Heliyon 9 (2023) e22092

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Review article

Immunotherapy combined with local therapy in the late-line treatment of repair-proficient (pMMR)/microsatellite stable (MSS) metastatic colorectal cancer

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ARTICLE INFO

Keywords:
Immunotherapy
Local therapy
Repair-proficient (pMMR)/microsatellite stable (MSS)
Colorectal cancer

ABSTRACT

Colorectal cancer (CRC) is one of the most common malignancies, and at the initial visit, most patients are diagnosed with metastatic CRC (mCRC). However, immunotherapy is only and highly effective in a very small proportion of patients with mCRC having mismatch repair defect (dMMR)/high microsatellite instability, and the majority of the patients with mCRC having mismatch repair proficient (pMMR)/microsatellite stability (MSS) cannot benefit from it. At present, many clinical studies of immunotherapy combined with tyrosine kinase inhibitors (TKIs) are trying to regulate the immune microenvironment of pMMR/MSS mCRC, transforming a "cold tumor" into a "hot tumor," which has not only surprising effects but also certain limitations, i.e., the response could not be specific to metastasis. Therefore, regarding the bottleneck encountered by immunotherapy in patients with patients pMMR/MSS mCRC, this study summarized current research and possible mechanisms of immunotherapy combined with local therapy for metastasis, including radiotherapy, ablation, and transcatheter arterial chemoembolization.

1. Introduction

Colorectal cancer (CRC) is one of the three most common malignancies worldwide [1], and most patients are diagnosed with metastatic CRC (mCRC) at the first visit. The late-line standard treatment for patients with mCRC includes regorafenib [2],fruquintinib [3], and trifluridine-tipiracil (TAS-102) [4]. Although they were beneficial for both progression-free survival (PFS) and overall survival (OS) compared with placebo, efficacies were still limited.

Microsatellite instability (MSI) refers to changes in the microsatellite (MS) sequence length and base composition during DNA

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https://doi.org/10.1016/j.heliyon.2023.e22092
Received 19 October 2023; Received in revised form 30 October 2023; Accepted 3 November 2023
Available online 10 November 2023

replication because of insertion and deletion mutations. This change is commonly due to abnormal DNA mismatch repair function, and MS replication errors, which were not corrected and accumulated [5]. MSI can be divided into three categories according to their degree, namely, microsatellite stability (MSS), microsatellite instability-Low (MSI-L), and microsatellite instability-High (MSI-H). The detection methods of MSI include immunohistochemical analysis of MMR protein expression, multiplex polymerase chain reaction capillary electrophoresis, and next-generation sequencing.

Recently, immunotherapy has been in full swing. Pembrolizumab and nivolumab ± ipilimumab have been approved for patients with mismatch repair defect (dMMR)/MSI-H mCRC. However, approximately 5 % of mCRC cases are dMMR/MSI-H [6], and the remaining 95 % are mismatch repair proficient (pMMR)/MSS. The KEYNOTE-016 phase II clinical trial [7] showed that the objective response rates (ORR) of patients with dMMR/MSI-H mCRC and those with pMMR/MSS mCRC were 40 % and 0 %, respectively. How to break through the resistance of immunotherapy in patients with pMMR/MSS mCRC has become a research hotspot.

In 2019, the REGONIVO(Japan) [8] study opened a new era in the immunotherapy of patients with pMMR/MSS mCRC. Subsequent studies of fruquintinib combined with sintilimab [9], LEAP-005 study [10], and CAMILLA study [11] all suggested the great potential of tyrosine kinase inhibitors (TKIs) combined with immunotherapy in patients with pMMR/MSS mCRC. However, the REGONIVO study (North America) [12], REGONIVO Plus study [13], REGOTORI study [14], and Regomune study [15] could not reproduce the satisfying ORR of REGONIVO (Japan), and the efficacy was uneven. The ORR of these studies ranged from 7 % to 27 %, the disease control rate (DCR) was 39%–80 %, and the median OS (mOS) was 7.5–15.5 months. Subgroup analyses of the REGONIVO (North America) study and REGONIVO Plus study further showed a near absence of remission in patients with pMMR/MSS mCRC having liver metastases, who did not respond to immunotherapy. Thus, for patients with pMMR/MSS mCRC having local metastasis, particularly liver metastasis, combined local therapy based on immunotherapy appears to be a research direction worth exploring.

Many recent studies on immunotherapy combined with local therapy in hepatocellular carcinoma have achieved good results, such as the Cal Era study [16] with an ORR of 80 % and the NASIR-HCC study with an ORR of 41.5 % [17]. However, it has not been well explored in patients with pMMR/MSS mCRC exhibiting local metastasis.

In this study, we present an overview of the progress in immunotherapy combined with local therapy, including radiotherapy, radiofrequency ablation, and transcatheter arterial chemoembolization (TACE), for patients with pMMR/MSS mCRC (Table 1, Fig. 1), and discuss prognostic biomarkers.

