Immune checkpoint therapy in liver cancer
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Abstract
Immune checkpoints include stimulatory and inhibitory checkpoint molecules. In recent years, inhibitory checkpoints, including cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein-1 (PD-1), and programmed cell death ligand 1 (PD-L1), have been identified to suppress anti-tumor immune responses in solid tumors. Novel drugs targeting immune checkpoints have succeeded in cancer treatment. Specific PD-1 blockades were approved for treatment of melanoma in 2014 and for treatment of non-small-cell lung cancer in 2015 in the United States, European Union, and Japan. Preclinical and clinical studies show immune checkpoint therapy provides survival benefit for greater numbers of patients with liver cancer, including hepatocellular carcinoma and cholangiocarcinoma, two main primary liver cancers. The combination of anti-PD-1/PD-L1 with anti-CTLA-4 antibodies is being evaluated in phase 1, 2 or 3 trials, and the results suggest that an anti-PD-1 antibody combined with locoregional therapy or other molecular targeted agents is an effective treatment strategy for HCC. In addition, studies on activating co-stimulatory receptors to enhance anti-tumor immune responses have increased our understanding regarding this immunotherapy in liver cancer. Epigenetic modulations of checkpoints for improving the tumor microenvironment also expand our knowledge of potential therapeutic targets in improving the tumor microenvironment and restoring immune recognition and immunogenicity. In this review, we summarize current knowledge and recent developments in immune checkpoint-based therapies for the treatment of hepatocellular carcinoma and cholangiocarcinoma and attempt to clarify the mechanisms underlying its effects.

Keywords: Immune checkpoint, Hepatocellular carcinoma, Cholangiocarcinoma, Immunotherapy, Epigenetics

Background
Globally, primary liver cancer accounts for 6% of all cancers and 9% of all death from cancer. It is the sixth most common cancer and the second leading cause of cancer death. The important primary liver cancers include hepatocellular carcinoma (HCC), accounting for approximately 75%, and cholangiocarcinoma, accounting for approximately 6%. Although either surgical resection or liver transplant can be used for the treatment of liver cancer, limitations are caused by high recurrence rates after resection and low-ratio eligibility for surgery and transplant because this cancer is often detected at a late stage [1, 2]. In the tumor microenvironment, cancer cells and host immune responses interact to promote or inhibit the pathologic progression of cancer. The immune system can identify cancer cells, and mobilizing the immune response is able to eliminate cancer [3].

Immunotherapy has emerged as a promising therapy and is being investigated in various tumors including liver cancer [4]. Emerging evidence supports that the blockade of immune checkpoints is among the most promising approaches in cancer immunotherapy [4–6].

The activity of the immune system is mostly regulated by immune cells called T cells. In the tumor microenvironment, T cells can recognize tumor antigens, which are presented to T cell receptors by antigen-presenting cells (APCs). Besides signal via T cell receptors, T cell response is fine-tuned by a group of cell surface molecules, named immune checkpoints. They can be either stimulatory or inhibitory, and participate in various stages of T cell response (Fig. 1) [6–11]. Many cancers are able to evade the immune system, mainly by overexpressing inhibitory ligands to damp T cell attack. As a result, fewer, and damaged T cells were found in patients with HCC, which contributed to the progression of this cancer [12].

Recently, in vitro and in vivo results show histone deacetylase inhibitors (HDACi) and DNA methyltransferase inhibitors (DNMTi), two important epigenetic drugs,
can up-regulate expression of inhibitory immune checkpoints in either immune or cancer cells [13–15]. Epigenetic modifiers function importantly in priming and enhancing the therapeutic effect of the host immune system on cancer [14, 15]. The purpose of this review is to give a brief overview of the role for immune checkpoints related to liver cancer progression. It also provides new insights into the epigenetic mechanism in checkpoint immunotherapy and checkpoint blocking – based therapeutic approaches for treatment of liver cancer.

**Immune checkpoints and hepatocellular carcinoma**

The most ex vivo studied and clinically relevant checkpoint proteins are CTLA-4, PD-1, and PD-L1 (Tables 1 and 2). The expression of inhibitory immune checkpoints can be dysregulated in a tumor microenvironment, which can lead to improvement of T cell-mediated immune response through cancer immunotherapy [16]. The PD-1 pathway is found to suppress T cell activation mainly within peripheral tissues at the later phase, whereas the CTLA-4 pathways are involved in regulation of T cell-mediated immune responses primarily in lymph nodes at the priming phase [17].

