Immunotherapy for Hepatocellular Carcinoma and Its New Development

Heping Li1, Idiko Gyory2, Xiaoli Zhang1, Mingyan Xu1 and Shiqiu Xiong4*

1Department of Medical Oncology, First affiliated hospital, Sun Yat-sen University, Guangzhou, China
2Department of Molecular and Cell Biology, University of Leicester, UK
3HaploX Biotechnology Co. Ltd., Shenzhen, China
4LGC Limited, Fordham, Cambridgeshire, CB75WW, UK

Abstract

Hepatocellular Carcinoma (HCC) is the most common primary liver cancer with high mortality. Because of the limitation on the conventional therapeutic options and immunological characters of this disease, variable immunotherapy strategies for HCC have been designed and investigated. A few of them look promising. In this review, we comprehensively summarized recent and novel immunotherapies for HCC, with a particular focus on clinical trials on the basis of their physiological rationales for these strategies. Insights into these clinical progress and their ideologies are essential to improve efficacy and clinical outcome of these immunotherapies.

Keywords: Hepatocellular carcinoma; Immunotherapy; liver tolerance; Clinical trial

Core Tip

This review article aims to connect the physiological characters of liver immunity with the rationales of HCC immunotherapies, provide the updated information about recent clinical progress, and summarize the advantage and disadvantage of each regimen, new vision on the potential research future of immunotherapies.

Introduction

Hepatocellular Carcinoma (HCC) is the most common histologic type of primary liver cancer, accounting for between 85% and 90% of liver cancers. HCC is one of the cancers with highest morbidity worldwide. There are about 750,000 new cases every year, accounting for 5.7% of all new cancer cases, 85% of these new cases reported in sub-Saharan Africa and East Asia. This geographic distribution of HCC overlaps with that of chronic viral hepatitis, according to epidemiological data [1]. It is widely accepted that HCC is developed from viral hepatitis B and C, and subsequent stage liver cirrhosis. The other less frequent causes are chronic exposure to toxins or hereditary liver diseases, such as non-alcoholic fatty liver disease [2].

HCC is the fifth most common malignant tumour, but in mortality it is ranked as third world-wide. Although there are great advances in medical diagnosis technology and surveillance programs for high-risk populations, only 20% of HCC patient can be diagnosed in phase I, and only these patients are amenable to curative therapy (liver transplantation, surgical resection or ablative therapies); most HCC patients with liver dysfunction, are not feasible to these aggressive treatments. Therefore, the only reliable therapeutic option is Sorafenib which yielded a median overall survival of 6.5-10.7 months [3].

There is a growing evidence to suggest that HCC may be considered an immunogenic tumour, arising from chronic inflammation in an immunosuppressive environment. The strong association between spontaneous regression of this disease and systemic inflammatory response sparked greater interest in the immunological pathogenesis in HCC [5]. Further reports on particular types of immunological cells, which associated with favourable prognosis of HCC, introduced novel immunotherapy concepts to the medical management of patients with HCC [6]. The characters of immunotherapy elicit non-toxic, systemic, memorial and specific anti-tumour activity makes it particularly promising in the setting of HCC.

The immunosuppressive microenvironment is an important factor that contributes to tumour initiation, progression and their therapeutic resistance. Immunological cells and molecules are fundamental components of the tumour microenvironment. All biological aspects of HCC are shaped by the immune system.

The rationale of immunotherapy for tumours is to counteract the immunosuppressive mechanisms and/or amplify tumour-specific and memory immunity, which is expected to improve clinical outcomes profoundly by causing long-lasting regression and preventing relapse in cancer patients [7].

Although HCC is assumed as a good disease model for immunotherapy, most current clinical trials on this disease achieved only marginally positive response, which attribute to the inherently tolerogenic character of the liver in both healthy and diseased states [8]. Understanding the characteristics of the immune system in HCC setting is crucial to improve future immunotherapy strategies.

Because of the vast array and constant flux of blood-derived antigens and bacterial molecules from the intestinal microbiota, human liver has evolved to respond with a plethora of immune-regulatory mechanisms to exhibit a distinctive form of immune privilege or tolerance, best exemplified by orthotopic liver transplantation resulting in systemic, donor-specific T-cell tolerance [9].

