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# Locoregional therapies combined with immune checkpoint inhibitors for liver metastases

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**Abstract**
Immune checkpoint inhibitors (ICIs) have achieved remarkable success in clinical research and practice. Notably, liver metastasis is not sensitive to ICIs. Liver locoregional therapies can cause irreversible damage to tumor cells and release tumor antigens, thereby providing a rationale for immunotherapy treatments in liver metastasis. The combination therapy of ICIs with locoregional therapies is a promising option for patients with liver metastasis. Preclinical studies have demonstrated that combining ICIs with locoregional therapies produces a significantly synergistic anti-tumor effect. However, the current evidence for the efficacy of ICIs combined with locoregional therapies remains insufficient. Therefore, we review the literature on the mechanisms of locoregional therapies in treating liver metastasis and the clinical research progress of their combination with ICIs.

**Keywords** Liver metastases, Immune checkpoint inhibitors, Locoregional therapies, Immune microenvironment

## Introduction

Liver metastasis originates from primary malignancies in other parts of the body, including melanoma, breast cancer (BC), non-small cell lung cancer (NSCLC), pancreatic cancer, and, more commonly, colorectal cancer (CRC). Epidemiologically, approximately 50% of individuals with various types of cancer are diagnosed with or will develop liver metastases [1]. Various treatment options have been explored, including surgery, systemic therapy, locoregional therapies, liver transplantation techniques, and their combinations [2]. Additionally, some scoring systems have been proposed to evaluate patient prognosis [3–6]. In recent decades, the rapid development of ICIs has significantly improved the clinical outcomes and prognosis of several tumor types [7, 8], especially in patients with microsatellite instability-high (MSI-H) or mismatch repair deficiency (dMMR) solid tumors [9, 10]. However, the use of a single ICIs, in the treatment of primary and secondary liver cancer has shown disappointing results [11, 12]. Understanding the mechanisms behind immunotherapy resistance in patients with liver cancer remains an urgent challenge. Notably, locoregional therapies in the liver, such as radiotherapy, ablation, chemoembolization, and radioembolization, are widely applied in hepatocellular carcinoma (HCC) patients. Moreover, ICIs combined with locoregional therapies play a crucial role in the treatment of various malignancies [13–15]. Currently, several combinatorial approaches of locoregional therapies and ICIs are being developed or have been published for HCC patients

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worldwide, detailing these treatments in the existing literature [16]. However, systematic reviews of immunotherapy combined with locoregional therapies in liver metastases are lacking. Therefore, this article describes the underlying mechanisms of liver-induced resistance and the effects of locoregional therapies on the immune microenvironment of liver metastases. Furthermore, we review clinical literature on the use of immunotherapy combined with locoregional therapies for the management of liver metastases.

## Mechanism of immune tolerance in liver metastasis

### Role of intrinsic properties in liver

The liver has a unique anatomical structure and distinctive physiological functions [17]. The blood supply of the human liver is extremely rich, with a flow of 1500 to 2000 ml per minute, originating from both the hepatic artery (systemic circulation) and the portal vein (gastrointestinal tract). The portal vein carries massive innocuous nutrients, commensal bacterial antigens, and pathogen products, to which the liver's immune system must maintain tolerance. Anatomically, the liver has a hexagonal lobular structure with fenestrated capillaries, allowing circulating immune cells to directly contact hepatocytes. In 1969, Calne and co-workers first described the unique induction of immunological tolerance in the liver through a series of experiments. They confirmed that liver allografts mismatched with major histocompatibility complex (MHC) antigens could be successfully performed [18]. Subsequently, a series of studies on this phenomenon were conducted. Available research suggests that the potential mechanisms of hepatic immune tolerance may be closely related to the unique immune microenvironment in the liver (Fig. 1).

Liver tissue mainly consists of parenchymal cells (hepatocytes) and nonparenchymal cells, including hepatic stellate cells (HSCs), Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), and antigen-presenting cells (APCs). Due to the presence of these liver-resident cells, allospecific T cells in the liver are either tolerized or deleted. In vitro models show that LSECs can cross-present ovalbumin, a mammalian protein with strong immunogenicity, to naive CD8 T cells, stimulating them but predisposing them to CD8 T cell tolerance through the initiation of a tolerogenic cytokine program [19]. Located on the inner surface of the hepatic sinusoids, KCs induce apoptosis of T cells through TNF-receptor (TNF-R) and Fas-ligand (Fas-L), both of which are caspase-3 mediated signals [20]. Similar to other APCs in the liver, HSC-presented antigens can suppress T-cell immune response through the PD-1/PD-L1 pathway and produce tolerogenic mediators, such as transforming growth factor-β (TGF-β) and interleukin-6/10 (IL-6/10) [21]. Additionally, LSECs, KCs, and HSCs produce