**CTLA-4**

CTLA-4 is a CD28 homolog and primarily located in intracellular compartments in resting naïve T cells. CTLA-4 inhibits T cell response by directly delivering an inhibitory signal to T cell, and interfering with the binding between B7 and CD28 [18]. In 31 HCC patients, it was found the addition of anti-CTLA-4 antibody resulted in an increase in the frequency of tumor-associated antigens (TAA)-specific cytotoxic T cells in 60% of HCC patients, accompanied with enhanced antitumor effect of tumor-specific T cells [19]. In addition, CTLA-4 is shown to be important for regulatory T cell (Treg) function. Tregs control functions of the effector T cells, and thus crucially maintain peripheral tolerance [20]. Unlike effector T cells, Tregs constitutively express CTLA-4 to exert their immune suppression [21, 22]. Treg-specific CTLA-4 deficiency was shown to affect in vivo Treg suppressive function and promote tumor immunity [21, 22]. In a rat liver transplantation model with tumor recurrence, hepatic expressions of CTLA-4, TGF-β and PD-L1 were increased in the tumor tissues from small-for-size liver graft group compared to whole graft group. The results suggested that up-regulation of CTLA-4 may mediate the mobilization of Tregs by small-for-size graft injury, contributing to HCC recurrence after liver transplantation [23]. HCC-derived Tregs down-regulated CD80/86 expression on splenic DCs in a CTLA-4 dependent manner, and inhibition of CTLA-4 could prevent the Treg-mediated suppression in anti-tumor immune response.
responses [24]. Thus, CTLA-4 could not only enhance the antitumor effect of effector T cells but also maintain self-tolerance and the suppressive function of Tregs in liver cancer immunity.

PD-1/PD-L1

PD-L1 is the main ligand for PD-1, which is crucial for tumor immunity. In addition, PD-L1 also interacts with B7-1 to inhibit T cell immunity, and the role of this interaction in cancer immunity is still unclear [25]. Binding of PD-L1 to its receptor can suppress T cell migration, proliferation, and secretion of cytotoxic mediators, and thus blocks the “cancer immunity cycle” [26]. In the HCC tumor microenvironment, PD-L1 expression is mainly expressed in Kupffer cells but is slightly expressed on other APCs or HCC tumor cells [27]. CD8+ T cells and Kupffer cells in human HCC tumor tissues expressed high levels of PD-1 and PD-L1, respectively. PD-L1+ Kupffer cells interact with PD-1+ CD8+ T cells and contribute to dysfunction of effector T cells in HCC. Elevated PD-L1 expression in HCC is indeed associated with poorer prognosis in HCC patients [27]. In 217 HCCs, PD-L1 was expressed by both neoplastic and intra-tumoral inflammatory cells, which are related to tumor aggressiveness. It also suggests that the PD-L1/PD-1 immune checkpoint could be targeted in the treatment of particular HCC variants [28]. More recently, 90 HCC patients with PD-L1 expression in peritumoral hepatocytes were demonstrated to have a significantly higher risk of cancer recurrence or metastasis and cancer-related death [29]. Immunohistochemistry data in 294 HCC tissue samples showed PD-1 and PD-L1 expression was significantly related to high CD8+ tumor-infiltrating lymphocytes (TILs). Only high Edmondson–Steiner grade was markedly related to high PD-1 expression. High PD-L1 expression was

| Table 2 Pre-clinical studies with immune checkpoints in therapy of liver cancers |
|---------------------------|-----------|-----------------|-----------------|----------------|---------------|---------------|---------------|-----------------|
| Cancer type | Number | TNM Stage (I + II / III + IV) | Tumor differentiation (I + II / III + IV) | Tumor size (cm) | Immune checkpoints | Therapy | Target cells | Year | Reference |
|-------------------------|--------|------------------------------|-----------------------------------|-----------------|-----------------|----------------|---------------|-----------------|-----------------|
| Human HCC | 71 | 57 / 14 | 58 / 13 | 36 (≤5) / 35 (>5) | PD-L1 and PD-1 antibodies | Kupffer cells and CD8+ T cells | 2009 [23] |
| Human HCC | NR | NR | NR | PD-L1 | Specific shRNA for PD-L1 and DNM1 | HCC cell lines | 2017 [57] |
| Human HCC | 31 | 22/9 | 21/10 | 9(≤5) / 22(>5) | CTLA-4 antibodies | CTLA-4 antibodies | Tumor-Associated Antigen-Specific T Cells | 2011 [16] |
| Mice HCC | NR | NR | NR | CTLA-4 | CTLA-4 antibodies | Regulatory T cells | 2017 [21] |
| Human HCC | 59 | 54 / 4 unknown, n = 1 | NR | LAG3, PD-1, Tim3 and CTLA4 | Blocking antibodies to LAG3, PD-1, TIM3 or CTLA4 | Tumor-infiltrating T cells | 2017 [30] |
| Human HCC | 21 | 8/13 | NR | GITR | GITR ligand | Tumor-infiltrating Treg | 2013 [33] |

HCC hepatocellular carcinoma, ICC Intrahepatic cholangiocarcinoma, NR not reported
demonstrated as an independent poor prognostic factor for disease-free survival in the high CD8+ TILs group. Further, combined high expression of PD-L1 and CD8+ TIL is an important prognostic factor related to the immune checkpoint pathway in HCC. Also, this result would be helpful in evaluating the applicable group of PD-1/PD-L1 blocking agent for HCC patients [30]. PD-L1 expression was significantly increased in tumors with a high number of tumor-infiltrating lymphocytes ($p = 0.533, \rho < 0.001$). High PD-L1 expression was associated with significantly shorter overall survival [31]. These clinic data further support that PD-L1 is an important mediator in the progression and an important target in the anti-tumor therapy for liver cancer.