*Corresponding author: Shiqiu Xiong, Senior Investigator, LGC Ltd, Molecular Biology, Fordham, Cambridgeshire, CB75WW.UK, United Kingdom, Tel: 00447749965484; E-mail: xiongshiqiu2008@yahoo.com

Received January 18, 2019; Accepted February 11, 2019; Published February 18, 2019


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The liver's pathway to immune tolerance is multifaceted. Structurally, the liver consists of repetitive hepatic lobules that are perfused by sinusoidal vessels. Hepatocytes are shielded from direct interaction with the bloodstream by densely populated non-parenchymal liver cells: including Liver Sinusoidal Endothelial Cells (LSECs); stellate cells; dendritic cells; Kupffer Cells (KC); natural killer cells; natural killer T cells and others. These non-parenchymal cells serve as a protective barrier between the hepatocytes and pathogens [10].

To protect the liver from autoimmune damage that could result from the continuous stimulation by intestinal antigens, effector T cells in the liver are kept in an induced state of tolerance; Kupffer Cells (KC) constitutively release inhibitory cytokines, such as Interleukin 10 (IL-10) and Transforming Growth Factor beta (TGF-β); hepatocytes, Hepatic Stellate Cells (HSC), KC, LSEC and intrahepatic leukocytes (LC) upregulate immune checkpoints, involving programmed death-ligand 1 or 2 (PD-L1 or PD-L2); regulatory T cells (Treg) highly express Cytotoxic T-lymphocyte Associated Protein 4 (CTLA-4). All these factors directly or indirectly suppress the immune effector cells CTL and NK (Natural Killer) cells [11]. The liver-specific physiological immune tolerance is beneficial in case of harmless antigens, however, it is potentially detrimental under hepatitis settings, and the chronic inflammation amplifies this immune suppression. Without proper immune surveillance, the initial lesions of HCC, which are induced by viral and immunologic factors, escape immune clearance and develop into solid tumours.

The summarized main components of immunological aetiology in HCC are: immunosuppressive tumour environment; negative regulation of PD-1/PD-L1 pathway; failure of Tumour Associate Antigen (TAA) processing and presentation; suppression of CD4+ and CD8+ T cells by Treg; insufficient help of CD4+ T cells; direct suppression on effector cells, such as CTL, NK, NKT. Hence, the rationale of immunotherapy in HCC is to interfere these immunosuppressive pathways and recover their immune potency (Figure 1).

Antigen-presenting cells (APC, such as DC, Kuffers, macrophage, B cells etc.) capture tumour antigens, process and present them, along with cytokines (including IL-12, IL-15 etc.), to effector CTL cells, to help them maturation and activation. Meanwhile, these APC also convey CTLA-4, PD-1 mediated inhibitory signals to balance CTL activation; activated CTL cells kill target tumour cells directly, or secret cytokine to amplify the anti-tumour response; APC also help NK cells maturation and function by secreted IL-12, IL-15; activated NK cells kill target tumour cells in a similar way of CTL.

On the basis of this rationale, HCC immune therapies were classified into 4 main regimens:

a. Blockade of T cells response checkpoint molecules PD-1and CTLA-4;

b. To perform the adoptive transfer with anti-tumour effector immune cells, such as CAR-T, CTL and NK [12];

c. To generally counteract immunosuppressive mechanisms by administrating cytokines, such as IFN, IL-12, IL-15 etc.;

d. Boosting antigen presentation by tumour antigen-pulsed DC cells;

We are trying to summarize the promising and recent immunotherapy for HCC respectively, mainly focus on clinical trials. Limited to our knowledge, we sincerely apologize to those colleagues whose important works have been missed in this report.

Immune checkpoint blockade

The field of cancer immunotherapy is undergoing a renaissance due to a deeper understanding of the immune system and immuno-surveillance. The balance between co-stimulatory signals and immune checkpoints determines T cell activation and the overall intensity of the immune response. Whenever CD80/CD86 from APC cells recognized by CD28 on effector T cells, they deliver co-stimulatory signals to synergize first activation signalling from TCR recognizing antigen/MHC (Major Histocompatibility Complex) complex; meanwhile, to control the intensity and remission of immune response tightly, T cells also present inhibitory receptors, such as CTLA-4 and PD-1. CTLA-4 compete CD28 binding to CD80/CD86 and thereby inhibits T-cell activation [13]. PD-1 receptors are expressed on several immune cells, including effector T and NK cells, which associate with PD-L1 from APC or tumour cells. Engaged PD-1 receptors deliver pro-apoptotic signalling to effector immune cells. After astonishing success of clinical trials on CTLA-4 and PD-L1 blockade to treat melanoma patients [14-16], similar positive results are highly anticipated in HCC patients [17].