2. Research progress on immunotherapy combined with radiotherapy

2.1. Antitumor mechanism of immunotherapy combined with radiotherapy

The radiotherapy-induced abscopal effect suggests that radiotherapy is closely related to the immune microenvironment and the patient's immune status [18]. Typically, cancer cell antigens are released following radiotherapy and recognized by dendritic cells (DC), which cross these antigens to T cells following activation, triggering T-cell activation, and circulating T cells infiltrate the tumor and bind to cancer cell antigens, triggering immune-mediated cell death. Therefore, the synergistic mechanism of radiotherapy combined with immunotherapy can be classified into three points, as shown below:

1. Radiotherapy enhances tumor-associated antigen release and presentation: Major histocompatibility complex (MHC)-1 is a key molecule for CD8+T cells that recognize antigens. Although its expression is significantly inhibited in tumors [19], radiotherapy can promote its expression [20]. In addition, local high-dose radiotherapy can enhance antigen presentation by DCs [21].
2. Radiotherapy triggers an immune response: The killing of tumor cells by radiotherapy leads to the formation of an "in situ vaccine", which activates the original immune response of the body [22]. DCs can present necrotic cell products such as DNA fragments to $CD8^+$ cells as antigenic substances, and cell necrosis and DNA damage induced by radiotherapy can enhance antitumor immune effects [23].
3. Radiotherapy modulates the tumor microenvironment: Radiation can regulate and reprogram the tumor microenvironment from an immunosuppressive phenotype to an immunostimulatory phenotype. Radiation can facilitate the infiltration of CD8+T cells, and low-dose radiation can promote the normalization of tumor vasculature and polarization of M2 macrophages to M1 iNOS+. iNOS + macrophages induce the expression of Th1 chemokines, which recruit $CD8^+$ and CD4+T cells into the tumor and promote the T cell-mediated antitumor effect [24]. Programmed cell death protein 1 (PD-1) combined with programmed death-ligand 1 (PD-L1) can transmit inhibitory signals and reduce the proliferation of CD8+T cells in the lymph nodes. A study reported that the expression of PD-L1 on the surface of tumor cells will increase significantly following radiotherapy [25]. Another study proved that following radiotherapy, the tumor releases fragments of genetic materials, such as double-stranded DNA (dsDNA), to trigger the cGAS–STING pathway and upregulate the expression of PD-L1 in the tumor cells, inducing tumor immune escape [26]. In pre-clinical CRC models, radiotherapy enhances single-agent immune checkpoint blockade [27], turning immunotherapy-resistant tumors into immunologically responsive ones [28].

A recent study showed that liver radiotherapy can eliminate immunosuppressed macrophages, improve the survival of hepatic T cells, and reduce the "siphoning effect" of hepatic T cells. Another study found that liver metastases may impair the efficacy of systemic immunotherapy [29]. In some cases, radiotherapy also enhances the functions of immunosuppressive factors. A study reported that radiotherapy can promote a significant increase in the number of regulatory T cells (Tregs) [30].

Table 1
Results of clinical trials of immunotherapy combined with local therapy in the late-line treatment of pMMR/MSS mCRC.

Study name	Fruquintinib + Tislelizumab + SBRT (FRIUT)	Fruquintinib + Sintilimab + RT	PD-1 inhibitor + RT + GM-CSF	Ipilimumab + nivolumab + RT (C2D1)	Ipilimumab + nivolumab + RT (C1D1)	durvalumab + tremelimumab + RT	durvalumab + tremelimumab + RFA/SBRT
Publish time	2023ASCO-GI	2023AACR	2022ASCO	2022.5.18	2023ASCO	2021.1.27	2023 ASCO-GI
MSI status	MSS	MSS	MSS	MSS	MSS	MSS	MSS
Line of therapy	≥Third-line	≥Third-line	/	≥Third-line	≥Third-line	≥Third-line	≥Third-line
PS status	/	/	/	0(65 %)/1(35 %)	0/1	0(25 %)/1(75 %)	/
Regimen	Fruquinitib (5 mg, QD, PO, d1-14); Tislelizumab (200 mg, d1, I.V.); SBRT (8–10Gy × 5F, QOD)	Fruquintinib (QD, PO, d1-14); anti-PD-1 (200 mg, d1, I.V.); Patients with isolated or localized metastasis will receive RT	RT (5 or 8Gy × 2-3f) for one metastatic lesion, PD-1 inhibitor dosing within 1 week RT, GM-CSF 200 μg subcutaneous injection QD for 14 days(d1-14), or GM-CSF 200 μg (d1-7), and then followed by IL-2200 million IU SC QD for 7 days (d8-14)	Ipilimumab (1 mg/kg every 6 weeks for the first 4 cycles); nivolumab (240 mg every 2 weeks on a 6-week cycle); RT with 24 Gy/3 fractions to one site starting on C2D1	Ipilimumab (1 mg/kg every 6 weeks for the first 4 cycles); nivolumab (240 mg every 2 weeks on a 6-week cycle); RT with 24 Gy/3 fractions to one site starting on C1D1	durvalumab 1500 mg plus tremelimumab 75 mg every 4 weeks plus RT	tremelimumab 75 mg and durvalumab 1500 mg for 4 cycles followed by durvalumab 1500 mg every 4 weeks. During cycle 1, RFA and SBRT were performed concurrently.
Enrolled number	23	25	9	27	30	24	21
ORR (%)	26 %	28.0 %	22.2 %	15 %	13 %	8.3 %	/
DCR (%)	83 %	80.0 %	66.7 %	37 %	33 %	/	/
mPFS	5.1 m	6.05 m	5.6 m	2.5 m	2.4 m	1.8 m	/
mOS	Not reach	/	/	10.9 m	10.6 m	11.4 m	2.2 m
Adverse Event	grade≥3 (17 %)	/	grade≥3 (11.1 %)	Grade ≥3(70 %)	Grade ≥3(53 %)	Grade ≥3(25 %)	Grade ≥3(30.8 %/50 %)

*Radiation Therapy (RT); Stereotactic Body Radiation Therapy (SBRT); Radiofrequency Ablation (RFA).