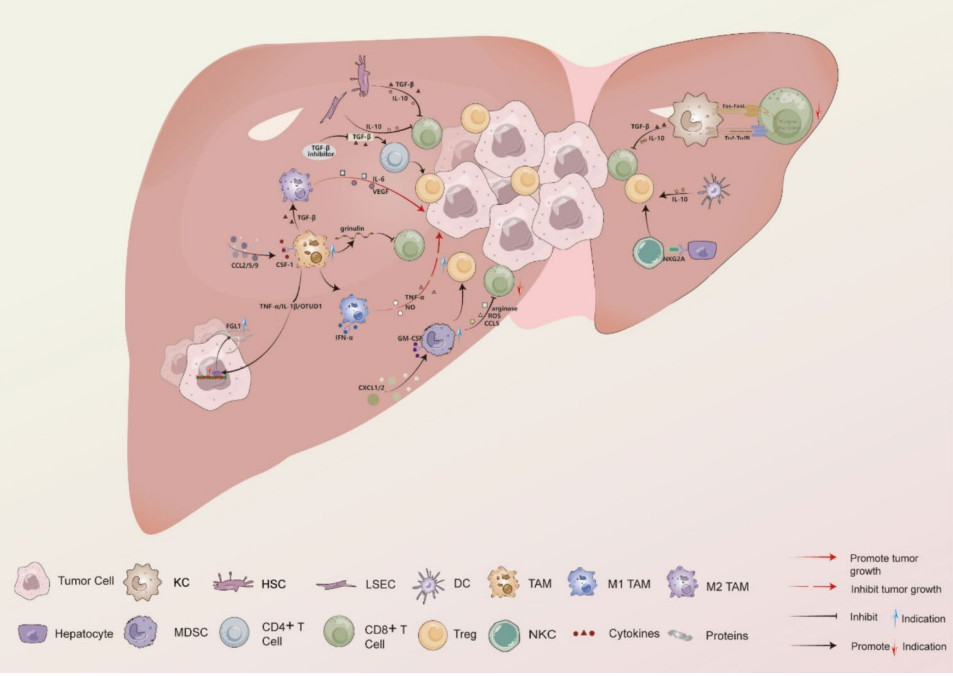


**Fig. 1** The tolerogenic immunity of the liver is achieved through an immunosuppressive network of multiple cell types. Liver tissue mainly consists of hepatocytes and NPCs, among which NPCs mainly include HSCs, KCs, LSECs and APCs. These immune cells have immune tolerance mechanisms that induce T cell apoptosis and promote the production of Tregs. *Abbreviations NPC* nonparenchymal cell; *HSC* hepatic stellate cell; *KC* kupffer cell; *LSEC* liver sinusoidal endothelial cell; *APC* antigen-presenting cells; *DC* dendritic cell; *TAM* tumor-associated macrophage; *M1 TAM* M1-type tumor-associated macrophage; *M2 TAM* M2-type tumor-associated macrophage; *MDSC* myeloid-derived suppressor cell; *Treg* regulatory T cell; *NKC* natural killer cell

IL-10 and TGF-β, promoting the polarization of regulatory T cells (Tregs) and suppressing T cell functions [1, 22, 23]. Moreover, natural killer cells induce Treg cells through NKG2A interaction with hepatocytes [24]. Dendritic cells (DCs) promote the generation of Tregs and T helper 2 cells by secreting high levels of IL-10 in the liver [25]. Notably, high levels of IL-10 suppress antigen presentation by professional APCs [26], resulting in the accumulation of type-1-like regulatory (Tr1) cells [27] and regulatory CD4+T cells [28, 29]. Additionally, liver parenchymal cells can make direct contact with T cells due to the fenestrated capillaries within the liver. Hepatocyte-activated T cells can result in premature T-cell death and tolerance in the absence of co-stimulation [30–32]. These data cumulatively indicate that the liver's tolerogenic immunity is achieved through an immunosuppressive network of multiple cell types.

## Liver metastasis environment

### *Immune cells*

**Tumor-associated macrophage (TAMs)** originating from blood monocytes, are recruited into liver metastases by pro-inflammatory cytokines such as CCL2, CCL5, and CCL9 [33]. Blocking the CCL2/CCR2 pathway can decrease macrophage infiltration, thereby reducing the liver metastatic burden in CRC [34]. TAMs can be divided into M1 and M2 phenotypes based on distinct activation pathways. M1-type TAMs play a role in anti-tumor effects by producing factors such as NO and TNF-α, while M2-type TAMs mainly promote tumor growth by producing IL-6 and growth factors such as vascular endothelial growth factor (VEGF) [35, 36]. In mice with liver metastases, metastatic lesions can polarize monocyte-derived macrophages into mature CD11b+F4/80+macrophages [37], facilitating angiogenesis by releasing VEGF and TGF-β [38, 39]. Additionally, CD8+cytotoxic T cells are well-known critical effectors in the immune response against cancer [40]. Tumeh et al. observed a decreased density of CD8+T cells in the periphery of aggressive tumors in biopsy samples from patients with liver metastases compared to those without liver metastases [7]. Unfortunately, immunotherapy relies on the function and state of tumor-infiltrating effector CD8+T cells. In preclinical models, activated antigen-specific CD8+T cells siphoned by liver metastases from systemic circulation undergo apoptosis after interacting with CD11b+F4/80+monocyte-derived macrophages via the Fas/Fas-L signal transduction pathway, contributing to the systemic absence of CD8+T cells and the formation of an "immune desert" [41]. Peranzoni et al. hypothesized that TAMs can limit CD8+T cell entry into tumor nests in lung squamous-cell carcinomas and tested this hypothesis in mouse tumor models using the colony-stimulating factor-1 receptor inhibitor (CSF-1RI) PLX3397 [42]. Similar to what was reported by Peranzoni and colleagues, Quaranta et al. found that macrophage-derived granulin, induced by CSF-1, contributes to cytotoxic CD8+T cell exclusion in liver metastasis. Blocking the CSF-1/CSF-1R axis could sensitize metastatic pancreatic ductal adenocarcinoma to αPD-1 [43]. Consequently, a potential immunotherapeutic strategy that blocks macrophages combined with ICIs may hinder the progression of liver metastasis, restoring CD8+T cell infiltration in metastatic liver lesions.