In a recent pilot clinical trial, 37 patients with HCC or HCV infection were injected with 15 mg/kg of the CTLA-4-blocking antibody tremelimumab for 90 days, to test the anti-tumour and antiviral effect of this antibody. In addition to demonstrating the safety of such approach in patients, a 17.6% partial response rate and a 76.4% disease control rate was achieved. A significant drop in viral load was also observed [18]. Another phase I clinical trial of tremelimumab with percutaneous Radiofrequency Ablation (RFA) or trans-arterial therapy is ongoing (NCT01853618) [19].

The anti-PD-1 antibody based therapy is even more promising than a CTLA-4 blockade. Nivolumab was the first immune drug approved as a second-line treatment for patients with HCC by FDA in September 2017. In the CheckMate040 study, 48 patients with HCC had received nivolumab 0.1-10 mg/kg intravenously for 2 years. The Objective Remission Rate (ORR) was 15%; median Overall Survival (OS) was 15.1 months and the 9 months OS rate was 67% [20]. In a dose-expansion study of nivolumab, 174 patients with HCC received nivolumab 3 mg/kg intravenously. The ORR was 20% and tumor burden was reduced In 68 patients (39%) [21]. Besides, in 80 HCC patients treated with nivolumab only, the ORR was 23%; Disease Control Rate (DCR) was 63%; and 40% of patients had stable disease over than 6 months. However, in the group of sorafenib-experienced patients that received nivolumab, 91% (166/182) of patients progressed on sorafenib treatment. The ORR was 16%-19% [22]. Most recently, the
efficacy and safety of anti-PD1 therapy of advanced HCC was evaluated on 11 cases, no related adverse effects were noted; the disease control rate reached 81.8% comparing to an objective response of 63.6%, this clinical trial proved promising application of PD-1 in HCC [23]. There are more clinical trials on other immune checkpoint blockade (Figure 2). Greten summarised clinical data of the therapies based on PD-1/PD-L1 blockade under study for the treatment of hepatocellular carcinoma [24].

Indeed, the combination of PD-1 and CTLA-4 blockade resulted in an objective response with limited toxicity in 40% of the patients [25]. In a recent clinical trial, 40 HCC patients received combination treatment with the anti-PD-L1 antibody durvalumab and the anti-CTLA-4 antibody tremelimumab to evaluate the efficacy. The ORR was 17.5, DCR at 16 weeks was 57.5%. In the 20 HCC patients without HCV infection, DCR at 16 weeks was 70% [26,27].

In immune checkpoint therapy, effective biomarkers could be used to assess efficacy and predict prognosis to predict survival benefits of patients with HCC. In the tumour microenvironment of HCC, PD-L1 is overexpressed, and this associates with CD8+ T cell immune infiltration, DCR at 16 weeks was 70.5%. In the 20 HCC patients without HCV infection, DCR at 16 weeks was 70% [26,27].

The evaluation of efficacy and potency of anti-checkpoint inhibitors in HCC is still in phase I/II trial. Large randomized phase III trials need to be designed and conducted to confirm the promising efficacy.

Given these evolutionary progress in tumour therapy, recent nobel prize rewarding the pioneers in this field is expected, which in turn will heat up these immunotherapy works. However, the resiliency and complexity of immune system and tumor cells may disappoint most scientists in the near future. Combination these agents with other immune regulators or complimentary drugs will shed light on the future in this field.

Antigen Presenting Cells (APC) capture tumour antigens, process and present them to T cells by recognizing TCR, which induced first activation signal. To be fully activated, T cells need co-stimulating signal mediated by CD28 binding with CD80/CD86. CTLA-4 competes CD28 binding to CD80/CD86 and thereby regulate intensity and remission of T-cell activation delicately. Activated T cells recognize and kill target tumour cells by TCR binding peptide/MHC specifically, this process is attenuated by PD-1/PDL-1 signalling. Specific Abs against CTLA-4 or PD-1 can terminate negative signals for T cells, therefore augment T cells mediated immunity.

