR/MSS CRC to monoimmunotherapy, reinforcing the exploration of combination strategies to modulate the TME and improve the efficacy of ICIs.

Chemotherapy combined with immunotherapy has emerged as a potential strategy to overcome this immune resistance. Cytotoxic chemotherapy induces direct tumor cell death and enhances tumor immunogenicity. The increased apoptotic burden promotes the release of tumor-associated antigens (TAAs), which are subsequently processed by antigen-presenting cells (APCs) and activated tumor-specific cytotoxic T-cell responses. This immunogenic effect enhances immune cell infiltration into the TME, potentially creating a more permissive milieu for ICIs to exert antitumor effects [29]. However, clinical trials evaluating chemoimmunotherapy in MSS CRC have yielded mixed results. The phase II CheckMate 9X8 trial evaluated the efficacy of nivolumab in combination with mFOLFOX6 and bevacizumab as first-line treatment for MSS CRC, failed to demonstrate a significant improvement in either PFS or OS compared to chemotherapy alone [30]. In contrast, the phase II AtezoTRIBE study reported a modest yet statistically significant improvement in PFS and OS with the addition of atezolizumab to FOLFOXIRI plus bevacizumab in predominantly MSS CRC patients [31, 32]. While these results support a potential role for combination strategies in overcoming immune resistance, they also underscore the complexity of modulating the immunosuppressive TME in MSS CRC. Overall, the mixed outcomes from CheckMate 9X8 and AtezoTRIBE indicate that although chemotherapy can enhance tumor immunogenicity, its combination with ICIs has yet to achieve transformative clinical benefit in MSS CRC.

For pMMR/MSS CRC, current research focuses extensively on identifying predictive biomarkers and developing effective therapeutic strategies to enhance immunotherapy efficacy. Due to their limited response to ICIs, various biomarkers are under rigorous investigation, including tumor-intrinsic factors such as immune checkpoint molecule expression, TMB, TIL composition, and alterations in key signaling pathways. PD-L1 expression remains the most widely used biomarker to predict ICI efficacy; however, prospective studies in advanced CRC have demonstrated inconsistent and limited predictive value of PD-L1 expression in this setting [33, 34]. Although MSS CRC generally exhibit low TMB, a subset of tumors with relatively higher TMB harbor sufficient neoantigens to enable immunotherapy responsiveness [35]. Additionally, detailed characterization of TIL density, phenotype (particularly CD8+ effector T cells and CD4+ helper T cells), and spatial distribution could further stratify MSS CRC patients most likely to benefit from immunotherapy [36]. Dysregulation of signaling pathways, notably WNT/β-catenin, MAPK, PI3K/AKT/mTOR, and TGF-β pathways, significantly contributes to immune evasion and therapeutic resistance, representing potential targets for rational combination therapies. The immunosuppressive TME in MSS CRC, involving myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and stromal elements

such as cancer-associated fibroblasts (CAFs), also represents critical therapeutic targets. Novel therapeutic approaches targeting these immunosuppressive populations (e.g., CCR2 inhibitors, anti-CD25 antibodies, CTLA-4 blockade) or stromal components (e.g., CXCL12/CXCR4 inhibitors, TGF-β antagonists) are actively explored to convert immunologically "cold" tumors into "hot" ones [36]. In parallel, the increasing recognition of HER2 overexpression/amplification in CRC has sparked significant interest in HER2-targeted therapeutic strategies, paving the way for novel approaches.

