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Review

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Cytotoxic immune cell-based immunotherapy for hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common solid tumors with poor clinical prognosis. Novel therapeutic regimens are urgently required for patients with advanced HCC. Both pre-clinical and clinical studies suggest immunotherapy as an attractive alternative for advanced HCC treatment. Natural killer (NK) cells and $CD8^+$ T cells are the most important cytotoxic immune cells involved in cancer treatment and elimination. Reinvigorating the anticancer activity of NK and $CD8^+$ T cells is the fundamental guarantee for the success of immunotherapy in advanced HCC treatment. Therefore, in this review, we aim to summarize the characteristics and roles of NK and $CD8^+$ T cells in HCC development, describe the frontiers of immunotherapy for advanced HCC based on immune checkpoint inhibitors and adoptive cell transfer, and discuss their limitations and scope for future improvement.

Keywords: Hepatocellular carcinoma, immunotherapy, natural killer cells, $CD8^+$ T cells

INTRODUCTION

Although hepatocellular carcinoma (HCC) is only the fifth-most common cancer worldwide, it ranks second in cancer-related mortality[1]. Local regional therapies such as surgical resection, cryoablation, radiofrequency ablation, transarterial chemoembolization, and liver transplantation are effective only for patients with early-stage HCC[2]. Multi-targeted tyrosine kinase inhibitors (TKIs) provide options for systemic treatment of

patients with advanced HCC. The first-line agent sorafenib modestly extends the survival in advanced HCC by about 3 months[3]. Lenvatinib has been approved for HCC treatment based on the results of a randomized phase III trial, in that lenvatinib is non-inferior to sorafenib in overall survival assessment in patients with advanced HCC, with similar safety and tolerability profiles as sorafenib[4]. Regorafenib and cabozantinib have been approved as second-line options for patients with progressive HCC despite on-going sorafenib treatment, in that they improve overall survival in such patients[5-7]. Nonetheless, the survival benefit from the TKIs is limited and unexpected. Therefore, novel clinical therapies are urgently required for treatment of early-stage and advanced HCC. Recently, immunotherapy with immune checkpoint blockade and adoptive immune cell transfer has been clinically tested in various types of cancers, which provides a novel therapeutic strategy for difficult-to-treat HCC cases. The present review aims to summarize the characteristics and roles of natural killer (NK) cells and $CD8^+$ T cells during HCC development, describe the frontiers of immunotherapy for advanced HCC based on immune checkpoint inhibitors (ICIs) and adoptive cell transfer (ACT), and discuss their limitations and scope for future improvement.

NK CELLS AND $CD8^+$ T CELLS IN HCC

The liver contains diverse types of immune cells such as T cells, NK cells, B cells, NKT, and Kupffer cells[8,9]. However, the liver is a tolerogenic immune organ in the physiological state, in that the liver remains tolerant to stimuli from the hepatic artery and portal vein, such as bacterial products, environmental toxins, and food antigens, to avoid tissue damage[10,11]. This immune-tolerant microenvironment of the liver contributes to the immunoescape of HCC[12]. Studies have extensively discussed the properties and contribution of immunosuppressive cells during HCC progression[13]. However, to our best knowledge, the characteristics and contribution of the two most important anticancer immune cells in the liver - NK cells and $CD8^+$ T cells - have not been well-documented.