Adaptive cell transfer

These immune cells are the immune effectors counteracting the tumour cell-mediated negative effects directly, the above-mentioned therapeutic approaches are all indirect and mediated by these ultimate cell types. The suppression of immune effector cells in the liver under physiologic and pathogenic conditions is well-documented. Both regulatory T cells [35-37] and γδ+ T cells [38,39] have been shown to mediate this suppression recently. Adoptive transfer of activated effector cells in vitro may hurdle this problem and trigger host immune response against tumour cells directly.

CAR-T

Chimeric Antigen Receptor T-Cell Immunotherapy (CAR-T) is the new and promising cell immunotherapy by combination of engineering T cells and Chimeric Antigen Receptor (CAR) which redirects T cell specificity and function [40]. CAR is composed of an extracellular antigen recognition, an extracellular spacer/hinge region, a trans-membrane domain, and an intracellular signaling domain. And the extracellular antigen recognition can identify TAA which are not restricted via HLA. Thus, CAR-T can overcome HLA-mediated tumor escape mechanisms [41]. CAR-T immunotherapy has been a great success in haematologic malignancies [42]. But the application to HCC is still in research stage. Glypican-3 (GPC3) is a potential target for CAR-T immunotherapy against HCC. GPC3 is a cell surface oncofetal proteoglycan, which was overexpressed in 75% of HCC [43]. Previous studies showed that anti-GPC3-CAR T cells could against HCC cell lines in vivo [44,45], and it efficiently suppressed tumor growth in the tumor cell in which GPC3 proteins were low expressed [43]. CAR-T immunotherapy are under way. In a recent clinical trial, 13 patients with refractory or relapsed HCC treated with GPC3 CAR-T. All 13 patients tolerated the treatment well. In the five patients who could evaluate the efficacy, 1 was partial response, 2 were stable disease and 2 were progressive disease. And 4 of them are still alive; the median OS was longer than 14 months [47].

The lack of specific antigens in HCC causes the CAR-T cells attack the normal tissues which express the same antigens. It leads to severe toxicity [48]. Dual-targeted CAR-T cells have been proposed as a potential way to reduce on-target, off-tumor toxicity. Chen et al. developed dual-targeted CAR-T cells, which co-express GPC3 and
Asialoglycoprotein Receptor 1 (ASGR1). They found that the dual-targeted CAR-T cells might reduce the risk of on-target, off-tumor toxicity while maintaining relatively potent anti-tumor activities on GP83+ASGR1+ HCC [49]. More clinical trials are required as CAR-T based immunotherapy is a new strategy for HCC treatment.

CTL

Adoptive immunotherapy of HCC is often hampered by the low frequency of tumour-specific T cells, therefore screening and choosing proper TAAAs to boost T cells. in vitro is critical to the success of CTL-based strategies [50]. Hiroshi and colleagues identified 3 HCC TAAAs, namely, glypican-3, NY-ESO-1, and Mage-A1 in their screen. They measured the magnitude of TAAA-specific CD8+ T-cell responses against these antigens in 20 HCC patients treated by radiofrequency ablation or Trans-catheter Chemo-embolization (TACE), and found that these responses represented the only significant prognostic factor to indicate a prolonged tumor-free Interval [51]. The responses to these antigens were compared and described in detail in subsequent publications [52]. These investigations highlighted the importance of CTL in HCC immunotherapy. Combining suitinib, a receptor tyrosine kinase inhibitor, with adoptive transfer of tumour antigen-specific CD8+ T cells led to the elimination of established tumours without recurrence in an orthotopic murine tumour model, highlighting the potential of a synergistic chemo-immunotherapeutic approach to HCC [53]. Qasim W and colleagues designed an experiment on one patient who had undergone liver transplantation for HBV-related HCC. Genetically modified autologous T cells that express an HBsAg-specific T cell receptor was introduced to this patient, with the rationale of HBV-DNA integration frequently occurring in HBV-related hepatocellular carcinoma. These T cells have been shown to mediate a reduction in HBsAg levels without causing an exacerbation of liver inflammation or other toxicity. This work marked the path to a novel adoptive immunotherapy in hepatitis B-associated HCC [54].