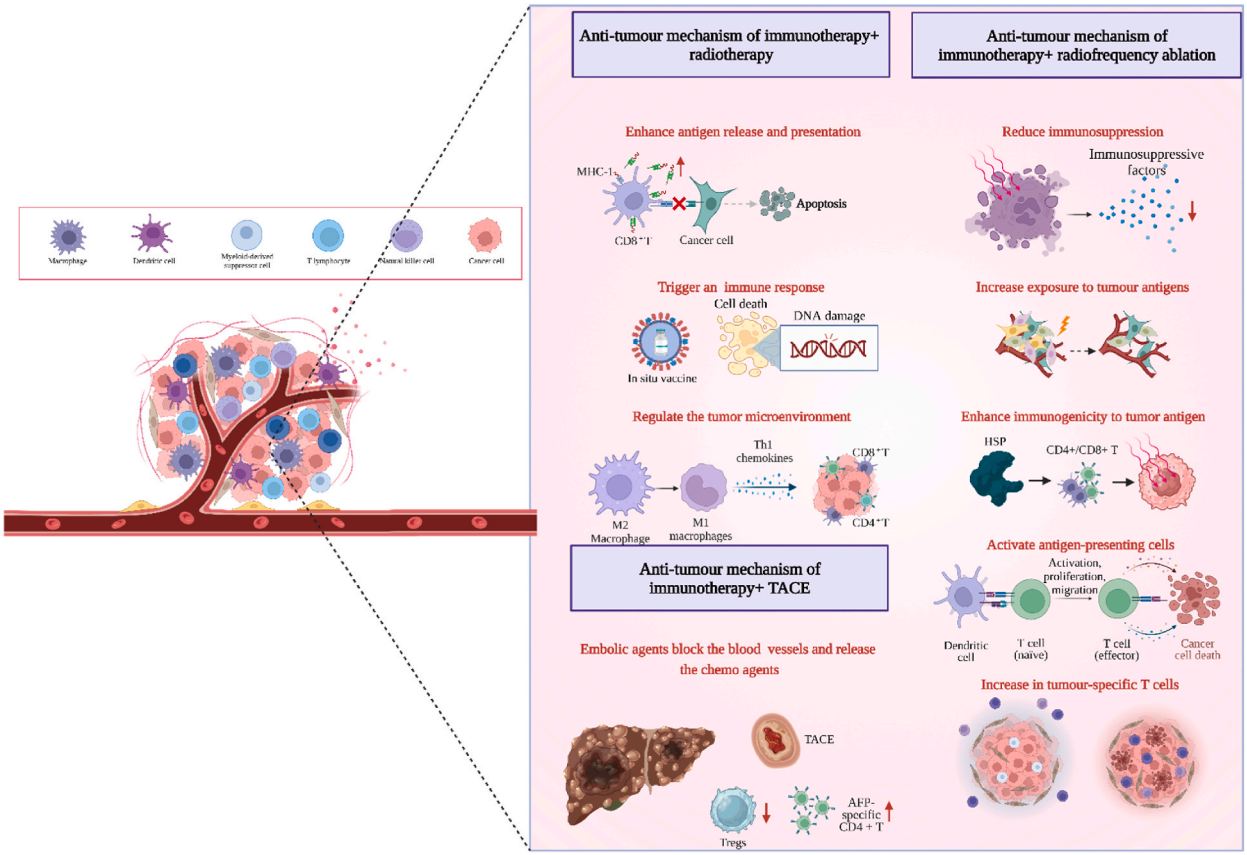

Fig. 1. The mechanism of immunotherapy combined with local therapy.

2.2. Clinical progress in immunotherapy combined with radiotherapy

A prospective cohort study of fruquintinib combined with sintilimab in the treatment of patients with pMMR/MSS mCRC [31] was presented at the 2023 American Association for Cancer Research (AACR) meeting. A total of 55 patients were recruited, of which 25 and 30 had and had not received radiotherapy, respectively. The ORR, DCR, and median PFS (mPFS) of the whole population were 16.4 %, 56.3 %, and 3.58 months, respectively. However, the results of the groups with and without radiotherapy were significantly different, with ORR of 28.0 % and 6.7 % (OR = 7.344, P = 0.039, with radiotherapy vs. without radiotherapy, the same below), DCR of 80.0 % and 36.7 % (OR = 7.991, P = 0.010), and mPFS of 6.05 and 2.60 months (HR = 0.286, P < 0.001), respectively. As regards adverse events, 49.1 % of the patients experienced grade 1 or 2 adverse events, and 12.7 % experienced grade 3 adverse events. The most common adverse events were hepatotoxicity, hypertension, and hand–foot syndrome. The multivariate Cox regression analysis identified radiotherapy as an independent factor that affects PFS. Patients with radiotherapy-exposed pMMR/MSS mCRC benefit more from the combination of fruquintinib and sintilimab than those without radiotherapy.