NK cells in HCC

Innate lymphoid cells (ILCs) function as the first line of immune defense against infections and cancers. Paralleling with T cell subsets, ILCs comprise NK cells, ILC1, ILC2, and ILC3, amongst which the NK and ILC1 cells are abundant in the liver[14,15]. Unlike helper ILC subsets, NK cells are classified as a cytotoxic ILC subset because of their direct killing of cancer cells and infected cells via cytotoxicity and cytokine secretion[16]. NK cells express activating receptors such as CD16, NKp30, NKp44, NKp46, NKp80, NKG2D, CD244, CD226; cytokine receptors such as IL-2R, IL-28R, IL-12R, IFNR, IL-15R, IL-18R, IL-1R8, IL-10R, and TGFR; and inhibitory receptors such as NKG2A, KLRG1, KIRs, TIGIT, TIM3, Siglecs, PD-1, LAG3, A2AR, LAIRs, and ILTs[17,18]. The activation of NK cells is determined by the net value of activating signal strength determined by the competition between activating and inhibitory receptors[17,19]. The abundance and activity of NK cells are modulated by multiple signals within the tumor microenvironment, which significantly influence cancer development. Compared with healthy controls, HCC patients have a dramatic reduction of tumor-infiltrating NK cells; their abundance in HCC tissues is positively correlated with patient survival[20]. NK cells in the HCC tissues of patients with advanced HCC show dysfunctional or exhausted state[20], suggesting that NK-cell exhaustion contributes to HCC progression. The regulators for NK cell exhaustion have been extensively investigated. For example, the up-regulation of inhibitory receptors on NK cells leads to NK-cell exhaustion and predicts poor prognosis in HCC patients[21]. TGF-β and IL-10 promoted NK-cell exhaustion in HCC[22,23]. Hypoxia-induced mitochondrial fragmentation limits NK-cell anticancer activity[24]. Additionally, immunosuppressive cells such as myeloid-derived suppressor cells, monocyte/macrophage, and HCC-associated fibroblasts in intratumor tissues of HCC contribute to NK-cell exhaustion[25-27]. The plasticity of NK cell activity provides the foundation of NK-cell-based cancer immunotherapy. Immunotherapeutic drugs triggering NK-cell activation are being developed and assessed in pre-clinical and clinical trials. Nevertheless, it is still crucial to decipher the mechanisms by which NK cells undergo exhaustion in patients with advanced HCC, in order to offer more patient benefits in terms of effective reinvigoration of NK-cell anticancer activity and precision therapy in HCC.

NK cells have been reported to account for 20%-40% of human hepatic lymphocytes and 10%-20% of murine hepatic lymphocytes, more than half of which *bona fide* comprise ILC1 or liver-resident NK cells[28]. The observations from the parabiosis model show that the liver contains conventional NK cells and liver-resident NK cells[29], further supported by the findings that hepatic irradiation could persistently eliminate liver-resident NK cells[30]. Early evidence has shown that liver-resident NK cells expressed higher levels of CD160, CD69, CD44, CXCR3, CXCR6, TRAIL, FasL, GM-CSF, and TNF-α[29]. CXCR6 is required for the retention of liver-resident NK cells within the liver[31]. $CD8^+$ T cells promote liver-resident NK cell maturation through the CD70-CD27 axis[32]. Liver-derived TGF-β maintains the property of liver-resident NK cells[33]. Functionally, liver-resident NK cells were originally found to mediate skin-contact inflammation[29]. Zhou *et al.*[34] reported that liver-resident NK cells inhibited T cell antiviral activity via PD-L1 during viral infection. Additionally, liver-resident NK cells can suppress autoimmune cholangitis by limiting the expansion of $CD4^+$ T cells[35]. These findings suggest that liver-resident NK cells play versatile roles in liver diseases. Human liver-resident NK cells are $CD56^{bright}$ $Eomes^{hi}$ $Tbet^{lo}$ $Hobit^+$ $TIGIT^+$ $CD69^+$ $CXCR6^+$ $CD49e^-$; express higher levels of NKG2D, NKp46, TRAIL, and FasL; and possess cytotoxicity against HCC cells[36]. However, liver-resident NK cells within the HCC tissue down-regulate NKG2D[37]. Moreover, liver-resident NK cells express more types of inhibitory receptors such as PD-1, CD96, and TIGIT[38]. Therefore, liver-resident NK cells undergo exhaustion during HCC progression. Fortunately, IL-15 could recover HCC-induced liver-resident NK-cell dysfunction[37]. In addition, the mTOR inhibitor - everolimus - enhances their anticancer activity through upregulation of TRAIL[39]. Thus, liver-resident NK cells have the potential for application in HCC therapy, although the complete underlying mechanism of liver-resident NK cells' exhaustion remains unclear.