NK

Apart from CTL, NK cells are the main effector immune cells, which can eliminate tumours or virus infected cells without specific antigen priming. NK cell activation depends on the signals from a repertoire of activating and inhibitory receptors, the integration of these distinct signals dictates the quality and the intensity of the NK cell response [55]. 25%-40% of liver lymphocytes are NK cells, this proportion is significantly higher than their 5% cellular abundance in peripheral blood [56]. This enrichment of liver NK cells indicates their potential clinical applications.

The therapeutic potential of NK cells has been most extensively explored in haematological malignancies, because NK cells' killing requires one-to-one target engagement and site-directed release of lytic granules. Nevertheless, an increasing number of studies already indicated that NK cell based immunotherapy is a promising therapeutic approach for HCC. Tsuchiyama and colleagues have shown that HSV thymidine kinase and MCP-1 transduced HCC cells can be eliminated by NK cells in a mouse model [57]. In a study of 37 HCC patients undergoing radiofrequency thermal ablation, their peripheral blood NK cells showed a more differentiated phenotype profile with a general increase of pro-activatory functional activities [58]. These may come from their ability to precisely kill cancer stem cells, antibody coated cells, and stressed cells specifically, which makes them appealing therapeutic effectors for all cancer forms, including metastases. Meanwhile activated NK cells produce pro-inflammatory cytokines, which can reverse the anti-inflammatory tumour microenvironment and augment adaptive immune responses [59,60].

In vivo and in vitro studies have been shown the involvement of TLR3 to contribute to the efficacy of NK cell mediated eradication of HCC, although the presence of ligands for this innate receptor in tumour cells has not been demonstrated [61]. Another report, which showed apoptosis of stressed liver cells by TNF-related Apoptosis-inducing Ligand (TRAIL) that was induced by activated NK cells. This interaction is mediated by the receptor DR5 which is expressed on these target cells at higher levels [62]. Apart from DR5, UL16-binding Protein (ULBP) expression level, which was regulated by proteasome, correlated well with HCC progression and early recurrence, suggested that signalling from NKG2D (ULBP1 receptor) in NK cells played a crucial role in immune response to HCC [63]. Another NKG2D ligand, MICA was proved to be a key molecule in tumour immune surveillance in the setting of HCC [64]. Intriguingly, a novel B7 family member B7-H6, the ligands of another NK activating receptor NKP30, proved to express highly on hepatocellular carcinoma samples [65]. IL-37 is a member of the interleukin 1 cytokine family. A new report implied that their expression level associated with the prognosis of HCC patients via stimulating the migration of the activated memory CD57+ NK cells [66].

In a recent clinical trial of 62 HCC patients, Injection of IL-2 and CD16 antibody induced NK cells helped to prevent the recurrence of HCC after radiofrequency ablation treatment [67]. Human allogeneic suicide gene-modified killer cells (aSGMKCs) exhibit a high, rapid, interleukin-2–dependent, and non–major histocompatibility complex class I-restricted in vivo and in vitro cytotoxicity to HCC. This, mainly NK and NK-like T cell mediated strategy opened a new perspective for immunotherapy of HCC [68]. Another promising NK cell mediated immunotherapy is the combination of adoptive transfer of NK cells with pharmacological STAT3 inhibitors as shown by two new reports demonstrating synergistic anti-tumour effects [69,70].

Moreover, two ongoing clinical trials are designed to assess NK cell therapy combined with liver resection (NCT02008929) or liver transplantation for HCC (NCT01147380).

Cytokine-induced killer cells

Cytokine-induced Killer (CIK) cells are mixed T and NK cell, which generated from human Peripheral Blood Mononuclear Cells (PBMC) activated with IFN-gamma, anti-CD3 antibody and IL-2. In murine tumour models, these CIK cells demonstrated significant antitumor effects against a number of hematopoietic and solid tumours, including HCC. The safety and antitumor effect of CIK cell-based therapy for patients also has been proved, it induced minimal toxicity and no graft-vs-host disease [71, 72]. In a recent multi-centre, randomized, open-label, phase III trial, IL-2 and CD3 antibody-induced killer (CIK) cells were injected into 230 HCC patients after conventional treatment. This immunotherapy resulted in increased recurrence-free (44.0 months vs 30.0 months, p=0.01) and overall survival [73]. Recent meta-analysis showed that immunotherapy based on CIK cells induces a significant increase in both Overall Survival (OS) and Progression-free Survival (PFS) of HCC patients [74,75]. PD-L1 expression could be as a predictive biomarker for CIK cell immunotherapy in patients with HCC. Previous studies showed that patients who treated with surgery and CIK with 5% PD-L1 expression had better OS and Recurrence-free Survival (RFS) than patients with <5% PD-L1 expression. However, PD-L1 expression did not show a direct impact on the survival of patients in the surgery alone group. High number of PD-1 positive
intra-tumoral lymphocytes also could predict survival benefit of CIK cells for HCC patients [76].