**Other inhibitory checkpoints**

Several other inhibitory receptors, including T-cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) and LAG-3, are also upregulated on TAA-specific CD8+ T-cells in various cancer types, and are also involved in progression of liver cancer. Tim-3 is strongly expressed on CD4+ and CD8+ T-cells obtained from HCC lesions in contrast to the surrounding liver tissue. Tim-3 is expressed on tumor-associated macrophages (TAM), which contributes to HCC growth [32]. Intriguingly, a high number of Tim3+ tumor infiltrating cells and Tim3+ TAM in HCC lesions are associated with a poor prognosis [33]. In 171 patients with hepatitis B virus (HBV)-related HCC, both PD-1 and Tim-3 expressions in liver infiltrating lymphocytes were significantly high in tumor tissues compared to tumor adjacent tissues. The up-regulation of PD-1 and Tim-3 were related to higher tumor grades [33]. There is a significant positive intercorrelation between the levels of PD-1 and Tim-3 expression in tumor tissues and tumor adjacent tissues. The expressions of PD-1 and Tim-3 in tumor tissues and tumor adjacent tissues were significantly associated with PD-1 and Tim-3 polymorphisms, with genotype AA of PD-1 rs10204525 and genotypes GT + TT of Tim-3 rs10053538 respectively [33]. LAG-3 is another important inhibitory immune check point and exerts synergistic effects with PD-1/PD-L1 on T cell activation in the tumor microenvironment. In HCC-vaccine-immunized mice, STAT3-blocked HCC vaccine downregulated expression of PD-1, TIGIT, and LAG-3, which could prevent cancer-induced dysfunction of CD8+ T and natural killer cells [34]. Recently, expression of LAG3 was found to be significantly higher on tumor-associated antigen (TAA)-specific CD8+ tumor-infiltrating T helper cells and CD8+ cytotoxic T cells in tumors than those in tumor-free liver tissues and blood of HCC patients [35]. Interestingly, blocking LAG-3 increased ex vivo proliferation of CD4+ and CD8+ TIL, and effector cytokine production. Combination of LAG-3 blocking antibody with PD-L1 blockade further augmented TIL responses to polyclonal stimuli and TAA [35]. This suggests that LAG-3 plays an important role in T-cell suppression in the HCC microenvironment and might be a promising immunotherapeutic target for HCC. Further clinical trials about Tim-3, Lag-3 or TIGIT blockers should be performed in liver cancer treatment.

**Co-stimulatory immune checkpoints**

The best characterized co-stimulatory ligands that have been investigated in hepatocellular carcinoma are B7-1 and B7-2. These two important immune checkpoints are mainly expressed on professional antigen-presenting cells. B7-1 and B7-2 can bind to both CD28 and CTLA-4, and thus regulate T cell activation via selective interacting with either CD28 or CTLA-4 [36]. Expression of costimulatory molecules, including B7-1 and B7-2, have been found to be down-regulated in HCC cells [37]. This down-regulation may lead to suppression of activation of effector T-cells mediated by B7/CD28. The glucocorticoid-induced tumor necrosis factor receptor (GITR) and the inducible T-cell co-stimulator (ICOS) are co-stimulatory checkpoints and regulate the immunosuppressive Tregs function. Importantly, GITR and ICOS are up-regulated in Tregs infiltrating HCC and may function as potential targets for immunotherapeutic interventions for antitumor therapy [38].

**Immune checkpoints and cholangiocarcinoma**

Intrahepatic cholangiocarcinoma (ICC) represents the second most common primary liver malignancy, accounting for 10–20% of all primary liver cancers [39]. Although ICC is traditionally viewed as a rare cancer, its incidence has been steadily rising, with recent reports showing the incidence of ICC in the USA has increased from 0.44 to 1.18 cases/100,000 over the past three decades [40]. The prognosis for ICC continues to be poor, with surgery as the only definitive option for cure. Median survival rate is low because most patients are not eligible for curative resection. As such, there is an increasing need for the development of novel adjuvant therapies for patients with ICC.

**PD-1/PD-L1**

In contrast to HCC, immunotherapy in cholangiocarcinoma has been limited and mostly ineffective [41]. However, a high frequency of tumor-infiltrating lymphocytes and PD-L1 expression suggest that checkpoint inhibition may prove effective [42]. Expression of PD-L1 was found both in tumor-associated macrophages and in the tumor front. Patients with tumors exhibiting PD-L1 expression around the tumor front had a lower overall survival than tumor front-positive patients [43]. In 31 surgically resected ICC samples from Asian patients, PD-L1
expression was significantly higher in tumor tissue than that in adjacent tissue [44]. High levels of PD-L1 expression were also found in Western patients with ICC, which resulted in tumor poor differentiation, higher malignant tumor stage and higher levels of apoptotic CD8+ TILs, and therefore led to lower chance of survival [42]. More recently, in occupational cholangiocarcinoma, PD-L1 expression was found in biliary intraepithelial neoplasia and intraductal papillary neoplasm. Cholangiocarcinoma cells expressed PD-L1 in a low number of cases of occupational cholangiocarcinoma, while carcinoma cells expressed PD-L1 in all cases. Moreover, PD-L1 and PD-1 were also expressed in tumor-associated macrophages and tumor-infiltrating T cells expressed. The number of PD-L1-positive monocellular cells, PD-1-positive lymphocytes, and CD8-positive lymphocytes infiltrating within the tumor was markedly high in occupational cholangiocarcinoma. Immunostaining with mAbs detected human leukocyte antigens (HLA) class I defects in 60% of ICC tumors and PD-L1 expression in 30%. Patients bearing tumors with HLA class I defects and PD-L1 expression had a significantly reduced survival rate. The results suggested PD-L1 up-regulation mediates immune escape in cholangiocarcinoma and could be potential biomarker of response to anti-PD-1/PDL1 immunotherapy [45]. The role of other immune checkpoints for cholangiocarcinoma is still not well established.