$CD8^+$ T cells in HCC

The abundance of tumor-infiltrating $CD8^+$ T cells and the frequency of IFN-γ^+ $CD8^+$ T cells were associated with improved survival of HCC patients[40,41]. $CD8^+$ T cells were enriched in early-stage HCC, but progressively reduced with tumor progression, accompanied with increased expression of checkpoints on tumor-infiltrating $CD8^+$ T cells[42]. Therefore, $CD8^+$ T cells in HCC tissues progressively underwent functional compromise during cancer progression, characterized by high levels of immune checkpoints, low effector cytokines, and impaired cytotoxicity and proliferation. In detail, however, $CD8^+$ T cells in HCC tissues expressed different PD-1 levels and displayed different anticancer capacity[43]. Among $PD\text{-}1^{high}$ $CD8^+$ T cells, $4\text{-}1BB^+$ $PD\text{-}1^{high}$ $CD8^+$ T cells displayed stronger anticancer activity and proliferative potential[44]. In recent times, several studies have reported that $TCF\text{-}1^+$ $PD\text{-}1^+$ T cells sustained the stemness and response to immune checkpoint blockade in certain types of cancers[45,46]. Therefore, the identification of functional tumor-infiltrating $CD8^+$ T cells for immunotherapy will likely benefit clinical outcomes and promote precision medicine for HCC patients.

The systemic, local, cellular, and molecular mechanisms of T-cell exhaustion in HCC have been extensively investigated. The hepatic inflammatory microenvironment had been confirmed to be critical for HCC development[47]. Lim *et al.*[48] found that HBV-related HCC microenvironment displayed more immunosuppression than non-viral-related HCC microenvironment, indicating increased difficulty in the immunotherapy of HBV-related HCC. Hepatoma cells, LSECs, suppressive immune cells, inhibitory receptors, and cytokines have been found to trigger tumor-infiltrating $CD8^+$ T-cell exhaustion[49]. For instance, myeloid-derived suppressor cells and T regulatory cells in HCC tissues had been found to impair T-cell functionality[50]. The inhibitory cytokine - IL-35 - dampened $CD8^+$ T cells activity in HCC patients[51]. 14-3-3ζ, a suppressor of apoptosis, is highly expressed in HCC and promotes epithelial-mesenchymal transition of HCC cells[52]. Wang *et al.*[53] reported that 14-3-3ζ delivered by HCC-derived exosomes contributed to impaired anticancer activity of $CD8^+$ T cells. The thymocyte selection-associated high mobility group box (TOX) transcription factor belongs to an evolutionarily conserved DNA-binding protein family and regulates the development of T cells[54]. Recently, several studies have confirmed that TOX was critical for $CD8^+$ T cell exhaustion[55,56]. Moreover, it was found that TOX could promote $CD8^+$ T-cell exhaustion in

HCC tissues by restraining PD-1 degradation[57]. The complicated immunosuppressive microenvironment in HCC tissues severely impairs the efficacy of immunotherapy. Therefore, the mechanisms of $CD8^+$ T-cell exhaustion in HCC needs to be further elucidated.

CYTOTOXIC IMMUNE CELL-BASED IMMUNOTHERAPY OF HCC

The increased understanding of $CD8^+$ T cells and NK cells promotes the development of effective immunotherapy. These two immune cell populations follow many similar patterns and/or complementary patterns to eliminate cancer cells. Moreover, their activities are regulated by common immune checkpoints. Here, we discuss the application and outcomes of cytotoxic cell-based ICIs and ACT in HCC treatment.

Immune checkpoint inhibitors

ICIs have displayed impressive efficacy in treating a variety of cancers. An increasing number of studies are being conducted on novel immune checkpoints and their inhibitors. Additionally, studies on ICI-based immunotherapy for advanced HCC treatment are also increasing, with some showing encouraging therapeutic effects.