**Myeloid-derived suppressor cell (MDSCs)** a heterogeneous population of CD11b+Ly6C+Ly6G+myeloid cells, exert immunosuppressive functions, including recruiting Tregs and suppressing CD8+cytotoxic T cells at sites of cancer growth [44]. They are also recruited by chemokines (CXCL1 and CXCL2) secreted by LSECs, KCs, and HSCs [45–47], and inhibit T-cell activation and proliferation by producing arginase, ROS, and CCL5. Of note, granulocyte-macrophage colony-stimulating factor (GM-CSF) has been proven to promote the recruitment and expansion of MDSCs in tumor models [48, 49]. MDSCs co-express GM-CSF receptor, indoleamine 2,3-dioxygenase (IDO), and PD-L1 in the liver [50]. GM-CSF drives IDO and PD-L1 expression in MDSCs by activating the signal transducer and activator of transcription factor 3 (STAT3) signaling pathway. Small molecules, such as Janus-activated kinase 2 (JAK2) and STAT3 inhibitors, can significantly diminish the expression of IDO and PD-L1 in hepatic MDSCs. Therefore, blocking the GM-CSF/JAK2/STAT3 axis may reverse immunosuppression and enhance intrahepatic antitumor immunity. Additionally, Milette and colleagues recently found that the recruitment of MDSCs and Tregs into CRC hepatic metastasis was TNFR2-dependent in female but not in male mice, and experimental liver metastasis was significantly reduced when tumor-bearing mice were treated with antisense oligonucleotides targeting TNFR2 [51]. Intriguingly, they also showed that estrogen regulates the infiltration and function of immune cells in the liver tumor immune microenvironment, including MDSC accumulation and production of interferon-γ (IFN-γ) and granzyme B (GB) in CD8+T cells [52]. Notably, several MDSC-targeting agents and the blockade of MDSC-inducing cytokines or chemokines have been confirmed to potentiate antitumor immune responses and reverse immune resistance to ICIs in patients with cancer [53–55]. In summary, interfering with these pathways may become a potential treatment strategy to enhance immunotherapy for liver metastasis.

**Regulatory T cell** As one of the several immunosuppressive cell types, Tregs are implicated in ICI resistance in liver cancer, despite the mechanisms of action of Tregs within the liver being unknown. Like MDSCs,

Tregs also suppress anti-tumor CTLs and curb the efficacy of immunotherapy. Several experimental cancer models have demonstrated that the removal of Treg cells is crucial in restraining tumor growth and progression [56–58]. Tregs can be classified into two types: natural (CD4+CD25+FOXP3+) Tregs and inducible (FOXP3+or FOXP3-) Tregs [59]. Among them, Tr1 cells are FOXP3-Tregs and are a significant source of IL-10 [60]. Additionally, in a preclinical study, CD29+ (Itgb1, integrin β1) Tregs, a liver-specific Treg subset, were found to be highly immunosuppressive in mice and mediated ICI treatment failure by doubling their number in response to anti-PD-1 [61]. Several Treg-targeting strategies are currently in development, such as using daclizumab (a CD25-neutralizing antibody) and blocking CCL22. However, these therapeutic strategies have shown promise mainly in preclinical research and clinical trials for breast cancer but have not yet been tested in liver metastasis models and clinical settings [62–64]. In addition, experimental liver metastasis induced the activation of CTLA-4, PD-1, and high inducible costimulatory molecule (ICOS-high) Tregs, which could induce coordinated immunosuppression [29].

#### *Cytokines*

Cytokines and chemokines are essential for orchestrating and shaping the tumor microenvironment. In the CT-26 murine model of colon cancer liver metastasis, an increase in the expression levels of IL-10 and TGF-β1 was observed compared to liver tissue in normal mice [65]. IL-10 exerts extensive immunosuppressive functions by interacting with its cognate receptors (IL-10R) and activating the downstream STAT3 signaling pathways. In human CRC patients with liver metastasis who are refractory to PD-1 blockade, blocking IL-10 induces carcinoma cell death by increasing the frequency of intratumoral CD8+T cells and the expression of IFN-γ genes [66]. TGF-β, a major driver of the immunosuppressive microenvironment, plays an important role in the angiogenic and growth phases of liver metastasis [67]. In the tumor microenvironment enriched with TGF-β, TAMs and tumor-associated neutrophils (TANs) can be polarized into immunosuppressive phenotypes (M2 and N2) [68, 69]. Blockade of TGF-βR signaling can augment the cytotoxic activities of CD11b+Ly6G+TANs by increasing the release of proinflammatory cytokines such as TNF-α, IFN-γ, IL-12, and CCL5 [70]. Additionally, TGF-β blockade can potentially suppress the differentiation of CD4+T cells into Tregs. Interferon-α (IFN-α) is a potent cytokine with pleiotropic immunoactivities that modulate the anti-tumoral functions of immune cells and target both neo-angiogenic endothelial cells and tumor cells concurrently [71]. In murine models of CRC and PDAC liver metastasis [72], Kerzel and colleagues described that IFN-α delayed the growth of liver metastasis by increasing the proportion of inflammatory phenotype in TAMs concomitant with the infiltration of tumor-associated CD8+T lymphocytes, although a higher percentage of LAG3+CD4+T cells was also observed. LAG3, an inhibitory co-receptor, has previously been reported as a surface marker of T cell exhaustion and also of human Tr1 cells [73]. Collectively, the co-administration of an anti-CTLA-4 antibody with IFN-α may reshape the liver metastasis TME and potentially overcome immunotherapy resistance in the future.

#### *Genes or proteins*

Extensive analysis of the genomic, epigenomic, and transcriptomic features of liver tumors has enhanced our understanding of liver tumor biology and its stromal immune microenvironment, laying the foundation for more targeted, subtype-based therapeutic approaches. Yang and co-workers discovered that the characteristics of T cells were distinctive in different liver cancer types [74]. They also revealed a potential mechanism where solute carrier family 2 member 1 (SLC2A1, encoding glucose transporter type 1 (GLUT1)) could promote immunosuppression and an immune desert by increasing the proportion of Spp1+macrophages, which can inhibit interactions with T cells in liver metastatic lesions. Recent research suggested that the presence of SPP1+TAMs indicated hypoxic areas due to the enrichment of GLUT1+cells [75]. Remarkably, hypoxia is a factor associated with tumor progression. Fibrinogen-like protein 1 (FGL1), also known as hepatocyte-derived fibrinogen-like protein 1 (HFREP1), was first identified in hepatocytes. High expression of FGL1 has also been found in cancer cells in recent years. Previous studies have shown that the level of FGL1 expression is closely associated with tumor therapy resistance [76]. Recently, Li and co-workers revealed that FGL1 expression levels were upregulated in primary gastrointestinal tumors and their liver metastases, which were associated with poor outcomes and predicted a diminished response to anti-PD-1/PD-L1 treatment. They also demonstrated that TAMs promoted the stabilization of FGL1 by activating nuclear factor kappa-B (NF-κB) through the secretion of TNF-α/IL-1β and OTU deubiquitinase 1 (OTUD1) [77]. Benzethonium chloride curbs FGL1 secretion, thereby inhibiting tumor cell progression in the liver microenvironment. Therefore, the TAM-OTUD1-FGL1 axis may be a potential target for the immunotherapy of liver metastases. Glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR), a co-stimulatory receptor belonging to the tumor necrosis factor receptor superfamily, has been widely studied in anti-cancer immunotherapy [78, 79]. Its activation has been shown to activate effector T cells and hamper Treg functionality

