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REVIEW

Immunotherapy for hepatocellular carcinoma patients: is it ready for prime time?

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Abstract Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and the second most common cause of cancer death worldwide. Current treatment options for patients with intermediate and advanced HCC are limited, and there is an unmet need for novel therapeutic approaches. HCC is an attractive target for immunomodulation therapy, since it arises in an inflammatory milieu due to hepatitis B and C infections and cirrhosis. However, a major barrier to the development and success of immunotherapy in patients with HCC is the liver's inherent immunosuppressive function. Recent advances in the field of cancer immunology allowed further characterization of immune cell subsets and function, and created new opportunities for therapeutic modulation of the immune system. In this review, we present the different immune cell subsets involved in potential immune modulation of HCC, discuss their function and clinical relevance, review the variety of immune therapeutic agents currently under investigation in clinical trials, and outline future research directions.

Keywords Hepatocellular carcinoma, HCC · Immunotherapy · Checkpoint blockade · Cancer vaccines · Review article · Cancer immunology

Abbreviations

CIK	Cytokine-induced killer
DFS	Disease-free survival
GPC-3	Glypican-3
HCC	Hepatocellular carcinoma
KC	Kupffer cells
LAG-3	Lymphocyte activation gene 3
MDSC	Myeloid-derived suppressor cells
NKT	Natural killer T
OS	Overall survival
PR	Partial response
SD	Stable disease
T-reg	T-regulatory
TACE	Transarterial chemoembolization
Tim-3	T-cell immunoglobulin mucin 3
TME	Tumor microenvironment

Introduction

Hepatocellular carcinoma (HCC) is the most common liver malignancy, constituting about 80% of primary liver tumors [1]. The incidence of HCC is rapidly rising in Japan, Europe, and North America due to increased incidence of HCV infection and non-alcoholic fatty liver disease, and in Africa and Middle East due to HBV infection [2, 3].

Accepted options for treatment of the early stage HCC include liver resection, liver transplantation, and ablation (radiofrequency or microwave). Patients with intermediate stage disease are selected for locoregional therapy including trans-arterial chemoembolization (TACE) and

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radioembolization. These patients have relatively high 5-year survival rates of up to 70% compared to a dismal 16% in patients with advanced stage disease [4]. The only available treatment for the latter group is sorafenib, a tyrosine-kinase inhibitor that has a limited survival benefit of 3 months [5]. Accordingly, a novel therapeutic approach is desperately needed.

HCC is a uniquely immunosuppressive cancer

There is a growing evidence to suggest that HCC may be considered an immunogenic tumor, arising in an immunosuppressive environment. The chronic inflammation, viral etiology, and cirrhosis underlying the formation of most HCC tumors highlight an intricate relationship between the immune biology and the development of this neoplasm [6]. The liver is constitutively immunosuppressive [6], as it promotes systemic tolerance to foreign antigens [7], which prevents excessive reactions to toxins and antigens draining from the enteric circulation [8]. The HCC tumor microenvironment (TME) has immunosuppressive features due to the chronic nature of the disease and to tolerogenic liver properties that include a combination of:

(a) Active T regulatory cell (T-reg) compartment [9] that will be amply discussed in this work.
(b) Underlying T-cell exhaustion due to the chronic inflammation of underlying chronic liver disease [10].
(c) Abundance of inhibitory myeloid cells or myeloid-derived suppressor cells (MDSC) due to their accumulation in the liver [11]. MDSCs are a heterogeneous population of immature myeloid cells [12, 13] that suppress T-cell responses through the depletion of L-arginine [14] and L-cysteine [15], release of reactive oxygen and nitrogen species [16], and the induction of T-regs [17]. They also inhibit NK-cell cytotoxicity and cytokine secretion [12]. A subset of MDSC ($CD14^{+}HLA\text{-}DR^{-/low}$) was found to inhibit immunity in HCC by inducing T-regs [17]. Increased MDSCs are reported as a negative prognostic indicator when found in the pre-treatment peripheral blood of patients receiving hepatic arterial infusion chemotherapy [18] and post-treatment in patients receiving radiation therapy [19]. Accordingly, MDSCs play a suppressive role in the development of HCC tumor immunity.
(d) Liver resident macrophages or Kupffer cells (KC) make up about 80% of the macrophages in the body [20]. During homeostasis, they maintain immune tolerance through anti-inflammatory functions [21]. KCs can secrete IDO [22] and IL-10 [8, 23] which suppress local immunity. In chemically induced murine HCC, KCs were found to promote carcinogenesis [24], and in HCC patient studies, KCs were found to inhibit anti-tumor $CD8^{+}$ T-cell killing through PD-L1 signaling [25, 26]. Therefore, KCs are pro-tumorigenic cells central to the liver's unique immunosuppressive function.
(e) The immunosuppressive enzyme arginase 1 is commonly expressed by HCC tumor cells [27]. Immunohistochemical detection of arginase 1 in unknown metastases is used to identify an HCC primary source [28].