Coincidentally, a real-world study [32] published in the 2023 American Society of Clinical Oncology (ASCO) meeting analyzed the effects of anti-angiogenic agents and immunotherapy on pMMR/MSS mCRC to investigate whether radiotherapy can further improve the prognosis. A total of 89 patients were included, of which 43 (48.3 %) had received and 46 (51.7 %) had not radiotherapy. The ORRs of the radiotherapy + anti-angiogenic drug + immunotherapy group and the anti-angiogenic drug + immunotherapy group were 18.6 % (8/43) and 10.9 % (5/46), and the DCRs were 83.7 % (36/43) and 67.4 % (31/46), respectively (P > 0.05). The mPFS of the radiotherapy + anti-angiogenic drug + immunotherapy group was slightly higher than those of the anti-angiogenic drug + immunotherapy group (5.0 vs. 4.3 months, p = 0.065). No treatment-related deaths were reported, and manageable radiation pneumonia developed in one patient in the combined radiotherapy group. This suggested that the addition of radiotherapy can significantly improve the survival benefit of antiangiogenic drugs combined with immunotherapy in patients with pMMR/MSS mCRC.

Meanwhile, Theodore S. Hong, a professor at Massachusetts General Hospital (MGH), found that patients with pMMR/MSS mCRC treated with radiotherapy combined with ipilimumab and nivolumab [33] had a significantly improved prognosis. A total of 40 patients with mCRC were enrolled (1 patient with CRC had disease control status before enrollment, and the other patients had progressive disease [PD]), with DCR of 25 % (10/40; 95 % CI 13–41 %), ORR of 10 % (4/40; 95 % CI 3%–24 %), mPFS of 2.4 months (95 % CI 1.8–2.5), and mOS of 7.1 months (95 % CI 4.3–10.9). The 27 patients who received radiotherapy had DCR of 37 % (10/27; 95 % CI 19–58 %), ORR of 15 % (4/27; 95 % CI 4–34 %), mPFS of 2.5 months (95 % CI 2.3–2.8), and mOS of 10.9 months (95 % CI 6.7–15.0).

Notably, a significant early exit (progression, toxicity, and decline in performance status) was noted in this trial. The radiotherapy in the above study was initiated at C2D1, and one-third of the patients did receive radiotherapy. To confirm this concern and address dropout before radiotherapy, a phase II study of nivolumab and ipilimumab with radiotherapy moved to C1D1 was conducted. Poster 3584(NCT04361162) in the 2023 ASCO meeting [34] showed the corresponding preliminary results, and a total of 30 patients with pMMR/MSS mCRC received nivolumab and ipilimumab combined radiotherapy (with 24 Gy/3 fractions to one site starting on C1D1). This trial reported an ORR of 13 % (4/30; 95 % CI 4%–31 %), DCR of 33 % (10/30; 95 % CI 17%–53 %), mPFS of 2.4 months (95 % CI 1.8–2.9), and mOS of 10.6 months (95 % CI 6.8–17.8). Moreover, 16 patients had grade $\geq$3 treatment-related serious adverse events, including lymphocytopenia (n = 3, grade 4), anemia, and diarrhea. The application of radiotherapy starting from C1D1 showed good results in patients with immune-resistant pMMR/MSS mCRC. Research on optimal radiotherapy strategies such as radiotherapy segmentation (large or conventional), dose (high or low), radiotherapy target selection (load and organ), and sequential sequence (induction or synchronization) is ongoing.

A pooled analysis of data from two phase II clinical studies [35] revealed certain efficacy of PD-1 inhibitors combined with radiotherapy and granulocyte–macrophage colony-stimulating factor with or without interleukin-2 in patients with pMMR/MSS mCRC. A total of 9 patients were enrolled. All patients completed at least two cycles of treatment and one assessment, and they had a median follow-up of 7.6 months (95 % CI 3.8–11.4), ORR of 22.2 %, DCR of 66.7 %, and mPFS of 5.6 months (95 % CI 1.5–9.7). Treatment-related adverse events occurred in 7 of 9 (77.8 %) patients. One patient (11.1 %) had grade 3 adverse events with renal insufficiency.

The FRUIT study published in the 2023 ASCO-GI meeting [36] enrolled 25 patients with pMMR/MSS mCRC who received fruquintinib (5 mg, qd, po, days 1–14, 21 days for a cycle) combined with tislelizumab (200 mg, IV, d1, 21 days for a cycle) and stereotactic body radiotherapy (SBRT; 8–10 Gy $\times$ 5F, qod, in 10 days). Moreover, 52 % of the enrolled patients had one metastatic site, and the rest had two or more metastases. With 7.8 months (95 % CI 4.31–11.29) of median follow-up, the mPFS reached 5.1 months (95 % CI 0.77–9.63), OS was not reached, and mOS was expected to have been $>$1 year. In addition, the ORR was 26 %, and the DCR was 83 %. Regarding safety, most adverse reactions were grades 1–2, and the incidence of grade 3–4 adverse reactions was only 17 %. The PFS and OS of this study represent an excellent result in the late-line treatment of mCRC, and the sample size must be further expanded to confirm the effectiveness of this regimen because of the limitations of a single-center phase II study.