Anti-PD-1 antibody and anti-PDL1 antibodies

PD-1 is mainly expressed on $CD8^+$ T cells, whose binding with PD-L1/PD-L2 induces $CD8^+$ T-cell exhaustion[58]. T cells from HCC tissues express high levels of PD-1[59,60]. Interestingly, $PD\text{-}1^{high}$ B cells in HCC tissues suppressed $CD8^+$ T-cell anticancer immunity by secreting IL-10[61]. Moreover, $PD\text{-}1^+$ dendritic cells (DCs) in HCC tissues also suppressed $CD8^+$ T-cell anticancer immunity[62]. In addition, PD-1 ligands are associated with aggressiveness and recurrence of HCC[63,64]. Wu *et al.*[65] reported that PD-L1 on Kupffer cell blocks $CD8^+$ T-cell anti-HCC activity. Besides, hepatoma cell-expressed PD-L1 induces apoptosis of $CD8^+$ T cells and promotes HCC recurrence[66]. Additionally, PD-L1-expressing monocytes induce polarization of Th22 cells through PD-1 in HCC tissues[67]. PD-L1 on intratumoral hepatic stellate cells or peritumoral neutrophils also contributes to the impairment of T cell-mediated anti-HCC immunity[68,69]. These findings indicate that blockade of PD-1/PD-L might be promising immunotherapy for HCC. Nivolumab and pembrolizumab gained approval for treatment of advanced HCC based on encouraging results from phase I/II studies in advanced HCC patients with objective response rates of 17%-20%[70,71]. However, results from two phase III clinical studies did not reveal statistically significant improvement in survival benefit[72,73]. There are several ongoing trials with monoclonal antibodies against PD-1, such as nivolumab, pembrolizumab, tislelizumab, and camrelizumab, in HCC patients, either as monotherapy or in combination with other treatments. A phase-I/II study reported that the combination of atezolizumab and bevacizumab resulted in a 62% response rate in advanced HCC patients[74]. The breakthrough therapy of atezolizumab in combination with bevacizumab is recently approved as a first-line treatment for patients with advanced or metastatic HCC. Moreover, a randomized phase III study demonstrated superior overall survival and progression-free survival compared to sorafenib in the first-line treatment of advanced HCC[75]. In addition, another antiangiogenic drug ramucirumab has been approved as a second-line therapy for advanced HCC following first-line therapy with sorafenib[76]. The clinical efficacy from the combination of anti-PD-L1 with antiangiogenic agents encourages researchers to extensively develop novel combination strategies to improve the clinical efficacy of HCC treatment.

Anti-CTLA-4 antibody

CTLA-4 is expressed on Treg cells and activated T cells and inhibits T-cell activation by competing for CD80/CD86 with CD28[77]. Liu *et al.*[78] found that CTLA-4 polymorphism may have negative effects on HCC. $CTLA\text{-}4^+$ Treg cells impair T cell-mediated anti-HCC immunity[50]. HCC-derived Treg cells limit DCs function by CTLA-4[79]. Furthermore, $CTLA\text{-}4^+$ DCs suppress T cell-mediated anti-HCC immunity by IL-10 and IDO[80]. Fortunately, CTLA-4 blockade with glucocorticoid-induced tumor necrosis factor receptor family-related protein (GITR) engagement completely abrogates Treg-mediated immunosuppression in

HCC[81]. These findings indicate that CLTA-4 is a promising target for HCC treatment. Recently, the CTLA-4 blockade agent, ipilimumab, has been tested in clinical trials of HCC treatment, with a partial response rate of 18% and a disease control rate of 76%[82]. Duffy *et al.*'s[83] study demonstrated that tremelimumab could achieve a partial response rate of 26% and a disease control rate of 84%. Furthermore, the combination therapy of ipilimumab and nivolumab could achieve objective response rates of 31% and a median duration of 17.48 months in advanced HCC patients[84]. As with other cancers, the combination regimens of nivolumab and ipilimumab led to grade 3-4 treatment-related adverse events occurring in 37.7% patients, which were more frequently observed with combination regimens than with single-agents, especially in patients who received higher dosages of ipilimumab. However, most of the adverse events were manageable with timely recognition, steroid treatment, and discontinuation of immunotherapy, with a very low rate of liver failure[73,85,86].

Other immune checkpoint inhibitors

The binding of inhibitory killer-immunoglobulin-like receptors (KIRs) expressed on NK cells with HLA class I molecules inhibit the activation of NK cells[87]. Antibodies against inhibitory KIRs enhance NK cell cytotoxicity. It was reported that KIR/HLA immunogenetic background influenced the evolution of HCC[88,89]. Anti-KIR antibodies - IPH2101 and IPH2102 - were well tolerated in patients with relapsed multiple myeloma[90,91]. However, little evidence emerged to confirm the efficacy of these anti-KIR antibodies against HCC. NKG2A is an inhibitor receptor expressed on both $CD8^+$ T cells and NK cells[92,93]. Therefore, anti-NKG2A mAb promoted anticancer immunity of both $CD8^+$ T and NK cells[94,95]. It has been shown that NKG2A mediated NK-cell exhaustion in patients with HCC[22]. However, the effect of anti-NKG2A mAb on HCC needs to be confirmed in clinical trials. Increasing inhibitory receptors such as IL-1R8, TIM3, TIGIT, and CD96 have been found to be important for regulating NK cell activity against tumors[96-100]. Clinical trials have been performed to evaluate the efficiency of antibodies against these checkpoints for cancer therapy. The efficacy of NK cell-based checkpoint inhibitors in HCC needs further preclinical and clinical studies.