**Epigenetic mechanism in checkpoint immunotherapy**

In cancer, two important epigenetic mechanisms include hypermethylation, which is mediated by DNMTs, and histone deacetylation, which is mediated by HDACs. Epigenetic dysregulation is a crucial mechanism underlying the progression of cancer [46–49]. Some epigenetic regulators can act negatively and positively in immune responses and lead to immune evasion [50], which provides a novel mechanism in immune checkpoint therapy for treatment of cancers.

Recently, epigenetic modifications of the key immune checkpoints including PD-1, PD-L1, and CTLA-4 were analyzed in non-small cell lung cancer tissues from 39 patients [51]. It was shown that CTLA-4 and PD-1, but not PD-L1, are hypomethylated in human lung tumors. This hypomethylation also led to increased expression of these two genes as shown by transcriptome analysis [51]. In a phase 2 trial, hypomethylating agents such as vorinostat and azacitidine upregulated mRNA expression of PD-L1, PD-L2, PD-1 and CTLA-4 in 61 patients with acute myeloid leukemia [52]. More recently, profiling DNA methylation in peripheral blood mononuclear cells and T cells from HCC patients show that a broad signature of DNA methylation intensifies with progression of HCC [53]. Importantly, HCC DNA methylation is highly enriched in immune function-related gene PD-1 [53]. Interestingly, Liu et al. found highly upregulated DNA methyltransferase 1 (DNMT1) is positively correlated with PD-L1 overexpression in sorafenib-resistant HCC cells. PD-L1 further induced DNMT1-dependent DNA hypomethylation and restored the expression of methylation-silenced Cadherin 1, a metastasis suppressor in HCC [54].

Accumulating evidence also shows histone deacetylation regulates immune checkpoint expression and plays an important role in cancer progression. HDAC is have been shown to sensitize cancer cells to immune checkpoint therapy by upregulating the immune checkpoints CTLA-4, PD-1, PD-L1, and PD-L2 on tumor cells and TILs [55]. For example, inhibition of the class I HDAC1, HDAC2 and/or HDAC3 led to acetylation of the PD-L1 and PD-L2 promoters, which augmented up-regulation of PD-L1/L2 protein and RNA transcription in melanoma patients, in melanoma cell lines and in a syngeneic mouse model of melanoma [56]. Interestingly, Lienlaf et al. [57] found HDAC6i (ACY-241) reduced PD-L1 production and increased co-stimulatory checkpoint (CD28) levels, and thus suppressed tumor growth in vivo. In the WM164 HDAC6KD cells, the expression of PD-L2, B7-H4 and TRAIL-R1 were largely diminished, while B7-H3, Galectin-9 and TRAIL-R2 were moderately decreased. In breast cancer cells, CD137, a co-stimulatory checkpoint, was found to be up-regulated by HDACi (SAHA) treatment [58]. Therefore, inhibitory and co-stimulatory checkpoints can be up-regulated or down-regulated by different HDAC isoforms in different tumor types. To date, the immune modulatory activity of HDAC inhibitors on tumor-specific immunity including immune checkpoints has not been well demonstrated or characterized in HCC.

Recent evidence suggests that noncoding RNAs, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), may also have direct epigenetic functions by recruiting specific protein complexes to genomic DNA, and specifically to some promoters modulating the expression of the corresponding genes. MiRNAs and lncRNAs play important roles in regulating expression of immune checkpoints in various tumors [59]. In human malignant pleural mesothelioma, the levels of miR-15b, miR-16, miR-193a-3p, miR-195, and miR-200c were significantly lower in the immune checkpoint PD-L1-positive samples. Likewise, PD-L1 and miR-138-5p levels were inversely correlated in human colorectal cancer tumors, and miR-138-5p inhibited PD-L1 expression in tumor models in vivo [60]. In lung cancer, it was demonstrated that the p53/miR-34/PD-L1 and miR-200/ZEB1/PD-L1 axis are novel mechanisms in tumor immune evasion [61, 62]. Moreover, it is recently...
demonstrated that transfection of human CD4+ T cells with miR-138 suppressed expression of CTLA-4, PD-1, and Foxp3 in glioma preclinical models [63]. Whether the association between miRNA expression and immune checkpoint levels in tumors can be translated into a predictive marker of checkpoint inhibitor therapy in liver cancer requires further investigation. Interactions among three kinds of RNAs were revealed in the 'IncRNA-miRNA-mRNA' competing endogenous RNA network. Several biomarkers were identified for diagnosis of diabetic pancreatic cancer, such as IncRNAs (HOTAIR, CECR7 and UCA1), hsa-miR-214, hsa-miR-429, CCDC33 and CTLA-4. Notably, interactions of 'CECR7-hsa--miR-429-CTLA4' were highlighted in the endogenous RNA network, which is very important in enhancing the progression of pancreatic cancer [64]. Some miRNAs and IncRNAs might be involved in the “cancer immunity cycle” regulated by immune checkpoints such as CTLA-4 and PD-L1-PD-1 and could be the subject of future investigations in liver cancer.