The aforementioned studies showed that immunotherapy combined with local radiotherapy can have improved efficacy on pMMR/MSS mCRC, and on this basis, the combination of anti-angiogenesis drugs may reach a better prognosis.

Other studies that show different results. A study analyzed 19 patients who had pMMR/MSS mCRC and liver metastases and treated with a priming dose of s.c vidutolimod and three intratumoral injections of vidutolimod and radiosurgery, combined with nivolumab and ipilimumab. None of the patients responded, except for one patient, and this was attributed to the high tumor mutation burden (TMB) [37]. A single-center phase II study [38] involving 24 patients who had pMMR/MSS mCRC and treated with radiotherapy combined with durvalumab and tremelimumab reported an ORR of 8.3 %, mPFS of 1.8 months, and mOS of 11.4 months. The combination regimen evaluated in this study did not meet the prespecified primary endpoint. A phase I clinical study [39] enrolled nine patients with pMMR/MSS mCRC and liver metastases who received Y90 radioembolization to the liver, followed by the administration of durvalumab and tremelimumab. All patients were evaluated as having PD within or after two cycles, the study was discontinued early, and associated flow cytometry results did not support the role of Y90 radioembolization in transforming “cold” tumors into “hot” ones. In a study of patients with mCRC treated with a PD-1 inhibitor (AMP-224) in combination with low-dose cyclophosphamide and SBRT [40], 5/15 patients with liver metastases were separated from the experimental group because of accelerated disease deterioration. Another study found that radiotherapy can cause immune escape while enhancing the effect of immunotherapy.

As neoadjuvant therapy is one of the main application areas of immunotherapy combined with radiotherapy, some advances are notable, which will be briefly mentioned here. Many studies have adopted immunotherapy combined with concurrent chemoradiotherapy (CCRT) and achieved good initial efficacy. In the VOLTAGE study [41], 39 patients with pMMR/MSS and locally advanced rectal cancer (LARC) were enrolled sequentially and received nivolumab following standard CCRT. Of these patients, 11 (29.7 %) achieved pathologic complete response (pCR). Subsequent molecular marker analysis showed the ratio of $CD8^+$ lymphocytes to CD45RA-FoxP3 + effector Tregs in tumor-infiltrating lymphocytes (CD8/eTreg$\geq$2) and PD-L1 expression $>$1 % in six patients, of which five achieved pCR and one achieved clinical complete remission without surgery. The ANAVA study [42] enrolled 101 patients with LARC sequentially receiving avelumab following standard CCRT. Only one patient refused surgery, and the pCR rate of the 100 patients who underwent surgery was 23 %, among which 39 of the 40 patients with microsatellite status information were MSS. The above two studies reported slightly higher historical pCR rates of neoadjuvant chemoradiotherapy (15%–18 %). Moreover, a phase II single-arm study used short-course radiotherapy followed by XELOX combined with camrelizumab for two cycles of neoadjuvant therapy. The pCR rate of 26 patients with MSS reached 46.2 %, which was also higher than previously reported.

However, the NRG-GI002 study [43] used the total neoadjuvant therapy model. The experimental group received capecitabine + pembrolizumab + radiotherapy, whereas the control group received capecitabine + radiotherapy. Consequently, the pCR rates of the control and experimental groups were 29.4 % and 31.9 %, respectively, with no statistical significance (P = 0.75). In the latest report in ASCO-GI 2023, the 3-year OS rate of the experimental group was higher than that of the control group.

In summary, exploring promising biomarkers associated with radiotherapy combined with immunotherapy is crucial.

3. Research progress on immunotherapy combined with radiofrequency ablation

3.1. Antitumor mechanism of immunotherapy combined with radiofrequency ablation

Radiofrequency ablation involves a current loop formed by an electrode needle inserted into the tumor tissue and an electrode plate attached to the patient's body. Once the generator is turned on, a high-frequency electricity is fired into the target tissue. It cannot only cause local coagulation necrosis of tumors but also produce numerous tumor fragments, which can induce an antitumor immune response [44].

Its synergistic mechanism can be classified into the following five points:

1. Reduce immunosuppression: Radiofrequency ablation can lead to tumor degeneration and necrosis and reduce tumor load and release of immunosuppressive factors, decreasing the immunosuppressive effect on the host [45].
2. Increase exposure to tumor antigen: Radiofrequency ablation can destroy tumor cells and expose numerous tumor antigens, enhancing the antigenicity of the tumor and playing a role similar to "tumor vaccines" [46].
3. Enhance immunogenicity to tumor antigens: Heat-shock proteins (HSPs), a class of peptide-binding proteins, can be used as carriers or peptide companions to combine with tumor antigens to form HSP complexes, present tumor antigens through MHC-I, activate CD4+/CD8+ T cells, and induce specific cellular immunity against specific tumor cells [47]. In tumor-bearing animals, radiofrequency ablation resulted in coagulation necrosis in the central ablation area, and the expression of HSP70 increased significantly in the surrounding nonfatal lesions. In patients with hepatocellular carcinoma, the expression of HSP70 after radiofrequency ablation is eight times higher than that before surgery. High temperatures can increase the expression levels of HSP70 and HSP90 in hepatocellular carcinoma cells and enhance the immune response to cancer cells [48].
4. Activate antigen-presenting cells: DCs are the most potent antigen-presenting cells in the body. Mature DCs can activate initial and resting T lymphocytes by presenting antigens and providing costimulatory signals, initiating antigen-specific T-cell killing of tumors. In tumors with few and nonfunctional DCs, tumor antigens cannot be effectively presented, and the local tumor-specific cytotoxic T-lymphocyte response cannot be effectively induced [49]. Radiofrequency ablation can activate DCs, enhancing the antitumor effect of specific T cells [50].
5. Increase the number of tumor-specific T cells: CD8+T and natural killer (NK), the main effector cells of tumor immunity, can directly kill tumor cells. Following tumor ablation, tumor-specific CD8+T and NK cells can accumulate in the necrotic lesion boundary tissue, which can further enhance the specific antitumor effect [45].