HCC exploits this immune tolerance to initiate and promote HCC carcinogenesis and progression. These characteristics of HCC may steer immunotherapeutic strategies to those that inhibit immune suppressive mechanisms, rather than directly increase immune effector function.

Biology of immune cells and molecules used in immunotherapy for HCC

T-regs are immune inhibitory cells that occur naturally and function mainly in self-tolerance and prevention of autoimmunity [29]. They are a subset of $CD4^{+}$ T-cells that are $FoxP3^{+}$ [30], and generally express the interleukin 2 receptor CD25 [31, 32] and the activation markers OX40 and CD69 [33]. More recently, three subtypes of $CD4^{+}FoxP3^{+}$ cells are characterized: a resting phenotype ($FoxP3^{low}CD45RA^{+}CD25^{low}$), an inhibitory phenotype ($FoxP3^{hi}CD45RA^{-}CD25^{hi}$), and a non-T-reg pro-inflammatory phenotype ($FoxP3^{low}CD45RA^{-}CD25^{low}$) [34]. T-regs reduce CD8 killer T-lymphocyte function by inhibiting their proliferation, activation, and degranulation [35], through secretion of IL-10 and TGF- β and the action of CTLA4 and CD39/CD73 [33, 36–39]. They also decrease NK-cell cytotoxic activity and IFNγ production [40]. T-regs are found in higher concentrations in HCC tumors and blood of HCC patients compared to those of healthy controls [9, 41]. T-regs are also thought to increase the chronicity of HCV and HBV infections promoting progression to HCC [42, 43]. Increased $FoxP3^{+}$ infiltration in HCC specimens is associated with worse patient survival [30, 35, 44, 45] and higher recurrence rates [46]. HCC induces T-reg formation and potentiates their effect by secreting TGF-β. A higher TGF-β expression in tumor cells is reported to be a marker of worse patient outcomes [47]. $CD8^{+}FoxP3^{+}$ T-regs were characterized in HCC and were found to be associated with more advanced tumor stage [48], suggesting a role in tumor progression. Inhibiting T-reg functions in HCC may be valuable addition to other immunotherapeutic strategies.

$CD8^{+}$ effector T-cells have been repeatedly associated with improved prognosis in several malignancies including breast, lung, colon cancers, and melanoma [49–52]. However, clinical data describing the effects of CD8 tumor infiltration on HCC patient outcomes are conflicting. A recent large study conducted in 499 patients with HCC, found CD8

tumor infiltration to be associated with better patient overall (OS) and disease-free survival (DFS) [53, 54]. The spatial distribution of $CD8^+$ TILs may influence their efficiency in tumor clearance, since their presence in the center of the tumor rather than its margin correlates with more favorable outcomes [53]. Thus, there is a basis for hypothesizing that T-cells recognizing specific HCC antigens may have a role in controlling HCC. Supporting this notion, T-cells were found to react to specific tumor antigens such as glipican-3, NY-ESO-1, hTERT, and p53 in patients with HCC [55–58]. On the other hand, it has been reported that CD8 infiltration in the center of tumors < 3 cm in diameter can be indicative of higher recurrence rates in HBV-associated HCC patients [59]. Several other studies did not identify a correlation between $CD8^+$ TILs and clinical outcomes in HCC [30, 45, 60, 61]. This could be due to the role that other immune cells may play in generating an immune response. In a cohort of 302 HCC patients, longer patient survival was observed in the group of patients with high activated $CD8^+$ TILs/T-reg ratio but not with high $CD8^+$ TIL alone [30]. There is no clear consensus on the prognostic significance of $CD8^+$ T-cell density in HCC tissue; this calls for a better characterization of the role of cytotoxic T-lymphocytes in HCC.

$CD4^+$ T-cells are essential for the establishment of an effective anti-tumor response. They include multiple subtypes that balance the activation of immune cells. Most prominently affecting $CD8^+$ T-cell upregulation and downregulation are the T helper 1 cells and the $FoxP3^+$ T-reg cells, respectively [62]. A recent experimental model described an association between the loss of $CD4^+$ T-cells and HCC development in mice with non-alcoholic fatty liver disease; this association was further validated in human HCC samples [63]. In another study, increasing numbers of $CD4^+$ T-cells expressing granzyme A, B and perforin called $CD4^+$ cytotoxic T-lymphocytes was found to have a positive effect on patient outcomes [64]. The available data indicate an anti-tumorigenic role of $CD4^+$ cytotoxic T-lymphocytes in HCC.