Taken together, a wave of translational research highlights the mechanistic and functional link between epigenetic regulation and immune checkpoints in the development and progression of primary tumors including liver cancer.

**Checkpoint-blocking based therapeutic approaches**

Over the last decade, there has been significant progress in our understanding of the immune system which has led to development of numerous immune checkpoints blockades that have altered the management and prognosis in some cancers including liver cancer (Table 2). As more such drugs are developed, we will have multiple additional options and indications for these inhibitors in the near future. Among these pathways, the PD-1/ PD-L1 and the B7-1/B7-2/CTLA-4 have been identified as clinically available inhibitors.

These immune checkpoint drugs such as nivolumab, pembrolizumab, and ipilimumab have already been FDA approved in non-small cell lung cancer, renal cell carcinoma, melanoma, Hodgkin lymphoma, and urothelial bladder cancer [65]. Trials investigating immune checkpoint blockades in HCC and cholangiocarcinoma are in progress and early signals of efficacy have recently been reported (Table 3). Encouraging clinical outcomes were reported from an ongoing phase I/II trial of the anti-PD-1 antibody nivolumab at the 2015 American Society of Clinical Oncology (ASCO) Annual Meeting held in Chicago [66]. Waterfall plots showed that the tumor size decreased to some extent in all cohorts including uninfected, HBV-infected, and hepatitis C virus-infected HCC patients. It was significant and stable in the response to the treatment of nivolumab in HCC patients. In another recent ongoing trial of nivolumab treatment in HCC patients, nivolumab showed a manageable safety profile, including acceptable tolerability. The objective response rate was 20% (95% CI 15–26) in patients treated with nivolumab 3 mg/kg in the dose-expansion phase and 15% (95% CI 6–28) in the dose-escalation phase [67]. Early data from the biliary tract cohort of Keynote-028 reported an objective response rate of 17% and a further 17% achieved stable disease in PD-L1 positive pretreated advanced cholangiocarcinoma [68].

Immunotherapy is promising for HCC and cholangiocarcinoma. However, even for those patients who respond to the single agent immunotherapy, combinational therapy may be more potent and lead to more durable response. At the 2016 ASCO meeting, an ongoing phase I trial showed trans catheter arterial chemoembolization. Radiofrequency, or cryoablation induced a peripheral immune response which may enhance the effect of anti-CTLA-4 treatment. This combination is safe and leads to the accumulation of intratumoral CD8+ T cells and activation of T cells in peripheral blood in responding patients. Encouraging clinical activity was seen with objective confirmed responses and a PFS of 5.7 months (NCT01853618) [65]. Another pilot study for the combined effect of immune checkpoint blocking and ablative therapies has been initiated in patients with advanced liver cancer (NCT02821754). Chemotherapy such as cisplatin can reduce PD-L2 expression on tumor cells [69, 70]. Both these studies show that chemotherapy can enhance antitumor immunity and thus may combine and augment immune checkpoint therapy for treatment of liver cancer.

As previously discussed, epigenetic modulators enhance cell surface expression of immune checkpoints. Several studies provided evidence to support increased expression of checkpoint inhibitors on tumor cells following epigenetic treatment, which enhances responses to immune checkpoint therapy [56, 71]. Recently, the role of HDACi and histone methyltransferases in tumor immunity and cancer therapy has been investigated. In melanoma-bearing mice, HDACi upregulated expression of PD-L1 and PD-L2 through increased histone acetylation. Further, combination of HDACi and PD-1 blockade led to higher efficiency in slowing tumor progression and improving survival rate than single agent therapy [56]. 3-Deazaneplanocin A and 5-aza-2’ deoxycytidine, two important DNMTi, enhanced the therapeutic efficacy of PD-L1 blockade in reducing tumor volume, increasing tumor infiltrating CD8+ T cells and Th1-type chemokine expression in ovarian cancer in C57/BL6 mice [72]. Chiappinelli et al. demonstrated that 5-azacytidine, sensitized tumors to anti-CTLA-4 immune checkpoint therapy compared to 5-azacytidine or anti-CTLA-4 alone in a mouse model of melanoma [73]. Enhancer of zeste homolog 2 blockade led to reduced PD-L1 mRNA levels and a
decrease in PD-L1+ Pax3+ in melanoma cells, which was maintained during concomitant IL-2cx or anti-CTLA-4 immunotherapy [74]. Taken together, these discoveries create a highly promising basis for combination studies using epigenetic and immune checkpoint therapy in patients with various cancers including liver cancer (Table 4).