[80, 81]. The overexpression of GITR was found on CD4+and CD8+tumor-infiltrating lymphocytes (TILs) from CRC liver metastases [82], and anti-GITR agonistic antibodies can induce efficient anti-tumor responses [83]. Overall, the complicated network associated with genes and proteins is worth further exploration to guide more therapeutic approaches.

## Role of the locoregional therapies in liver metastasis

To improve the prognosis of patients with liver metastasis, multidisciplinary treatment approaches have been employed, incorporating systemic and locoregional therapies. Currently, surgical resection is recommended as the first-line treatment. However, for patients with inoperable disease, alternative therapeutic strategies may be effective in improving clinical outcomes. Although local therapies have been extensively studied in liver tumors, their role in immunotherapy, particularly checkpoint inhibitors, remains not fully understood.

### *Radiation therapies for liver metastasis*

Radiotherapy has been demonstrated to control hepatic tumors and stimulate antitumor immunity in both preclinical and clinical research [84–86] (Fig. 2).

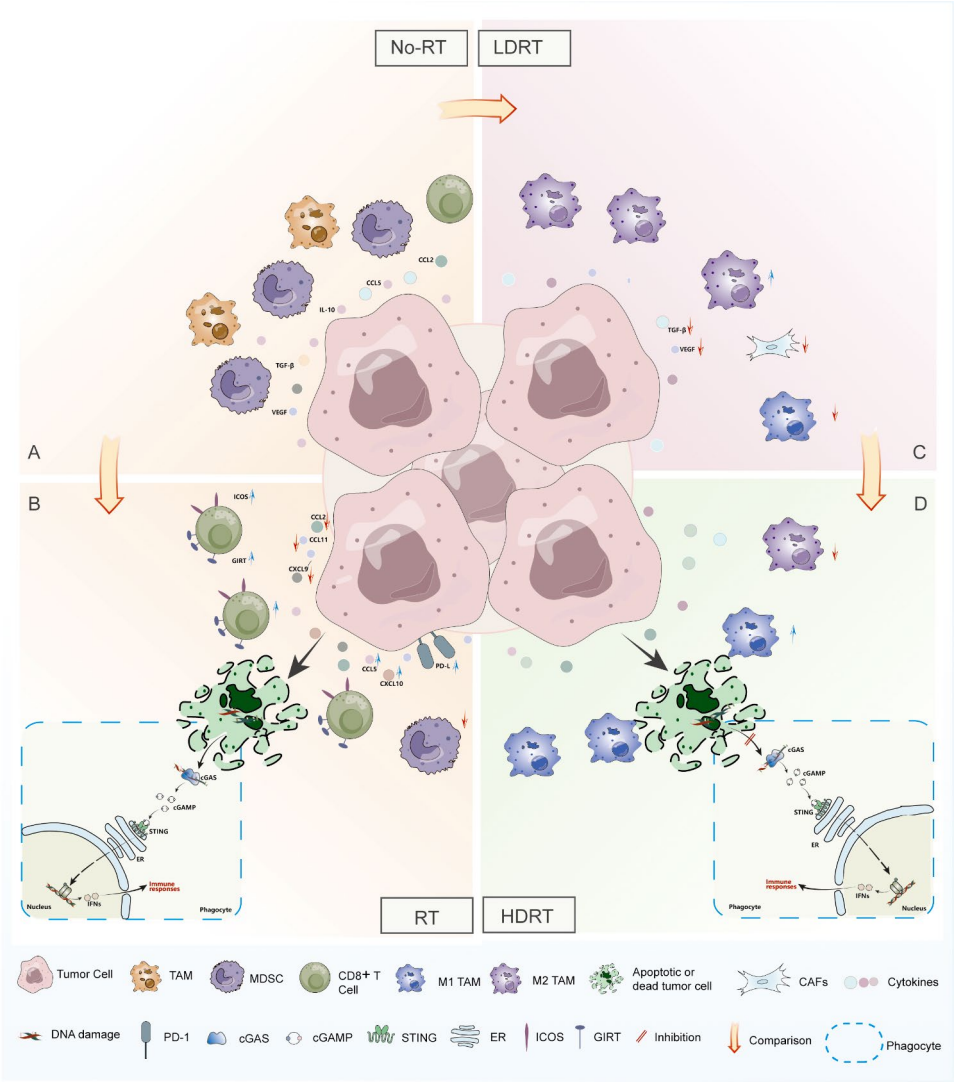


**Fig. 2** Radiotherapy stimulates antitumor immunity. *Legend***A**: Local microenvironment of liver metastases. **B**: Radiotherapy activates the cGAS /STING signaling pathway to induce IFN-stimulated genes to induce cancer cell death. Additionally, liver-directed radiotherapy can increase hepatic CD8+T cell infiltration and diminish the number of MDSCs. **C**: LDRT with immunotherapy increased the M1-to-M2 macrophage ratio in comparison with HFRT and decreases CAFs, TGF-β, and VEGF. **D**: HDRT could lead to a significant reduction in multiple T-cell populations and also may result in regulatory inhibition of the cGAS/STING signaling pathway. *Abbreviations TAM* tumor-associated macrophage; *MDSC* myeloid-derived suppressor cell; *M1 TAM* M1-type tumor-associated macrophage; *M2 TAM* M2-type tumor-associated macrophage; *CAFs* cancer-associated fibroblasts; *cGAS* cyclic GMP-AMP synthase; *STING* stimulator of interferon genes; *ER* endoplasmic reticulum; *ICOS* inducible costimulatory molecule; *GIRT* Glucocorticoid-induced tumor necrosis factor receptor-related protein