Role of ADCs in CRC treatment

ADCs represent an emerging class of targeted therapies composed of three key elements: a mAb that selectively binds to a TAA, a linker that connects the antibody to the cytotoxic payload, and a payload that exerts potent antitumor effects upon internalization. This structure enables ADCs to precisely deliver cytotoxic agents to tumor cells while minimizing off-target toxicity, thereby enhancing therapeutic efficacy. The concept of ADCs was validated in solid tumors with the 2013 FDA approval of ado-trastuzumab emtansine (T-DM1) for HER2-positive metastatic breast cancer (mBC), marking the first ADC approval for a solid malignancy [37]. T-DXd is another HER2-targeting ADC that shares the same mAb component as T-DM1 (trastuzumab) but differs in its linker and payload. T-DXd utilizes a cleavable tetrapeptide linker and a highly potent exatecan-derived topoisomerase I inhibitor payload, with a drug-to-antibody ratio (DAR) of 8:1, which is significantly higher than T-DM1 [38]. This structural difference contributes to the enhanced cytotoxic potency of T-DXd. The FDA granted accelerated approval for T-DXd at a dose of 5.4 mg/kg every 21 days for patients with metastatic HER2-positive breast cancer who have received ≥2 prior anti-HER2 therapies, based on the breakthrough activity observed in the phase II DESTINY-Breast01 trial [39]. A notable advantage of T-DXd over T-DM1 is its ability to induce a stronger bystander effect, whereby the cytotoxic payload diffuses beyond HER2-positive target cells to neighboring antigen-negative tumor cells. This phenomenon is due to the cleavable linker, which allows the free cytotoxic moiety to spread beyond the HER2-expressing tumor cells, potentially improving efficacy in tumors with heterogeneous HER2 expression. While ADCs have revolutionized HER2-targeted therapy in breast cancer, their therapeutic potential extends beyond this tumor. In CRC, HER2 overexpression or amplification occurs in approximately 3-5% of patients, providing a compelling rationale for exploring HER2-targeting ADCs in this setting [9].

The DESTINY-CRC01 trial was the first global, multicenter phase II study to evaluate the feasibility of T-DXd in HER2-expressing mCRC [12, 40]. A total of 78 patients with unresectable, recurrent or metastatic colorectal adenocarcinoma who had received at least two lines of systemic therapy were enrolled to receive T-DXd at a dose of 6.4 mg/kg every three weeks. Patients were stratified into three cohorts based on HER2 expression status: cohort A, HER2 IHC 3+ or IHC 2+/FISH+; cohort B, HER2 IHC 2+/FISH-; and cohort C, HER2 IHC 1+. The trial demonstrated promising efficacy in cohort A, with an ORR of 45.3% and a disease control rate (DCR) of 83.0%. The median duration of response (DoR) was 7.0 months, while median PFS and OS were 6.9 months and 15.5 months, respectively. However, no objective responses were observed in cohorts B and C, with median PFS of 2.1 months and 1.4 months, and median OS of 7.3 months and 7.7 months, respectively. Biomarker analysis further confirmed the positive association between *HER2* amplification and response to T-DXd [41]. In addition, the exploratory analysis suggested that patients with *RAS* mutations (n=6) had numerically lower response rates and survival outcomes than *RAS* wild-type patients (n=46), with an ORR of 33.3% *vs*. 47.8%, median PFS of 4.1 *vs.* 7.6 months, and median OS of 11.6 *vs.* 17.3 months. A similar trend was observed in *PIK3CA* mutant patients (n=6), who had a numerically lower ORR (33.3% *vs.* 47.8%), median PFS (4.1 *vs.* 7.3 months), and median OS (11.6 *vs.* 17.3 months) compared to *PIK3CA* wild-type patients (n=46) [41]. These results highlight the potential impact of *RAS* and *PIK3CA* mutations on treatment response, which warrants further investigation in larger cohorts.

The DESTINY-CRC02 study further confirmed the clinical benefit of T-DXd in patients with HER2-positive mCRC [42]. Notably, this study included 20 patients with *RAS* mutations.

Among *RAS*-mutant patients in the 5.4 mg/kg cohort, 4 achieved a PR, corresponding to an ORR of 28.6%, compared to an ORR of 39.7% in *RAS* wild-type patients (n=68). However, in the 6.4 mg/kg cohort, none of the 6 *RAS*-mutant patients achieved an objective response, whereas *RAS* wild-type patients (n=34) had an ORR of 32.4%. The DESTINY-CRC01/2 trials suggest that HER2-positive, *RAS*-mutant CRC patients may derive clinical benefit from T-DXd treatment, but the small sample size and numerically lower response rates compared to *RAS* wild-type patients highlight the potential limitations of T-DXd monotherapy in this subset.