Combination therapy with immune checkpoint inhibitors

Immune checkpoint blockade leads to recovery of immune response against HCC cells and suppression of tumor growth in HCC. However, most HCC patients still do not achieve clinical benefit from ICI immunotherapy, highlighting the need for creative strategies to improve therapeutic efficacy. First, novel checkpoints need to be identified in HCC. For example, B and T cell lymphocyte attenuator has been found to participate in suppressing $CD4^+$ T cell function in HCC[101]. Siglec-15 has been confirmed to be an immune suppressor and displays promising efficacy in cancer immunotherapy[102]. The roles of novel checkpoints in HCC have not been addressed. Second, novel combinations of checkpoints need to be designed. Zhou *et al.*[42] found that T cells isolated from HCC tissue expressed high levels of PD-1, CTLA4, TIM3, and LAG3, suggesting the involvement of multiple checkpoints in T-cell exhaustion in HCC. The efficacy achieved by combining blockade of checkpoints was better than that by single checkpoint alone[42]. Therefore, a combination of ICIs might achieve better results than just monotherapy for HCC treatment. Moreover, the individualized combination for HCC patients can be designed based on omics-data to achieve precision medicine. Third, novel comprehensive combination needs to be tested. Wehrenberg-Klee *et al.*[103] reported that combining radioembolization with nivolumab could enhance the ICI-induced anticancer immune response. Shigeta *et al.*[104] found that dual anti-PD-1/VEGFR-2 therapy enhanced $CD8^+$ cytotoxic T cell anticancer immune response in HCC. PD-1/PD-L1 double blockade increased anticancer immune response of vaccine-induced $CD8^+$ T cells in advanced HCC patients[105]. To improve the benefits of ICI, it is necessary to integrate ICI therapy with targeted agents, locoregional therapy, vaccines, or other forms of therapy. Of note, the clinical outcomes of such integration require further investigation in future studies.

Adoptive cell transfer

NK cells and $CD8^+$ T cells eliminate cancer cells by direct cytotoxicity. In advanced HCC, the scarcity of NK cells and $CD8^+$ T cells in HCC tissues eliminates the ICI-induced anticancer efficacy. In this setting, it is absolutely necessary to adaptively transfer cytotoxic immune cells into patients with advanced HCC.

NK cell therapy

NK cells have potent anticancer capacity. HLA class I molecule-independent activation endows NK cells with more potential for extensive applications. HLA class I molecules block the NK cell killing though interaction with KIRs or CD94/NKG2A/B on NK cells[106]. Meanwhile, stress-induced ligands on cancer cells can activate NK cells by interacting with activation receptors on them[107]. However, NK cell function is impaired and hardly restored in advanced cancers. Hence, adoptive transfer of NK cells is a valuable option for cancer therapy. However, adoptive transfer of autologous lymphokine-activated NK cells with IL-2 into patients with metastatic cancer led to a poor clinical outcome[108], which might be attributed to high levels of HLA class I molecules on cancer cells and the exhausted function of patients' NK cells. To overcome these defects, allogeneic NK cells - especially allogeneic haploidentical NK cells - are harnessed to treat various malignancies[109]. Encouraging clinical efficacy has been observed in trials of acute myeloid leukemia[110]. Moreover, cryoablation combined with allogeneic NK cell therapy markedly improved the progression-free survival of patients with advanced HCC[111]. Besides autologous and allogeneic NK cells, NK-92 cells, an NK cell line, is also used in clinical trials of cancer therapy, with encouraging results observed in patients with advanced lung cancer[112]. To enhance the targetability of NK cells, Chimeric antigen receptor (CAR)-NK cells have also been developed and pre-clinically evaluated. NK-92 cells with CD19-CAR display potent ability to kill $CD19^+$ leukemia cell lines and lymphoblasts from patients with leukemia[113]. NK-92 cells with GPC3-CAR show significant *in vitro* cytotoxicity to $GPC3^+$ HCC cells and potent anticancer activity in HCC xenografts[114]. Accumulating evidence indicates that NK cell therapy is a potential approach for HCC treatment with technical improvements in the activation and expansion of NK cells.