In addition to inducing cancer cell death by mediating DNA damage [87] and promoting the release of dsDNA (double-stranded DNA), thereby activating the cGAS/STING signaling pathway to induce IFN-stimulated genes [88], radiotherapy can also modulate immunogenicity by releasing inflammatory mediators and increasing tumor-infiltrating immune cells (Fig. 2A). In preclinical models, liver-directed radiotherapy has been found to increase hepatic CD8+T cell infiltration and diminish the number of MDSCs. The concentrations of CCL2, CCL11, and CXCL2 were diminished after irradiation, which may reduce the recruitment of myeloid cells into the liver, whereas increased concentrations of chemokines CCL5 and CXCL10 were observed, potentially recruiting effector T cells to the irradiated liver. Moreover, the expression of ICOS and GITR in inducible T cells and the level of PD-1/PD-L1 on the surface of tumor cells are higher in tumor tissue following liver metastasis radiotherapy, suggesting that radiotherapy combined with immunotherapy may be a potential therapeutic strategy for patients with liver metastases [89] (Fig. 2B). Radiotherapy combined with αPD-L1 enhanced the levels of Ki67+, IFN-γ+, and GB+CD8+T cells in the liver [41]. Unfortunately, this study only demonstrated that hepatic radiotherapy may offer a potential approach to overcoming the tolerance of liver metastasis to immunotherapy efficacy, but did not specify the radiation dose and its effect on the tumor immune microenvironment. Indeed, both high-dose radiotherapy (HDRT) and low-dose radiotherapy (LDRT) exert an impact on the local immune microenvironment in the liver and systemic immunogenicity. A study by Monjazeb and colleagues demonstrated that the combination of LDRT with immunotherapy increased the M1-to-M2 macrophage ratio compared to HDRT patients [90]. In addition to inducing macrophage repolarization, LDRT decreases cancer-associated fibroblasts (CAFs), TGF-β, and VEGF [91] (Fig. 2C). However, despite its pro-immunogenic properties, radiotherapy may also produce a variety of immunosuppressive effects [85]. It is well known that lymphocyte depletion is one of the major adverse consequences of irradiation. HDRT can result in a significant reduction in multiple T-cell populations in peripheral blood [90]. Higher radiation doses are not only associated with augmented side effects such as lymphocyte depletion, but they may also result in regulatory inhibition of the cGAS/STING signaling pathway [85] (Fig. 2D). Therefore, more studies are needed to find the balance between radiation dose and clinical benefit.

#### *Ablation therapies for liver metastasis*

Percutaneous radiofrequency ablation (RFA), microwave ablation (MWA), and cryoablation are widely used to treat primary and secondary hepatic malignancies. The effects of these treatments on the immune microenvironment of the liver are illustrated below (Fig. 3).

Coagulation necrosis occurs in hepatic lesions treated with thermal ablation when local temperatures reach 60–65 °C. The capacity of HCC and CRC cells to stimulate CD4+and CD8+T cell-specific immune responses significantly increased following RFA, including the quantity and cytotoxic activity of tumor-specific circulating CD4+and CD8+T cells [92]. Additionally, RFA can strongly stimulate the proliferation of circulating B cells in patients with metastatic liver cancer [93]. The levels of IFN-γ, C-reactive protein, and VEGF markedly increased after RFA therapy [93, 94]. The number of CD3+T cells, CD56+NK cells, and CD68+monocytes significantly increased in microwave-ablated tumor tissue in HCC patients [52, 95, 96]. Zhao and colleagues observed a significant elevation in serum levels of specific cytokines, including IL-2 and IL-6, following MWA treatment for liver malignant tumors [97]. On one hand, IL-6 plays a crucial role in facilitating T-cell infiltration into tumors [98], potentially benefiting immunomodulatory therapy. On the other hand, IL-6 can stimulate the expansion of MDSCs [99], impede the development and maturation of DCs [100], and hinder Th1 cell polarization [101], ultimately exerting an adverse immune regulatory effect. Shi and colleagues found that in patients with liver metastasis treated with RFA, T cell infiltration and tumoral PD-L1 expression increased in CRC tissues [15]. However, on day 8 after RFA, the ratio of CD8+T cells to Treg cells significantly decreased compared to day 3. Nevertheless, it can be assumed that anti-PD-1/PD-L1 therapy may potentially benefit from RFA. When considering liver local ablation combined with ICI treatment, it is important to take advantage of the positive effects of related cytokines while avoiding their negative effects. Another mode of ablation, cryoablation, can lead to direct cell death, releasing abundant antigens and cytokines such as IL-2, IL-12, IFN-γ, and TNF-α, which facilitate the immune response [102]. After three weeks of combined treatment with transarterial infusion of pembrolizumab and cryoablation, the population of NK cells (CD3- CD16+CD56+) was significantly elevated, whereas the frequency of Treg cells (CD4+CD25+) was markedly reduced in the serum of patients with melanoma and multiple liver metastases [103]. Strikingly, no direct comparison of the different ablation modalities has been performed, and existing studies primarily focus on the assessment of immune cell and cytokine infiltration in peripheral blood following ablation, while the underlying mechanisms influencing the local liver tumor remain elusive.