Because of the small HCC patient numbers in above clinical trials, further validation of the efficacy of CIK immunotherapy for HCC is needed in the near future, by increasing the number of evaluating HCC patients.

**Interferon**

Interferons (IFN) are cytokines, named after their ability to “interfere” with viral replication and thereby protecting cells from viral infections. They are widely applied in clinical anti-viral therapy, including HBV and HCV infections. It is proven that IFNs also have various immune regulatory functions, such as activation of natural killer cells and macrophages, up-regulation of antigen presentation by increasing the expression of Major Histocompatibility Complex (MHC) [77].

In a clinical trial enrolled a large cohort of post-operative HCC patients (236 patients in total), IFN-α treatment had been applied in long-term, randomized and observation-controlled settings. In these patients, Sun and his colleagues reported significant beneficial effect of IFN-α treatment, represented as improved overall survival (63.8 vs. 38.8, p=0.0003) and longer cancer-free survival period (31.2 vs. 17.7 months) [78]. However, other clinical trials did not confirm this significant improvement [79-83]. Most of these clinical trials concluded with just a marginal beneficial effect of IFN-α or a combination of IFN-α and IFN2β on overall survival and/or partial response; at the expense of severe side effects such as flu-like symptoms (chills, fever, headache, myalgia, malaise), myelosuppression, depression, and hepatotoxicity.

Interestingly, a recent clinical trial applying PEGIFN α-2β/ribavirin (RBV) to a cohort of 54 randomized patients with HCV-associated cirrhosis showed very promising outcome. The 3 year overall survival rate in the experimental group was 90.2% contrast to 61.2% in the control group [84]. Because systemic immune-stimulation by IFN is usually bad tolerated, cytokine treatment in combination with other antiviral drugs or immunotherapy could be a future direction.

**IL-12**

The other cytokine, besides interferones, which has been clinically investigated is IL-12. Produced by DCs and macrophages, this cytokine activates immune effector cells, such as CTLs and NKs. To avoid the severe side effects that were previously reported in mouse models, Sangro B and his colleagues injected an adenoviral vector encoding IL-12 directly into gastrointestinal carcinomas, including primary liver tumours. This vector introduced the exogenous IL-12 gene directly to the autologous DC cells of the patients. Adenoviral vector delivered IL-12 alone exerts only mild antitumor effects; this approach elicited only partial response in only 1 patient out of 9 [85,86]. Kayashima H and colleagues achieved more promising results using combined IL-12 gene and dendritic cell therapy in a mouse model, however, this combination is yet to be introduced to patients [87]. Although it’s direct boost immune effector cells, the strong side effect of IL-12 limited its application in HCC.

**IL-15**

IL-15 is a cytokine produced by monocytes, macrophages and DCs. IL-15 is firstly recognized by IL-15Ra and upon association of the ligand with this receptor subunit, it is trans-presented to the cells expressing the β and γ subunits of the IL-15R. IL-15 regulates development, proliferation, maintenance and activation of T and NK cells [88]. IL-15 is widely anticipated to be the most promising therapeutic cytokine in HCC on the basis of its safety [89]. In rhesus macaques, sub-optimal IL-15 administration was associated with increased numbers of circulating NK and CD8+ central and effector-memory T cells [90]. In a recent clinical trial, IL-15 has been safely administered to patients with metastatic malignancy. This cytokine markedly altered the homeostasis of the patients’ peripheral blood lymphocyte subsets, with NK cells and γδ+ T cells most dramatically affected, followed by CD8+ memory T cells. Five out of eight patients showed a 10% to 30% volume-decrease in their marker lesions, while two of them experienced clearing of the lesion [91]. Scientists are quite optimistic in IL-15 for HCC immunotherapy.