Immunotherapy enhances the antitumor immune response by reversing the depletion of T-cell function and restoring immune recognition and immune attack. Therefore, radiofrequency ablation combined with immunotherapy exerts a significantly enhanced antitumor effect, and the combined treatment will play a $1 + 1 > 2$ therapeutic effects.

3.2. Clinical progress in immunotherapy combined with radiofrequency ablation

Shi et al. [51] subjected mouse models to a combination of radiofrequency ablation and PD-1 monoclonal antibody to treat CRC, which showed significantly increased expression of tumor antigens, enhanced killing function of T cells, and significantly increased proportion of effector T cells in the distal tumor, achieving a good antitumor effect.

A nonrandomized phase II clinical study [52] enrolled 26 patients with pMMR/MSS mCRC who were given pembrolizumab in combination with palliative radiotherapy (cohort 1) or radiofrequency ablation (cohort 2), the intermediate ORR in cohort 1 was 9 % (1/11), and no response was observed in cohort 2. Moreover, 73 % of the patients experienced a drug-related adverse event; however, all were grade 1 or 2. A phase II clinical study (Poster 141 of ASCO-GI 2023) [53] analyzed 23 patients with pMMR/MSS mCRC who were treated with durvalumab and tremelimumab combined with radiofrequency ablation or SBRT. With a median follow-up of 11 months, the mPFS was 2.2 months, 55 % of the patients died during follow-up, and the trial was discontinued early. Of the 13 patients who received radiofrequency ablation, 30.8 % had grade 3 toxicities.

A retrospective study [54] did not find an objective response with the combination of regorafenib plus anti-PD-1 antibody, suggesting its little clinical activity in unselected Chinese patients with pMMR/MSS mCRC. However, one of the patients who received radiofrequency ablation to treat liver and abdominal wall metastases before combination therapy achieved a PFS of 9.2 months with stable disease (SD). Thus, for patients with pMMR/MSS mCRC and liver metastases, the application of radiofrequency ablation based on TKIs and immunotherapy appears to be a more effective treatment approach.

Although the results of clinical studies appear to indicate the unsatisfying effect of immunotherapy combined with radiotherapy ablation, whether continued combination with TKIs can further improve survival remains to be further explored.

4. Research progress on immunotherapy combined with TACE

4.1. Antitumor mechanism of immunotherapy combined with TACE

TACE exerts antitumor effects mainly by selectively embolizing tumor arteries and increasing the concentration of local antitumor drugs in the lesion to maximize the occurrence of ischemic necrosis. According to embolic agents, TACE is divided into conventional TACE (cTACE) and drug-eluting bead-TACE (DEB-TACE). cTACE involves embolizing tumor arteries with iodized oil drugs and

granular embolizers, whereas DEB-TACE requires embolizing tumor arteries with drug-eluting microspheres preloaded with chemotherapy drugs.

Similar to radiofrequency ablation, TACE-induced death of necrotic cells can also trigger a systemic immune response. Studies have suggested that TACE can cause a reduction of Tregs in liver cancer [55] and an increase in AFP-specific CD4 + T cells [56], which can promote immune response and inflammatory response in the microenvironment. Moreover, the systemic immune response can be activated by changing the phenotype of peripheral immune cells [57]. Liver tumor cells are more sensitive to radiation. Under radiation, in addition to causing changes in the tumor microenvironment, tumor cells will undergo apoptosis and develop immunogenicity, further promoting the killing effect of immune cells on tumor tissues [58,59].

4.2. Clinical progress in immunotherapy combined with TACE

Although TACE combined with immunotherapy and TKIs has led to good results in advanced liver cancer [60], similar studies in patients with mCRC and liver metastases are scarce.

At present, some studies have reported results of TACE combined with TKIs. A single-center observational study [61] evaluated the efficacy and safety of regorafenib combined with DEB-TACE in patients with CRC and liver metastases (MSI status not defined). The regorafenib group included 42 patients, whereas the regorafenib + DEB-TACE group included 34 patients. The PFS (median: 7.6 versus 4.1 months, $P < 0.001$), OS (median: 15.7 versus 9.2 months, $P < 0.001$), ORR (35.3 % versus 7.1 %, $P = 0.002$), and DCR (76.5 % versus 47.6 %, $P = 0.011$) were all better in the regorafenib + DEB-TACE group than in the regorafenib group. Regorafenib combined with DEB-TACE could improve the response rate and prolong the survival of patients with CRC and liver metastasis. In the 2021 ASCO meeting, a study reported the use of hepatic arterial infusion chemotherapy (HAIC) combined with regorafenib in treating patients with mCRC and liver metastasis (MSI status not defined) [62], with an OS of 22.2 months and an ORR of 51.3 %, suggesting that this regimen is effective in the treatment of liver metastasis in mCRC.