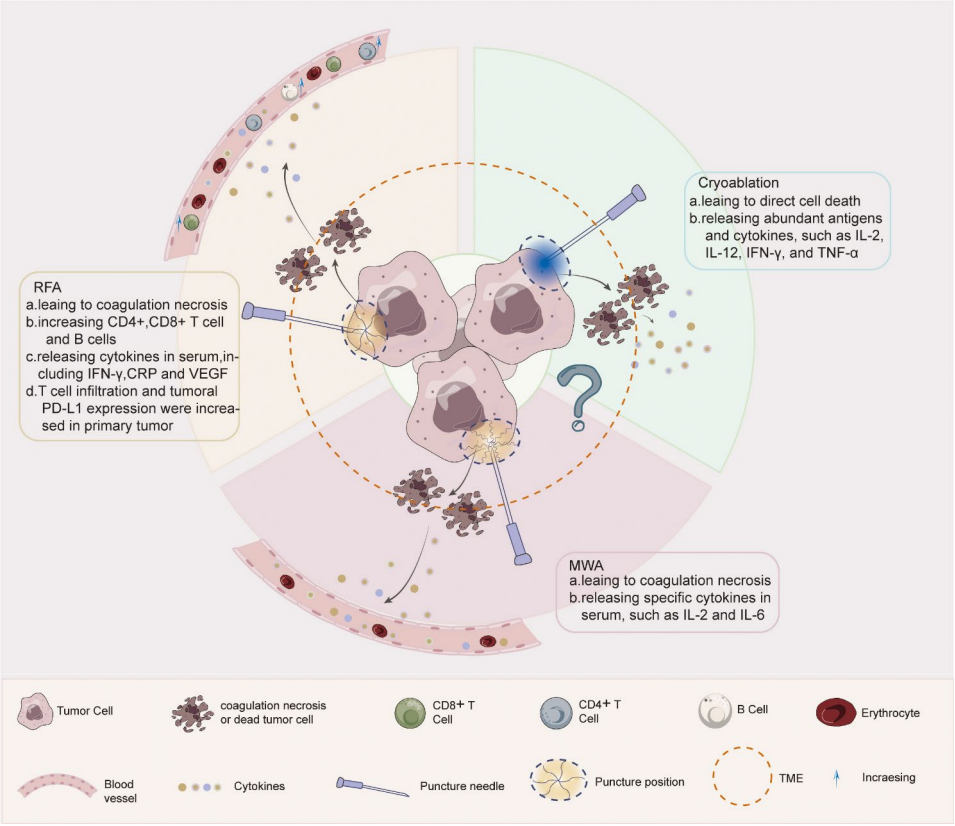


**Fig. 3** Whether ablation can enhance the sensitivity of liver metastases to ICI drugs is unclear. RFA, MWA and cryoablation all regulate the liver immune microenvironment, such as regulating immune cells and releasing cytokines. However, the function of cytokines is complex and may have dual regulatory effects of activating immunity and suppressing immunity. *Abbreviations RFA* radiofrequency ablation; *MWA* microwave ablation; *TME* tumor immune microenvironment

***Chemoembolization/radioembolization for liver metastasis***

Similar to ablation, transarterial chemoembolization (TACE) is also an effective treatment approach for localized or regional liver cancer. Studies have shown that TACE may decrease the number of immunosuppressive Tregs in the peripheral blood of HCC patients [104]. Interestingly, TIL numbers and subsets were not significantly modified in preoperative TACE compared to surgery without preoperative treatment [105]. Additionally, transarterial radioembolization(TARE), especially Yttrium-90 radioembolization (Y90-RE), also known as selective internal radiation therapy (SIRT), combined with ICIs has been successfully applied to achieve disease control or surgical resectability in HCC and hepatic metastases. After undergoing Y90-RE, an enrichment of CD4+T cells, CD8+T cells, NK cells, and NKT cell subsets was observed in HCC tumor tissue [106]. In addition, the expression of immune markers such as GB and Tim-3 was also found to be elevated in TILs. Despite substantial tumor cell necrosis, the intra-tumor expression of CD8+TILs, CD4+TILs, and GB showed no significant difference between TACE and the spontaneous condition in tumors. This indicates that the induction of ischemic cell death by TACE does not significantly modify the inflammatory/immunogenic tumor microenvironment. Interestingly, therapeutic combinations of TARE and dual immune checkpoint blockades may overcome some resistance mechanisms, at least partially [107]. Nevertheless, the majority of the aforementioned data comprises HCC-based studies, necessitating further investigation into the impact of TACE and TARE on liver metastatic tumor tissue. The safety and efficacy of combining immune checkpoint blockade with either TACE or TARE remains.

## ICIs clinical shreds of evidence of liver metastasis treated with ICIS

### ICIs alone

Immune checkpoint blockers have provided significant long-term clinical benefits across multiple tumor types since their introduction as a treatment for metastatic or unresectable melanoma in 2011 [108]. Compared with vaccines or chemotherapy, improved OS with the anti-CTLA-4 monoclonal antibody (ipilimumab) was demonstrated in phase II/III clinical studies on patients with metastatic melanoma [109, 110].