Table 3 Clinical trials with immune checkpoints therapy in liver cancers

| Cancer type | Number | Study arms | Stage | Status | Design | Primary outcome | Estimated completion | Trial NCT |
|-------------|--------|------------|-------|--------|--------|----------------|----------------------|----------|
| HCC         | 35     | Nivolumab (anti PD-1 Ab) + LRT (Yttrium 90Y glass microspheres) | Phase 1 | Recruiting | Single Group Assignment | July 2019 | July 2019 | NCT02837029 |
| HCC         | 154    | PD0001 (anti PD-1 Ab) + NIS793 (anti TGF-β Ab) | Phase 1 | Recruiting | Non-Randomized | January 12, 2020 | January 12, 2020 | NCT02947165 |
| HCC         | 114    | Durvalumab (anti PD-1L Ab) + ramucirumab (anti-VEGF-R2 Ab) | Phase 1 | Recruiting | Non-Randomized | March 2018 | September 2018 | NCT02572687 |
| HCC         | 51     | Durvalumab (anti PD-1 L Ab) + AZD4635 | Phase 1 | Recruiting | Non-Randomized | November 9, 2017 | November 9, 2017 | NCT02740985 |
| HCC         | 61     | Tremelimumab (anti CTLA-4 Ab) | Phase 1 | Active, not recruiting | Non-Randomized | December 31, 2017 | December 31, 2018 | NCT01853618 |
| Liver cancer| 60     | Ipilimumab (anti CTLA-4 Ab) + MGN1703 (Toll-like receptor agonist) | Phase 1 | Recruiting | Non-Randomized | May 2019 | May 2019 | NCT02668770 |
| HCC         | 120    | Ipilimumab (anti CTLA-4 Ab) + stereotactic body radiation | Phase 1 | Recruiting | Randomized | August 2019 | August 2019 | NCT02239900 |
| HCC         | 75     | Nivolumab (anti PD-1 Ab) + ganitumab (TGF-β inhibitor) | Phase 1/2 | Recruiting | Non-Randomized | April 2018 | March 2019 | NCT02423343 |
| HCC         | 620    | Nivolumab (anti PD-1 Ab) + ipilimumab (anti-CTLA-4 Ab) | Phase 1/2 | Recruiting | Non-Randomized | July 22, 2018 | July 9, 2019 | NCT01658878 |
| HCC         | 108    | PD0001 (anti PD-1 Ab) + INC280 (c-Met inhibitor) | Phase 1/2 | Recruiting | Non-Randomized | December 24, 2018 | December 24, 2018 | NCT02795429 |
| HCC         | 50     | Pembrolizumab (anti PD-1 Ab) + dendritic cells, cytokine-induced killer cells | Phase 1/2 | Recruiting | Single Group Assignment | September 2019 | October 2019 | NCT02886897 |
| HCC         | 15     | Pembrolizumab (anti PD-1 Ab) | Phase 1/2 | Recruiting | Single Group Assignment | December 2019 | December 2019 | NCT02940496 |
| HCC         | 50     | Nivolumab (anti PD-1 Ab) + CC-122 (immunostimulatory pathway modifier) | Phase 1/2 | Recruiting | Single Group Assignment | June 23, 2020 | June 23, 2020 | NCT02859324 |
| HCC         | 90     | Durvalumab (anti PD-1 L Ab), Tremelimumab (anti CTLA-4 Ab) + LRT | Phase 1/2 | Recruiting | Non-Randomized | April 30, 2020 | April 30, 2021 | NCT02821754 |
| HCC         | 620    | Nivolumab (anti PD-1 Ab), Nivolumab + ipilimumab, Nivolumab + cabozantinib, Nivolumab + ipilimumab + cabozantinib | Phase 1/2 | Recruiting | Non-Randomized | September 4, 2018 | July 9, 2019 | NCT01658878 |
| HCC         | 28     | Pembrolizumab (Keytruda) (anti PD-1 Ab) | Phase 2 | Recruiting | Single Group Assignment | April 2018 | April 2019 | NCT02658019 |
| HCC         | 440    | Durvalumab (anti PD-1 L Ab) + Tremelimumab (anti CTLA-4 Ab) | Phase 2 | Recruiting | Randomized | March 20, 2020 | March 20, 2020 | NCT02519348 |
| HCC         | 726    | Nivolumab (anti PD-1 Ab) | Phase 3 | Recruiting | Randomized | October 1, 2018 | June 22, 2019 | NCT02576509 |
| HCC         | 408    | Pembrolizumab (anti PD-1 Ab) | Phase 3 | Active, not recruiting | Randomized | February 1, 2019 | February 1, 2019 | NCT02702401 |
| HCC         | 1200   | Durvalumab (anti PD-1 L Ab) + tremelimumab (anti CTLA-4 Ab) | Phase 3 | Non yet recruiting | Randomized | February 27, 2020 | March 29, 2021 | NCT03298451 |
| Cancer type                                        | Number | Immune checkpoint inhibitors | Epigenetic drugs                  | Stage | Status               | Design               | Trial NCT          |
|---------------------------------------------------|--------|------------------------------|-----------------------------------|-------|----------------------|----------------------|-------------------|
| HCC                                               | 90     | Durvalumab                   | Guadecitabine                      | Phase 1 | Recruiting           | Single Group Assignment | NCT03257761     |
| Unresectable NSCLC                                | 41     | Nivolumab                    | ACY-241                            | Phase 1 | Recruiting           | Single Group Assignment | NCT02635061     |
| Metastatic unresectable HER2-negative breast cancer| 45     | Pembrolizumab                | Entinostat                          | Phase 1 | Recruiting           | Single Group Assignment | NCT02453620     |
| Advanced solid tumors                             | 30     | Pembrolizumab                | Entinostat                          | Phase 1 | Recruiting           | Randomized           | NCT02909452     |
| Unresectable stage III/IV melanoma                | 17     | Ipilimumab                   | Panobinostat                        | Phase 1 | Recruiting           | Single Group Assignment | NCT02032810     |
| Advanced CRC                                      | 30     | Pembrolizumab                | Romidepsin and/or 5-AZA             | Phase 1 | Recruiting           | Randomized           | NCT02512172     |
| MSS advanced CRC                                  | 30     | Pembrolizumab                | Romidepsin and/or 5-AZA             | Phase 1 | Recruiting           | Randomized           | NCT02512172     |
| MDS following DNMTi-failed therapy                | 27     | Pembrolizumab                | Entinostat                          | Phase 1 | Recruiting           | Single Group Assignment | NCT02936752     |
| Advanced solid tumors or lymphomas                | 45     | Nivolumab                    | RRx-001                            | Phase 1 | Active, not recruiting | Single Group Assignment | NCT02518958     |
| MM                                                | 19     | Ipilimumab                   | SGI-110                            | Phase 1 | Recruiting           | Single Group Assignment | NCT02608437     |
| MDS                                                | 73     | Durvalumab with or without tremelimumab | Azacytidine                      | Phase 1 | Recruiting           | Non-Randomized       | NCT02117219     |
| Advanced cell carcinoma                           | 62     | Atezolizumab                 | Entinostat                          | Phase 1/2 | Recruiting           | Single Group Assignment | NCT03024437     |
| Breast cancer                                     | 88     | Atezolizumab                 | Entinostat                          | Phase 1/2 | Recruiting           | Randomized           | NCT02708680     |
| DLBCL                                             | 5      | Rituximab                    | Belinostat                          | Phase 2  | Active, not recruiting | Single Group Assignment | NCT01686165     |
| Metastatic uveal melanoma                         | 29     | Pembrolizumab                | Entinostat                          | Phase 2  | Recruiting           | Single Group Assignment | NCT02697630     |
| DLBCL                                             | 42     | Rituximab                    | Panobinostat                        | Phase 2  | Active, not recruiting | Randomized           | NCT01238692     |
| Advanced solid tumors and NSCLC                   | 119    | Durvalumab                   | Mocetinostat                        | Phase 1/2 | Recruiting           | Single Group Assignment | NCT02805660     |
| NSCLC and melanoma                                | 202    | Pembrolizumab                | Entinostat                          | Phase 1/2 | Recruiting           | Non-Randomized       | NCT02437136     |
| HNSCC and SGC                                     | 49     | Pembrolizumab                | Vorinostat                          | Phase 1/2 | Active, not recruiting | Single Group Assignment | NCT02538510     |
| Stage IV NSCLC                                    | 100    | Pembrolizumab                | Vorinostat                          | Phase 1/2 | Recruiting           | Randomized           | NCT02638090     |
| DLBCL                                             | 83     | Rituximab                    | Vorinostat                          | Phase 1/2 | Active, not recruiting | Single Group Assignment | NCT00972478     |
| Lymphoma/leukaemia                                | 40     | Rituximab                    | Vorinostat                          | Phase 1/2 | Active, not recruiting | Single Group Assignment | NCT00918723     |
| Advanced renal or urothelial cell carcinoma        | 42     | Pembrolizumab                | Vorinostat                          | Phase 2  | Recruiting           | Non-Randomized       | NCT02619253     |
| Hormone therapy-resistant breast cancer            | 87     | Pembrolizumab                | Vorinostat                          | Phase 2  | Recruiting           | Randomized           | NCT02395627     |
| AML                                               | 182    | Nivolumab                    | 5-AZA                              | Phase 2  | Recruiting           | Non-Randomized       | NCT02397720     |
| Metastatic CRC                                    | 31     | Nivolumab                    | 5-AZA                              | Phase 2  | Active, not recruiting | Single Group Assignment | NCT02260440     |
| Advanced/metastatic NSCLC                         | 100    | Nivolumab                    | 5-AZA                              | Phase 2  | Active, not recruiting | Randomized           | NCT02546986     |
| MDS                                               | 120    | Nivolumab and/or ipilimumab | 5-AZA                              | Phase 2  | Recruiting           | Non-Randomized       | NCT02530463     |
therapies targeting liver cancers. Therefore, immuno-
therapy offers hope to liver cancer patients with a dismal
prognosis that has not seen significant changes in ther-
apy for a long time.