Cytokine-induced killer cell adjuvant therapy

Cytokine-induced killer (CIK) cells generated from blood mononuclear cells cultured with IFN-γ, anti-CD3, and IL-2 show potent anticancer activity[115]. Jia *et al.*[116] reported that CIK cells improved overall survival in HCC. CIK cell adjuvant therapy also reduces the recurrence in HCC patients undergoing curative treatment[117]. Lee *et al.*[118] found that the efficacy of CIK cells in patients with HCC lasted over 5 years. Chang *et al.*[119] reported that the high number of $PD\text{-}1^+$ tumor infiltrating lymphocytes could predict the response and clinical benefits of CIK cell adjuvant immunotherapy in HCC patients. Pan *et al.*[120] reported that CIK cell cytotoxicity is a predictive biomarker for adjuvant CIK cell immunotherapy of HCC patients after surgery. Collectively, increasing evidence suggests that CIK cell-based adjuvant immunotherapy shows modest efficacy in early-stage HCC. Although Wang *et al.*[121] showed that intraperitoneal perfusion of CIK cells with local hyperthermia was safe for patients with advanced HCC, more clinical data on the efficacy of CIK cell therapy in advanced HCC is currently lacking. Further detailed studies on the characteristics of CIK cells and their recognition and effector function are required to improve the clinical outcomes of CIK cell adjuvant immunotherapy in HCC.

CAR-redirected T cell therapy

CAR-T cells have shown tremendous clinical efficacy in the therapy of hematological malignancies[122]. Moreover, CAR-T cell therapy is expected to convert cold tumors into hot tumors, which represents a promising immunotherapeutic option for HCC treatment. Glypican-3 (GPC3) is a membrane heparan sulfate and is highly expressed in HCC tissues[123,124]. Unfortunately, the GPC3-targeted antibody - GC33 - was unsuccessful in bringing about clinical benefit to patients with HCC[125]. However, the anti-GPC3/anti-CD3 bispecific antibody - ERY974 - could activate T cells and convert the microenvironment of a cold tumor to that of a hot one[126]. Therefore, GPC3 is a promising target of CAR-T cells in HCC. Indeed, GPC3-CAR-T cells could eliminate $GPC3^+$ HCC cells and tumors in a patient-derived xenograft model[127]. GPC3-CAR-T therapies have been registered for clinical trials. To overcome T-cell exhaustion induced by checkpoints, an enhanced version of CAR-T cells is being currently designed. For instance, PD-1 is disrupted via CRISPR/Cas9 to enhance the activity of GPC3-CAR T cells against HCC[128]. A soluble PD-1-CH3 fusion protein is expressed to increase anticancer activities of GPC3-CAR-T cells[129]. Co-expressing GPC3-CAR and co-

stimulatory molecule ICOSL-41BB promotes CAR-T cell proliferation and tumor rejection[130]. Besides GPC3, MUC-1, EpCAM, AFP, and CEA might be potential targets of CAR T cells for HCC treatment, which have been registered for clinical trials on the applicability of CAR-T therapy as a treatment strategy for HCC[131]. Moreover, these classical tumor-associated antigens and ligands for receptors expressed on T cells also act as the targets for HCC recognition. For instance, NKG2D-based CAR-T cells could potently eliminate NKG2DL$^+$ HCC cells[132]. A CD147-targeted inducible CAR-T cell system has been developed for HCC treatment[133]. Although clinical trials of CAR-T therapy against HCC have not been completed, CAR-T therapy might provide effective therapeutic modalities for HCC treatment. Nonetheless, to date, the therapeutic efficacy of CAR-T cells remains limited owing to the lack of cancer-specific targets, weak expansion, poor infiltration, and induced exhaustion of CAR-T cells. Hence, smarter optimization strategies and more clinical trials are required for the confirmation and improvement of clinical outcomes of CAR-T cells in HCC treatment.

T cell receptor-genetically engineered T cell therapy

The success of T cell receptor-genetically engineered T (TCR-T) cells in melanoma treatment has encouraged the use of TCR-T cells in HCC treatment. Autologous T cells forced to express an HBV-specific TCR recognized HBsAg$^+$ HCC cells and decreased HBsAg levels in a patient who underwent liver transplant[134]. T cells genetically engineered with HCV NS3:1406-1415-reactive TCR recognized the naturally processed antigen and led to suppression of HCV$^+$ HCC *in vivo*[135]. T cells genetically engineered with AFP-specific TCR specifically recognized and killed AFP$^+$ HepG2 cells, both *in vitro* and *in vivo*[136]. Although there remain many challenges such as off-target cross-reactivity and low TCR affinity that need to be overcome before successful translation into clinical practice[137,138], increasing findings suggest that TCR-T therapy might be an attractive alternative immunotherapeutic modality for HCC treatment.