DCs are pivotal antigen-presenting cells in the initiation of host defense against immune insults. They prime the adaptive immune response against HCC cells [65]. Mouse models suggest that tumor antigen pulsed DC vaccines decrease HCC size and increase survival through the activation of natural killer T (NKT) cells and $CD8^+$ and $CD4^+$ T-cells [66]. DC vaccines also potentiate a reduction in immune suppressive T-regulatory cells and tumor growth factor secretion in the TME [67].

NK cells are part of innate immunity, yet they share some features with adaptive immune cells [68]. NK cells can recognize specific ligands on tumor cells such as MHC class I related chains A and B, and support the development of adaptive immunity [68–70]. Their physiological localization in liver sinusoids predisposes them to be more prevalent in liver tumors including HCC than in other cancers. Hepatic NK cells tend to be more cytotoxic than the hematogenous ones and may have a role in immune modulated response to metastasizing tumor cells [71]. Furthermore, the decreased expression of CD155, a modulator of NK-cell function, was found to be associated with worse HCC patient outcomes [72, 73]. NK cells may have a role in the defense against HCC tumors.

Natural killer T (NKT) cells express receptors common to T-cells including CD4 and CD3 [74]. Enhanced reactivity of NKT cells (as identified by combinations of CD8 and NK1.1 markers or CD3 and DX-5 markers) to tumor antigens proved to be effective in suppressing HCC [66, 75]. Activated NKT cells, or cytokine induced killers (CIK), have been investigated in the treatment of HCC patients as will be discussed later.

PD-1 and its ligand PD-L1 constitute an immune regulating checkpoint with a well-established role in cancer progression. Their role in HCC progression is currently being characterized based on their involvement in the development of chronic HBV [76] and HCV [77] infections. PD-L1 expression on HCC tumor cells is a marker of shortened patient DFS [78] and OS [79, 80]. HCC cells tend to minimally express PD-L1 [25] which regulates the interaction between hepatic macrophages and $CD8^+$ $PD\text{-}1^+$ T cells [25, 26]. Provocative results from ongoing clinical trials testing the efficacy of PD-1/PD-L1 blocking antibodies in HCC are presented further along this article.

CTLA4 inhibits T-cell function by competing with an activating surface molecule (CD28) for the binding of CD80 and CD86 on antigen-presenting cells [81]. CTLA4 is also essential for the production of the immune suppressors IL-10 and IDO when expressed on DC in the HCC TME [82]. CTLA4 is active in the HCC TME, making it a popular target for cancer treatment.

Lymphocyte activation gene (LAG-3) is a CD4-like molecule [83] expressed on activated T and NK cells [84]. LAG-3 binds to MHC-II or Galectin-3 and negatively regulates T-cell function [85, 86]. It is an attractive immunotherapeutic target in many cancers alone or in combination [87], especially that LAG-3 does not compete with CD4 for MHC-II binding, and, therefore, does not affect CD4 T-cell-mediated effector functions [88]. In HCC, increased LAG-3 expression correlates with a decrease in the activity of anti-HBV-specific $CD8^+$ T-cells [89]. LAG-3 is currently being investigated as a single target or in combination with anti-PD-1 and PD-L1 in HCC patients.

TGF-β signaling regulates cell differentiation, proliferation, motility, death [90], and angiogenesis [91]. It plays a potent immunosuppressive role in HCC by inhibiting T- and NK-cell activation [92]. Furthermore, it has a central role in epithelial–mesenchymal transition that contributes to HCC metastasis [93, 94]. Increased TGF-β expression in HCC

tumor cells [95] and patient serum [96] are associated with worse patient outcomes. Targeting TGF-β signaling is an attractive strategy for treatment of HCC patients and is currently in clinical trials as discussed later.

A summary of the immune landscape in HCC is illustrated in Fig. 1.

Immunotherapy in HCC management (Fig. 2)

The unique immunosuppressive HCC tumor microenvironment described above makes it an attractive target for immunotherapy, particularly immune checkpoint inhibitors. In this section, we present ongoing clinical trials and currently available results.

(a) *Cancer Vaccines* are conceptually attractive for cancer therapy due to their limited adverse effect profile and potential for antigen-specific anti-tumor effects [97]. Prophylactic vaccines such as the HBV vaccine prevent viral infection and development of chronic liver disease, thus, dramatically decreasing the risk of developing HCC [98]. Therapeutic cancer vac-