Similarly, clinical benefits were confirmed with anti-PD-1/anti-PD-L1 monoclonal antibodies (nivolumab, atezolizumab, pembrolizumab, durvalumab) in NSCLC [111], triple-negative breast cancer [112], colorectal cancer with dMMR-MSI-H [113], urothelial carcinoma (UC) [114, 115], and squamous cell carcinoma of the head and neck [116]. Regardless of the primary site, tumors with high tumor mutational burden (TMB-high) have shown positive responses to ICIs based on findings from the phase II KEYNOTE-158 trial [117]. A recently published case reported that a woman diagnosed with TMB-high gallbladder carcinoma with liver metastasis achieved a partial response after pembrolizumab treatment [118]. Nevertheless, it was first observed in 2015 that the immune-modulatory effect of PD-1 blockade was counterbalanced in patients with liver metastasis [119]. In a large sample study conducted by Goldinger and colleagues, multivariate analysis confirmed these findings [120]. Preclinical mouse models and patients with liver metastasis have indicated that liver metastasis is associated with a reduced response to anti-PD-1 monoclonal antibody and diminished OS and PFS [7, 41]. Given the immune tolerance of the liver, the combination of CTLA-4 blockers and PD-1 blockers is promising for a synergistic effect. In several cancer types including melanoma [121, 122], NSCLC [123], malignant pleural mesothelioma [124], renal cell carcinoma [125, 126], and colorectal cancer (MSI-H/dMMR) [127], combination therapy with anti-CTLA-4 and anti–PD-(L)1 antibodies showed higher response rates than monotherapy. Unfortunately, in the CheckMate 277 clinical study, nivolumab combined with ipilimumab or chemotherapy in the liver metastasis subgroup did not benefit patients with advanced NSCLC [123]. Collectively, these publications underscore the diminished clinical efficacy of ICIs in patients with liver metastases across various tumor types.

#### ICIs combined with chemotherapy

ICI monotherapies can achieve durable responses. Even though combining ICIs with anti-CTLA-4 and anti-PD-1/PD-L1 has shown higher response rates than single-agent treatment, only a small fraction of cancer patients respond to ICIs in the clinic. Consequently, the identification of novel combination strategies is pressing and necessary. Notably, chemotherapy, as a traditional anti-tumor treatment, has been combined with other treatment methods in almost all tumors. Given that chemotherapy can inhibit immunosuppressive cells in the TME, increase tumor antigen exposure by inducing cancer cell death, and promote immune cell infiltration and dendritic cell maturation, thereby enhancing the antitumor immune response [128–130], ICIs combined with chemotherapy have been tested in various clinical trials. In a phase 3 trial, nivolumab plus fluorouracil-cisplatin demonstrated significantly longer OS compared to chemotherapy alone in patients with metastatic esophageal squamous cell carcinoma [131]. Similarly, two additional phase 3 studies showed that the nivolumab plus platinum-based chemotherapy group and the carboplatin-etoposide with atezolizumab group had significantly longer PFS and OS compared to chemotherapy-alone groups in lung cancer [132, 133]. A reported pooled analysis that included KEYNOTE-021 cohort G, KEYNOTE-189, and KEYNOTE-407 found that the addition of pembrolizumab to first-line chemotherapy improved survival outcomes for patients with liver metastases; however, the observed benefit was comparatively attenuated compared to patients without liver metastases [134].A recent meta-analysis including eight randomized controlled trials showed that PD-1/L1 inhibitors combined with chemotherapy reduced the risk of disease progression by 40% in lung cancer patients without liver metastases, compared to only 31% in patients with liver metastases [135], suggesting comparable efficacy in lung cancer patients with and without liver metastases. However, the conclusions are inconsistent. Two phase 3 clinical studies, IMpower130 [136] and IMpower132 [137], did not show significant OS benefit in the subgroup of patients with liver metastases who received ICIs plus chemotherapy. Another study found that Cox regression analyses showed the HR for OS was 1.55 for UC patients with liver metastasis treated with platinum-based chemotherapy followed by three different PD-L1 inhibitors (durvalumab, atezolizumab, and avelumab) in the second-line treatment [138]. Some scholars consider that cytotoxic drugs may potentially compromise the anti-tumor functionality of immune cells, thereby diminishing the efficacy of ICIs. Consequently, novel strategies are needed to increase response rates in liver metastases. A case published online in 2022 indicated that a man with MSI-L/p-MMR mCRC showed a partial response to Tislelizumab (an anti-PD-1 drug) after systemic therapy failure and SBRT (targeting liver metastasis) [139]. Therefore, the combination of systemic therapies and locoregional therapies may open new avenues for the treatment of liver metastasis. An ongoing phase II clinical trial is examining SIRT of liver metastasis in combination with a PD-L1 inhibitor, chemotherapy, and anti-angiogenesis therapy in CRC [140].

#### ICIs combined with radiotherapy

Preclinical studies in subcutaneous tumor models have shown that checkpoint blockade and radiotherapy can synergistically promote an enhanced ICI immune response [141]. However, the sequence and timing of immunotherapy and tumor irradiation therapy [142], as well as the dose of radiation [143], appear to be important. Administering anti-PD-1 antibodies before

radiation appears to be deleterious due to the destruction of immune cells induced by αPD-1 [144]. Preclinical data have suggested that abscopal responses are observed when αPD-1 antibody is administered after SBRT in MC38 colorectal tumor mice [142]. The KEYNOTE-001 clinical trial also showed that patients with metastatic NSCLC who received radiotherapy before immunotherapy had better PFS [145]. Unfortunately, combining radiotherapy with immune checkpoint blockade (ICB) failed to improve clinical efficacy in mNSCLC [146] and Merkel cell carcinoma [147].In a recently published clinical trial, liver SBRT combined with ICB appears to be safe [148]. A subgroup analysis of a randomized, controlled trial suggested that irradiated liver metastasis achieved a higher clinical benefit compared to irradiated extrahepatic disease (30.8% vs. 15.8%) [149]. A recently reported representative case involved a patient diagnosed with stage-IV melanoma with multiple metastases, including lung, bone, liver, and brain, who received ipilimumab and nivolumab followed by liver and lung radiation treatment, resulting in durable and complete responses for liver metastasis [150]. However, in a randomized controlled trial, Monjazeb et al. observed that combining PD-L1 and CTLA-4 inhibition with targeted low-dose or hypofractionated radiation for liver metastasis in mCRC had limited effects on improving mPS and mOS [90]. Despite promising data from preclinical and clinical trials indicating that radiation can induce tumor-specific immune responses [151], the clinical efficacy of radiation for liver metastasis combined with systemic immunotherapy is indeterminate and incompletely understood. In addition to radiation doses, advanced tumor burden and prior extensive treatments may also impair patients' immune systems. Thus, optimizing immunotherapy and radiotherapy regimens, including the dose and sequence of ICI and irradiation, requires further experimental studies.