DC-vaccines adjuvant immunotherapy

Briefly, DCs are professional antigen-presenting cells with the capacity to prime antigen-specific T-cell immunity. DC vaccines are recognized as promising agents for activating T cells to eliminate cancer cells; their role and functions have been evaluated in some malignancies in clinical trials, including HCC. A phase II study using intravenous vaccination with DCs pulsed with HepG2 lysate was found to be safe and showed evidence of anticancer efficacy in some patients with advanced HCC[139]. Another phase I/II study reported that vaccination with DCs pulsed with AFP peptides induced strong T-cell immunity against AFP but no clinical responses in HCC patients[140]. Other phase I/IIa studies also reported that subcutaneous vaccination with DC pulsed with multiple antigens such as AFP, glypican-3 (GPC-3), and melanoma-associated antigen 1 (MAGE-1) enhanced anticancer immunity and prolonged time-to-recurrence and recurrence-free survival in HCC patients[141-143]. Interestingly, Lu *et al.*[144] showed that exosomes derived from AFP-expressing DCs elicited potent anticancer immune responses and cancer regression in HCC mice, thus providing a novel option for vaccine-based immunotherapy of HCC. Pang *et al.*[145] reported that DCs fused with cancer stem cells could efficiently stimulate T lymphocytes to generate specific CD8 T cells against cancer stem cells. Collectively, several studies indicate that DC vaccine-based adjuvant therapy enhances anticancer immunity and improves the survival of patients with HCC. Nonetheless, further improvements such as specific immunogenic neoantigens for HCC, safe and feasible DC source, potent adjuvant, and access to vaccination are required for future success of DC-based HCC immunotherapy.

Combination therapy of immune checkpoint blockade and adoptive cell transfer

The existence of cancer immunosuppressive microenvironment limits the effector function of adoptive immune cells. Therefore, it is reasonable to improve the cancer immunosuppressive microenvironment to enhance the curative efficacy of adoptive immune cells on HCC. Kodumudi *et al.*[146] reported that adoptive transfer of tumor infiltrating lymphocytes from tumors with anti-PD-L1 antibody treatment led to a significant delay in tumor growth, suggesting that pretreatment with immune checkpoint blockade could be

an effective strategy to improve tumor infiltrating lymphocyte infiltration and function. Moreover, CAR-T cell therapy in combination with PD-1 blockade overcomes PD-L1-mediated cancer immunosuppression and leads to enhanced therapeutic efficacy[147]. The combination of CTLA-4 blockade with ACT also generates greater therapeutic efficacy[148]. Besides systemic delivery of checkpoint blockade, the knockout of immune checkpoint in CAR-T cells by gene-editing technologies improves anti-tumor efficacy of CAR-T cells in various cancer models by enhancing effector function and survival of T-cells[149]. It is understood that the immunosuppressive trait of the HCC microenvironment requires combinational therapeutic modalities for effective outcome[150]. These findings support combination immunotherapy with immune checkpoint blockade and ACT for cancers, which is expected to result in greater anticancer immune response than with either intervention alone.

CONCLUSION

Following significant therapeutic progress made on the basis of basic research, immunological studies offer a new era of clinical application. Immunotherapy brings a new hope to depressed patients with chronic infection, autoimmune disease, or cancers. More importantly, cytotoxic immune cell-based immunotherapy has markedly improved survival in patients with advanced cancers. The high mortality of patients with advanced HCC owing to resistance to chemotherapy highlights the importance and value of immunotherapy in HCC treatment, although the clinical efficiency has not been as promising as expected. The development of novel ICIs, cytokines, tumor-specific antigens, gene-modified/CAR NK cells, and TCR/CAR $CD8^+$ T cells is expected to improve the curative effect. Furthermore, the flexible combination of immunotherapy and other therapies might offer the much required breakthrough in clinical efficacy of HCC treatment.

DECLARATIONS

Authors' contributions

Contributed to conception and design of the study and manuscript writing: Li J, Tao L, Wang X
Final approval of manuscripts: Li J, Tao L, Wang X

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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