Limitations and perspectives of immune
checkpoint therapy

Resistance to immune checkpoint blockades is still com-
monly observed in most cancer patients [75]. Failure of
immune checkpoint inhibitors therapy can result from
three categories: (1) mutations of the immunogenicity of
cancer itself. The mutations influence expression of
components of antigen-processing and presentation ma-
chinery (e.g., transporter associated with antigen pro-
cessing, HLA class molecules, and β2 microglobulin),
novel tumor-associated antigens (e.g., cancer-testis anti-
gen, neoantigens), and cytokines; (2) expression of al-
ternative immune checkpoint ligands on tumor cells
(and/or immune cells). Expression of alternative
co-inhibitory immune checkpoints (e.g., CTLA-4,
TIM-3, LAG-3, and VISTA) has been associated with re-
sistance to PD-1 blockade [76, 77]; or (3) defects in T
cell infiltration. Diminished infiltration of T cells led to
resistance to PD-1 blockade in melanoma patients [78].
However, epigenetic modifying agents including
demethylating agents and histone deacetylase inhibitors
may enable re-expression of immune related thera-
peutic genes, especially in combination of immunother-
apy [79, 80]. They can also increase expression of
immune checkpoints to synergize with immune check-
point blockade therapy, leading to improving
anti-tumor responses [81].