A single-center retrospective study presented in the 2023 ASCO meeting evaluated the efficacy of DEB-TACE in combination with HAIC for unresectable CRC with liver metastases. For patients who were refractory to second-line and above systemic therapy, the hepatic PFS and PFS were 6.2 (95 % CI 4.899–7.501) and 5.2 (95 % CI 3.682–6.718) months, respectively, and OS was not yet

Table 2
Ongoing clinical trials of immunotherapy combined with local therapy in the late-line treatment of pMMR/MSS mCRC registered on clinicaltrials.gov.

Study name/ ID	Intervention	Target	CRC study population	Phase	Status
NCT04535024	SABR + Sintilimab	PD-1; Ablation	MSS Oligometastatic CRC	II	Recruiting
NCT04030260	Regorafenib + PD-1 antibody + Radiotherapy ± SABT	PD-1; VEGFR; Radiotherapy	MSS Metastatic CRC	II	Recruiting
NCT04575922	ipilimumab + nivolumab, +Radiation therapy	PD-1; CTLA-4; Radiotherapy	Metastatic MSS CRC	II	Active, not recruiting
NCT03104439	Nivolumab + Ipilimumab + Radiation Therapy	PD-1; CTLA-4; Radiotherapy	MSS and MSI High CRC	II	Recruiting
NCT05292417	Sintilimab + GM-CSF + Fruquintinib + Radiotherapy	PD-1; VEGFR; Radiotherapy	MSS Metastatic CRC	II	Recruiting
NCT05160727	Tislelizumab + Irinotecan + Radiotherapy	PD-1; Radiotherapy; Chemotherapy	MSS inoperable recurrent and metastatic CRC	II	Recruiting
NCT04659382	SIRT + Xelox + Bevacizumab + Atezolizumab	PD-1; VEGFR; Radiotherapy; Chemotherapy;	MSS mCRC with predominantly non-operable liver metastases	II	Recruiting
NCT04108481	Yttrium-90 radioembolization (Y90-RE) + durvalumab	PD-1; Radiotherapy;	liver-predominant, MSS Metastatic CRC	I/II	Suspended (Working on revisions)
NCT03802747	Durvalumab/Durvalumab and Tremelimumab + SIRT/SBRT	PD-1; CTLA-4; Radiotherapy	liver metastases for patients with MSS CRC	I	Withdrawn (PI left the institution)
NCT05438108	SBRT + CapeOX + Bevacizumab + Sintilimab	PD-1; VEGFR; Radiotherapy; Chemotherapy;	MSS Metastatic CRC	II	Recruiting
NCT02437071	Pembrolizumab + Radiotherapy/Ablation	PD-1; Radiotherapy; Ablation	Metastatic CRC	II	Active, not recruiting
NCT04924179	Fruquintinib + Tislelizumab + SBRT	PD-1; VEGFR; Radiotherapy;	Metastatic CRC	II	Active, not recruiting
NCT05635149	Fruquintinib + PD-1 antibody + CRT/SBRT	PD-1; VEGFR; Radiotherapy;	MSS Metastatic CRC	Observational Cohort Study	Recruiting
NCT02888743	Durvalumab + Tremelimumab + High or low dose RT	PD-1; CTLA-4; Radiotherapy	MSS Metastatic CRC	II	Active, not recruiting
NCT05894837	Serplulimab + Regorafenib + Hepatic Artery Bicarbonate Infusion	PD-1; Chemotherapy; Hepatic Artery Bicarbonate Infusion	Metastatic CRC liver Metastases	II	Active, not recruiting

*Radiation Therapy (RT); Stereotactic Body Radiation Therapy (SBRT); Selective Internal Radiation Therapy (SIRT); Stereotactic Ablative Radiotherapy (SABR); Conventional radiotherapy (CRT).

achieved. This suggests that DEB-TACE and HAIC are promising local treatments that may improve prognosis.

More studies are expected to explore the combination of immunotherapy with TACE/HAIC in patients with pMMR/MSS mCRC and liver metastasis in the future.

5. Exploration of potential biomarkers

5.1. Gut microbiota

Studies have shown characteristic changes in the gut microbiota of patients with CRC and an increase in the abundance of bacterial genera such as Fusobacterium nucleatum and porphyromonas in CRC. The pathogenic mechanism of gut microbiota may be related to the regulation of the immune microenvironment by certain signaling pathways [63]. The gut microbiota is also expected to be a biomarker of prognostic prediction, and a study reported the prognostic value of gut microbiota in the treatment of CRC [64]. In the prospective cohort study of fruquintinib combined with sintilimab for pMMR/MSS CRC presented in the 2023 AACR meeting [31], researchers collected pretreatment stool samples from 20 patients for 16S rRNA sequencing to investigate the relationship between gut microbiota characteristics and patient response. Among the 20 patients who underwent microbiota sequencing, the mPFS in the group with radiotherapy (n = 8) was significantly different from those in the group without radiotherapy (n = 12) (6.1 vs. 3.1 months, P = 0.002). At the genus level, bifidobacterium and lactobacillus were significantly enriched in the radiotherapy group, and the receiver operating characteristic curves showed that co-enrichment of bifidobacterium and lactobacillus predicted greater DCR (AUC = 0.909), enhancing their potential as response biomarkers. A strong correlation was found between high lactobacillus abundance and prolonged PFS (P = 0.043).