The combination of ADCs with ICIs represents a hopeful strategy to boost antitumor activity, since the cytotoxicity mediated by ADCs enhances tumor immunogenicity and might overcome the immune resistance. Several clinical trials are exploring this approach. The trials NCT03523572 and NCT03742102 evaluated the anti-tumor activity of T-DXd in combination with either nivolumab or durvalumab in patients with urothelial carcinoma and breast cancer [43-46]. In breast cancer, the combination of T-DXd and nivolumab achieved an ORR of 60.4%, while T-DXd plus durvalumab achieved an ORR of 56.9%. In urothelial cancer, T-DXd plus nivolumab demonstrated an ORR of 36.7%. Notably, clinical benefit was observed in all patients regardless of PD-L1 expression status. Our case of T-DXd plus serplulimab in CRC further supports the potential role of ADC-ICI combinations in overcoming immune resistance. Future studies should focus on identifying biomarkers that predict response, optimizing combination regimens, and evaluating safety profiles.

Beyond T-DXd, several other ADCs have been investigated for efficacy and safety in CRC, though the results have been largely disappointing. Sacituzumab govitecan (SG) is a trophoblast cell surface antigen-2 (Trop-2)-targeting ADC conjugated to SN-38, the active metabolite of irinotecan and a topoisomerase I inhibitor, with a DAR of 7.6. The phase I/II IMMU-132-01 basket trial evaluated SG in 31 CRC patients, reporting a limited ORR of 3.2%, with only 1 patient achieving a PR [47]. The median PFS was 3.9 months, underscoring the limited clinical benefit in this population. Similarly, MRG003, an EGFR-targeting ADC, is conjugated with monomethyl auristatin E (MMAE) via a valine-citrulline linker. The phase Ib NCT04868344 trial evaluated MRG003 in 8 CRC patients, but no objective responses were observed (ORR=0%) [48]. Despite major improvements in ADCs across multiple tumor types, CRC remains a challenging disease with high tumor heterogeneity, unclear resistance mechanisms, and variable antigen expression levels.

HER2 testing in CRC

The DESTINY-CRC01 trial established HER2 overexpression or amplification as a key predictive biomarker for response to T-DXd in CRC. Currently, HER2 positivity in CRC is defined using criteria adapted from breast and gastric cancer, with IHC and FISH serving as the gold standards for assessment. According to the HERACLES study criteria, a CRC tumor is considered HER2-positive if ≥50% of tumor cells exhibit IHC 3+ staining or if IHC 2+ is observed with concurrent FISH positivity (HER2:CEP17 ratio ≥2.0 in ≥50% of tumor cells) [49]. Notably, these criteria are more stringent than those for HER2-positive breast and gastric cancers and are endorsed by both the National Comprehensive Cancer Network (NCCN) and Chinese Society of Clinical Oncology (CSCO) guidelines. The DESTINY-CRC02 trial also used this HER2 definition for patient selection (HER2 IHC 3+ or IHC 2+/FISH+). However, it is important to recognize that the current standard for HER2 interpretation in CRC is based solely on phase II clinical trials, including HERACLES and DESTINY-CRC02. While HER2 positivity is well established in breast and gastric cancer, HER2 testing in CRC is evolving and requires further refinement.