Conclusions

Most liver cancers are diagnosed at an advanced stage,
while the therapy is limited. Immune checkpoint therapy
provides survival benefit for liver cancer treatment. Epi-
genetic regulation mechanistically and functionally links
with immune checkpoints. Epigenetic mechanisms of
checkpoint blocking prove to be promising in treat-
ing liver cancers and determining patient prognosis. Fur-
ther investigations are required to explore the clinical
potential in combination with epigenetic and immune
checkpoint therapy for liver cancer treatment.

Abbreviations

APC: Antigen presenting cell; ASCO: American Society of Clinical Oncology;
BTLA: B- and T-lymphocyte attenuator; CTLA-4: Cytotoxic T lymphocyte
associated antigen 4; DNMT: DNA methyltransferase inhibitor, MM Multiple myeloma, DLBCL Diffuse large B cell lymphoma,
HNSCC head and neck squamous cell carcinoma, SGC salivary gland cancer, AML Acute myeloid leukaemia, OC ovarian cancer

Table 4 Ongoing clinical trials combining epigenetic drugs and immune checkpoint blockade therapy in cancers (Continued)

| Cancer type | Number | Immune checkpoint inhibitors | Epigenetic drugs | Stage | Status | Design | Trial NCT |
|-------------|--------|-------------------------------|------------------|-------|--------|--------|----------|
| Refractory/relapsed AML | 37 | Lirilumab | 5-AZA | Phase 2 | Active, not recruiting | Single Group Assignment | NCT02399917 |
| MDS | 12 | Lirilumab and nivolumab | 5-AZA | Phase 2 | Active, not recruiting | Non-Randomized | NCT02599649 |
| Metastatic melanoma | 71 | Pembrolizumab | 5-AZA | Phase 2 | Recruiting | Non-Randomized | NCT02816021 |
| NSCLC | 120 | Nivolumab | 5-AZA and/or entinostat | Phase 2 | Recruiting | Randomized | NCT01928576 |
| NSCLC | 60 | Nivolumab | 5-AZA- CdR/ tetrahydrodouridine | Phase 2 | Recruiting | Randomized | NCT02795923 |
| Advanced solid tumors | 60 | Durvalumab | 5-AZA | Phase 2 | Recruiting | Single Group Assignment | NCT02811497 |
| Advanced/metastatic NSCLC | 100 | Pembrolizumab | Oral azacytidine | Phase 2 | Active, not recruiting | Randomized | NCT02546986 |
| PR recurrent OC | 38 | Pembrolizumab | Guadecitabine | Phase 2 | Recruiting | Single Group Assignment | NCT02901899 |
| PR recurrent OC | 20 | Pembrolizumab | Oral azacytidine | Phase 2 | Recruiting | Randomized | NCT02900560 |
| MDS | 120 | Durvalumab | Oral azacytidine | Phase 2 | Recruiting | Randomized | NCT02281084 |
| MDS, AML | 213 | Durvalumab | Azacytidine | Phase 2 | Active, not recruiting | Randomized | NCT02775903 |
| Refractory/recurrent epithelial OC | 138 | Avelumab | Entinostat | Phase 2 | Recruiting | Randomized | NCT02915523 |
| DLBCL | 304 | Rituximab | 5-AZA | Phase 3 | Recruiting | Randomized | NCT02951156 |

HCC hepatocellular carcinoma, NSCLC Non-small cell lung cancer, HER2 human epidermal growth factor receptor 2, CRC colorectal cancer, 5-AZA Azacitidine, MSS Microsatellite stable, MDS Myelodysplastic syndromes, DNMTi DNA methyltransferase inhibitor, MM Multiple myeloma, DCC Diffuse large B cell lymphoma, HNSCC head and neck squamous cell carcinoma, SGC salivary gland cancer, AML Acute myeloid leukaemia, OC ovarian cancer
immunoglobulin-like receptors; LAG-3: Anti-lymphocyte activation gene-3; IncRNAs: long noncoding RNAs; miRNAs: microRNAs; PD-1: Programmed cell death protein-1; PD-L1: Programmed cell death ligand 1; TAA: Tumor-associated antigens; TAM: Tumor-associated macrophages; TILs: Tumor-infiltrating lymphocytes; Tim-3: T-cell immunoglobulin- and mucin-domain-containing molecule-3; Tregs: Regulatory T cells; VISTA: V-domain Ig suppressor of T-cell activation

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Authors’ contributions

FX, YZ and CD contributed to study conception and design. FX and TJ wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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