Bifidobacterium and lactobacillus may be potential predictive markers for the therapeutic efficacy of pMMR/MSS CRC treated with immunotherapy combined with radiotherapy and TKIs, and the exact conclusion must be confirmed by further studies.

5.2. TMB

Previous studies have shown that patients with pMMR/MSS CRC having plasma TMB of $\geq$28 mut/Mb have greater OS benefit [65]. After finding that the prognosis of patients with pMMR/MSS mCRC treated with radiotherapy combined with ipilimumab and nivolumab was significantly improved, Professor Theodore S. Hong comprehensively examined whether the prognosis of the two groups correlated with the TMB before and after treatment. TMB was detected by whole exome sequencing (WES) in tumor biopsies before treatment, immediately before radiotherapy, and after radiotherapy completion. Surprisingly, the sequencing results revealed that the TMB before, during, and after treatment were all very low (<10 mut/Mb) and did not change significantly [33]. Whether TMB can be also applied to determine potential patients who may benefit from immunotherapy combined with radiotherapy and TKIs needs further study.

5.3. DNA damage and repair pathway genes

Professor Theodore S. Hong further analyzed nonsynonymous gene mutations [33] and identified expected genes that frequently mutate including *KRAS*, *TP53*, and *APC* in CRC. In addition, significant mutations were found in DNA damage and repair pathway genes with shared frequent mutations in DDX11(4/13) and FANCD2(2/13). No specific DNA damage and repair pathway gene mutation was particularly enriched in patients with response or disease stability. Notably, patient 4 (PR) had six mutations in DNA damage and repair genes, which suggests their potential importance in predicting response; however, this trial had no sufficient samples to make any clear conclusions.

5.4. NK cells and HERV-K

To further identify the mechanisms underlying the heterogeneity of tumor prognosis, Prof. Theodore S. Hong also conducted single-cell RNA sequencing [33]and found that the expression of genes involved in the epithelial–mesenchymal transition (EMT) was significantly higher in patients with SD/partial response (PR)/complete response (CR) (n = 5) than in patients with PD (n = 7). Previous studies have shown that the number of NK cells [66] and expression of HERV_RNA [67] correlated with the effect of immunotherapy. To confirm this, transcriptome and immune cell infiltration were analyzed, and the results showed a high number of NK cells in the tumor tissues of patients with SD/PR/CR, who had significantly higher RNA repeats of AluYb9, HERVK, HERV_LTRa, LTR35, and MER34C2 than those with PD. However, according to existing research data, among these repeats, only the HERVK repeat has protein-coding capacity. Therefore, NK cells and HERV-K in tumors may serve as potential predictive biomarkers for immunotherapy, and their functional relationship between innate immune sensing pathways and related immune cell responses is worthy of deeper investigation.

6. Perspectives

First, global experts are trying to transform cold tumors into hot tumors by adding immunotherapy to chemotherapy plus target therapy. Unfortunately, none of these studies have yielded satisfying results so far. We have summarized some breakthroughs in immunotherapy combined with local therapy; however, whether it can achieve positive results and prolong patient survival must be

considered with caution.

Second, many local therapy approaches are available, and which one is the most suitable remains unexplored. Different sites of metastases respond differently to the same combined treatment. At present, most clinical studies do not disclose specific stratified data; thus, a long time is needed to achieve better precision. The exploration of biomarkers is crucial for precision therapy; however, only a few clinical studies of immunotherapy combined with local therapy have explored biomarkers. In the future, more studies are expected to perform further exploration from phase II clinical trials.

Third, in addition to the late-line treatment, whether such kind of combined treatment could be a breakthrough in first-line therapy, perioperative, and conversion therapy is also worth studying.

7. Conclusions

Some small-sample studies of immunotherapy combined with local therapy as the late-line treatment are ongoing (Table 2), in which combined radiotherapy appears to be a direction worth exploring. These clinical trials could answer vital questions and further assist clinical work.

Immunotherapy is expected to play a role in the treatment of pMMR/MSS mCRC in the future. In addition to considering the basic disease molecular types, the benefiting group can be also further identified by mining biomarkers. Therefore, real-world studies and clinical trials with larger samples are necessary.

Funding

This work was supported by the Fundamental Research Funds for the Central Universities), China (226-2023-00088) and the Provincial Key R&D Program of Zhejiang Province), China (2021C03125).

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Yuwei Ding: Writing – original draft, Conceptualization. **Shanshan Weng:** Writing – original draft, Conceptualization. **Ning Zhu:** Data curation. **Mi Mi:** Data curation. **Ziheng Xu:** Data curation. **Liping Zhong:** Data curation. **Ying Yuan:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Thanks to Hangzhou Qianjiang special expert, for the help and support.

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