#### ICIs combined with ablation

RFA, MWA, and cryoablation, as minimally invasive approaches for treating primary and secondary liver cancer, have demonstrated clinical utility [152]. Much of the data regarding immune responses to ICIs and ablation are based on preclinical studies. Several studies have reported that ICIs combined with RFA or cryoablation synergistically promote systemic antitumor immunity in colon cancer, melanoma, and renal cell carcinoma murine models [15, 153, 154]. Similar results were reported by Chen et al.; the combination of MWA and TIGIT blockade increased the anti-tumor immune response [155]. Furthermore, the clinical efficacy of argon-helium cryosurgery combined with nivolumab in the treatment of advanced NSCLC is better than that of argon-helium cryosurgery alone [156]. Similarly, case reports have shown that cryoablation combined with PD-(L)1 inhibitors may enhance the synergistic anti-tumor immune response in clear cell carcinoma and HCC [157, 158]. Nevertheless, in a phase Ib/II trial, combining RFA targeting liver metastasis with ipilimumab demonstrated limited clinical activity in UM [159]. In another phase II trial, durvalumab and tremelimumab combined with RFA for unresectable liver metastasis failed to demonstrate clinical feasibility in mCRC [160]. Based on these data, rational treatment strategies deserve further exploration to improve ICI responses in more clinical settings. An ongoing non-randomized phase II clinical trial is testing the potential abscopal effect of radiotherapy or thermal ablation followed by pembrolizumab in patients with mismatch repair-proficient mCRC [161].

#### ICIs combined with transarterial chemoembolization/radioembolization

TACE and TARE are widely utilized liver-directed treatments for regional liver tumors. Yttrium-90 (Y-90) radioembolization has been extensively used for both primary and secondary liver tumors [162, 163]. Preliminary retrospective and clinical trials have shown that the sequential use of ICIs and Y-90-labeled microspheres achieves high local tumor control rates in HCC cases [164–166]. Similarly, in cases of uveal melanoma with synchronous liver metastasis (UMLM), the sequential administration of Y-90 microspheres to hepatic lesions and ICIs achieved high hepatic disease control rates in both retrospective and prospective studies [167, 168]. Interestingly, a prospective, single-center clinical trial by Wang et al. on patients with microsatellite-stable colorectal cancer liver metastasis found that durvalumab and tremelimumab, following Y-90 liver radioembolization, did not enhance immune responses to liver metastasis, and all eligible patients experienced progressive disease [169]. Therefore, while Y-90 microspheres targeting liver metastasis are safe and effective, and may improve survival in HCC and UMLM, they did not increase the sensitivity of colorectal cancer liver metastasis to ICIs. It is reasonable to assume that the liver-dependent immune contexture of different malignant tumors, including the functional status and spatial distribution of immune cells, may underlie the inconsistent responses to the combination regimens described above. Additionally, percutaneous hepatic perfusion with melphalan (M-PHP), a locoregional treatment modality for malignant liver tumors, is a promising novel approach. An ongoing study is currently recruiting volunteers to evaluate the safety and efficacy of M-PHP combined with ipilimumab and nivolumab in individuals with unresectable UMLM [170]. In summary, the safety and efficacy of TACE in combination with ICIs remain unclear, and further studies are needed to validate these findings.

## Summary and outlook

Immunotherapy has introduced new hope for achieving prolonged survival in cancer patients. However, emerging evidence indicates that immunotherapy has limited efficacy in patients with liver metastases. Treating liver metastasis remains an unmet clinical need.

There is no consensus on the safety and efficacy of combining ICIs with locoregional therapies for liver metastases. Multiple mechanisms have been proposed to explain liver-associated immune tolerance, and researchers continue to explore new treatment methods to improve the prognosis of patients with liver metastasis. In this review, we elaborate on the influence of locoregional therapies on the liver immune microenvironment. Given the potential synergies of different treatments, preclinical studies have shown that combining immunotherapy with radiotherapy, ablation, and TACE/TARE holds significant promise for treating liver metastases.

Clinically, identifying clinical predictive biomarkers is crucial for determining which patient groups are likely to benefit from ICIs. In this review, we shown that interactions among various cells in the immune microenvironment of liver metastases lead to a reduction in CD8+T cells and an increase in Treg cells, accompanied by an abundance of immunosuppressive factors such as TGF-β and IL-10. The potential of these immune cell factors and immune cells in liver metastases as predictive markers warrants further investigation. Additionally, the regulatory mechanisms of the immune microenvironment in the liver may vary depending on the different locoregional therapies applied, and this requires further exploration. Currently, ICIs are mostly used in the later-line treatment of patients with liver metastases. It is expected that more appropriate treatment opportunities will be found to use ICIs to prolong the survival of patients in the future. However, our work has limitations. Although our paper provides substantial preclinical evidence supporting the role of local treatment in promoting liver immunotherapy, there is relatively little direct clinical evidence for the combined treatment overcoming liver-induced immune tolerance. Most prospective studies are still ongoing, and the treatment of liver metastases continues to pose a significant challenge.

In conclusion, given the current scarcity of clinical studies on the combination of ICIs and locoregional therapies for liver metastases, it is crucial to conduct more prospective clinical and translational studies. These studies will help optimize immunotherapy strategies through a systematic review of treatment regimens.

#### Author contributions

Xing-Chen Zhang and Yu-Wen Zhou designed and wrote this manuscript. Gui-Xia Wei and Yi-Qiao Luo drew the figures. Meng Qiu offered valuable insights and revised the manuscript. All authors contributed to the initial draft. All authors have approved the final version of the manuscript.

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#### Data availability

No datasets were generated or analysed during the current study.

## Declarations

#### Ethical approval

Not applicable.

#### Competing interests

The authors declare no competing interests.

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