**Tumour antigen pulsed DC vaccine**

DCs are the most potent antigen-presenting cells, with the capacity to take up, process, and present tumour antigens to T cells and stimulate an immune response, thus providing a rational platform for vaccine development. Failure of Tumour Associate Antigen (TAA) processing and presentation by DCs is one of the main pathogenic factors in HCC [92,93], rendering the development of tumour antigen-pulsed DC vaccines to be a good immunotherapeutic strategy [94].

On an autochthonous hepatocellular carcinoma mouse model, a plasmid, expressing Alpha-feto protein (AFP), induced AFP-specific response and this led to a significant (65%) reduction in tumour size [95]. In a pilot phase I clinical trial, Butterfield and colleagues showed that 4 immuno-dominant peptides, derived from alpha-fetoprotein, along with incomplete Freund’s adjuvant induced specific T-cell responses in 6 HCC patients [96]. Furthermore, these peptide-pulsed DC cells induced significant antigen-specific T cell responses in a follow-up phase I/II trial in hepatocellular carcinoma patients [97].

Encouraged by these results, a series of clinical trials on HCC patients with tumour antigen-pulsed DC vaccines were carried out. Of 31 patients with advanced HCC, autologous tumour lysate-pulsed dendritic cells have increased the 1-year survival rates readily (63.3% against 10.7%; P < 0.001) [98]. In the follow-up phase II clinical study, thirty-five patients with advanced HCC were vaccinated intravenously with mature autologous Dendritic Cells (DCs) pulsed ex vivo with a liver tumour cell line lysate (HepG2). In this study, 28% of the disease control rate was observed without obvious toxicity and autoimmunity [99]. To augment the interaction between DCs and antigen-specific T cells, Gonzalez et al. transduced TAA-pulsed DCs with a CD40 L-encoding adenovirus, which increased the stimulatory capacity of DCs significantly. Intra-tumoral injection of DCs activated both acquired and innate immunity in mouse models, elicited complete regression of established tumours and long-term immunity was established against tumour recurrence [100]. DCs from HCC patients have been proven to strongly inhibit liver NK and NKT cells via an NK activating receptor NKP30 to boost these effector cells, α1, 3-galactosyl epitope-pulsed dendritic cells were injected into 9 HCC patients in a new clinical trial. Comparing to the 9 patients in the control group, this therapy significantly prolonged the survival of treated patients (17.1 ± 2.01 month vs 10.1 ± 4.5 month, p=0.00121) [101]. A new phase I clinical trial to evaluate the safety of an allogeneic dendritic cell vaccine COMBIG-DC in HCC patients is now recruiting participants (NCT01974661).

Because of the potent antigen-presenting capacity and feasibility of cells culture and expansion of autologous DCs, DC vaccines are one the most promising candidates for HCC immunotherapy. Frustratingly, current clinical trials haven’t demonstrated expected therapeutic
efficacy. Since the efficacy of DC-vaccines critically depends on techniques for the priming antigen loading onto DCs efficiently and their ability to migrate to the draining Lymph Nodes (LN). Therefore, improving DC-vaccines homing to draining LNs and increasing the antigen-loading efficacy appears as critical next steps in this field [102].

Future Perspectives

1. CAR-T and checkpoint blockage antibodies are stepping into the centre of immunotherapy stage, both strategies gained great success in treating other tumours. There are good reasons to keep optimistic of their efficacy in HCC.

2. Adoptive transfer in vitro modified TIL, NK or DC cells showed efficacy in several cases. They still have a lot to learn from CAR-T before they reach clinical satisfaction.

3. Administering cytokines in management HCC is still hugely challenging. Combination with chemotherapy or other therapy may be their future.

4. The combination of immunotherapy with local ablative therapies such as RFA or cryoablation, or the combination of two different immunotherapy modalities seems to be particularly promising approaches.

5. Development of additional animal models that reflect characteristics of different HCC subtypes would be necessary; magnificient genetic information from NGS helps better understanding and improving immunotherapy of HCC; Investigation of biology-driven biomarkers in response to different treatment strategies will help to follow treatment efficacy.

Acknowledgement

This work was sponsored by Nature Science Foundation of China (No. 81602701), Nature Science Foundation of Guangdong Province (No. 2017A030313547), and Nature Science Foundation of Guangdong Province (No. 2018A030313716).

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