The 2024 NCCN guideline update reflects the increasing significance of NGS in CRC molecular profiling. While previous guidelines stated that HER2 testing was unnecessary in *RAS/RAF*-mutant tumors, the updated recommendations suggest that NGS-based tissue or liquid biopsy testing can detect rare, actionable mutations and fusions, including *HER2* amplification. In this case, NGS identified *HER2* gene amplification, further supporting its role as a complementary diagnostic tool. Compared to IHC/FISH, NGS allows the simultaneous detection of multiple genomic alterations and has

demonstrated high concordance with traditional HER2 testing in mCRC. In gastric cancer, the FDA-approved threshold for *ERBB2* amplification is a tumor diploid copy number (CN) ≥5 [50]. In CRC, tumors with a HER2 copy number variant (CNV) ≥5.0 are definitively classified as HER2-positive, while those with CNV 4.0-4.9 require confirmatory IHC/FISH testing [51]. However, due to the limited number of HER2-positive cases in CRC studies, further research is needed to determine the optimal NGS cutoff for HER2 positivity in CRC.

Liquid biopsy using circulating tumor DNA (ctDNA) represents another promising approach for HER2 testing, particularly when tumor tissue is unavailable or difficult to obtain in advanced-stage patients [52]. The feasibility of ctDNA testing in mCRC has been confirmed. In the BREAKWATER trial, more than two-thirds of CRC patients had complete clearance of *BRAF* V600E mutant DNA from the circulation after treatment with chemotherapy plus cetuximab and encorafenib [53]. This supports that liquid biopsy could serve as a real-time, non-invasive method for monitoring tumor molecular profiles and response to therapy. As testing technologies continue to advance, HER2 detection in CRC is expected to become increasingly diverse, accurate and clinically accessible.

In addition to HER2 overexpression/amplification, HER2 low expression in CRC remains an area of active investigation. NGS can identify HER2-positive cases that may be missed by IHC, particularly in tumors with low HER2 expression. In breast and gastric cancer, T-DXd has demonstrated efficacy in low HER2 tumors, prompting investigation into similar therapeutic potential in CRC [54, 55]. However, the DESTINY-CRC01 trial reported limited response rates to T-DXd in HER2-low CRC patients, highlighting the need for larger prospective studies to establish validated HER2-low diagnostic criteria and explore effective treatment strategies in this population.

Conclusion

Metastatic HER2-positive, RAS-mutant, MSS CRC represents a rare and refractory subset with limited treatment options. Our case describes a durable response with T-DXd plus serplulimab, indicating that ADC-ICI combinations may represent a promising therapeutic strategy in these patients.

While ADCs have demonstrated efficacy in HER2-positive CRC, their benefit in RAS-mutant and MSS tumors remains underexplored. The preclinical evidence suggests that ADCs may modulate the tumor immune microenvironment through immunogenic cell death, macrophage reprogramming, and bystander effects, potentially enhancing the efficacy of ICIs. However, the precise mechanisms underlying ADC-ICI synergy in MSS CRC remain to be elucidated. In addition, HER2 testing methodologies continue to evolve. Although IHC/FISH remains the gold standard, NGS and liquid biopsy are emerging as complementary tools for the detection of HER2 amplification. However, optimal cutoff values for HER2 positivity in CRC remain undefined, and further standardization is needed.

Disclosure of conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

MSS, microsatellite stable; CRC, colorectal cancer; mCRC, metastatic colorectal cancer; T-DXd, trastuzumab deruxtecan; ADC, antibody-drug conjugate; PFS, progression-free survival; PR, partial response; ICI, immune checkpoint inhibitor; mAbs, monoclonal antibodies; TKIs, tyrosine kinase inhibitors; ORR, objective response rate; ECOG, Eastern Cooperative Oncology Group; PS, performance status; DpR, depth of response; AE, adverse event; G-CSF, granulocyte colony-stimulating factor; RTK, receptor tyrosine kinase; CN, copy number; CNV, copy number variant; ctDNA, circulating tumor DNA; TMB, tumor mutational load; EV, Enfortumab Vedotin; DV, Disitamab Vedotin; DAMPs, danger-associated molecular patterns; DCs, dendritic cells; ADCC, antibody-dependent cellular cytotoxicity; FOLFOX, 5-fluorouracil, leucovorin, and oxaliplatin; FOLFIRI, 5-fluorouracil, leucovorin, and irinotecan; XELOX, capecitabine plus oxaliplatin.

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