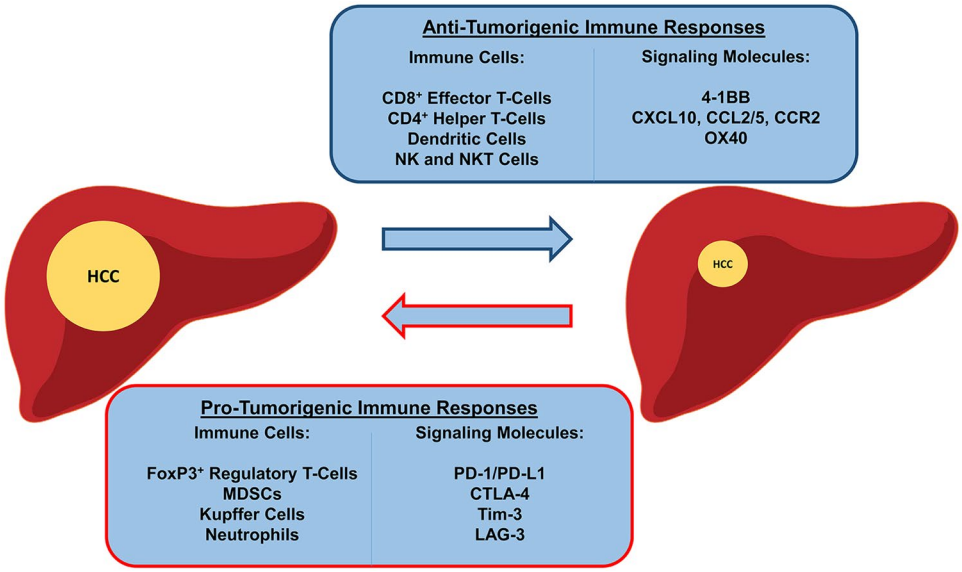

Fig. 1 Immune landscape in HCC. The immune cell subsets involved in tumor promotion and tumor suppression. *CCL* chemokine ligand, *CCR* chemokine receptor, *CXCL* chemokine C-X-C motif ligand, *LAG-3* lymphocyte activation gene, *OX40* CD134, *Tim-3* T-cell immunoglobulin mucin 3

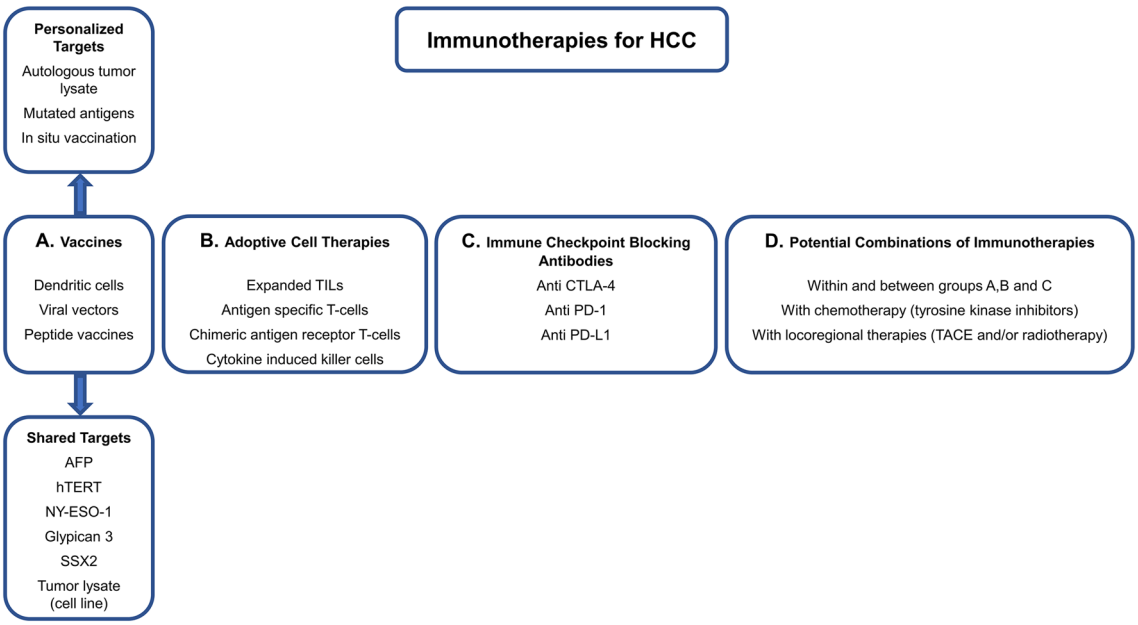

Fig. 2 Current immunotherapeutic strategies. Immunotherapeutic opportunities available for current and future clinical trials, and possible combinations. *TACE* transarterial chemoembolization

cines are designed to target TAA which are specific for tumor cells or overexpressed on cancer cells. HBV and HCV antigens are unique to the HCC malignancy and present attractive therapeutic targets [99]. Several vaccination strategies are used to target cancer cells including tumor lysate that contains all TAAs and peptide vaccines that contain specific TAAs or full-length proteins. Those targets can also be pulsed on dendritic cells and some can be encoded in DNA or viral vectors (Table 1). These vaccines prime the immune system to target TAAs presented by MHC-I or MHC-II molecules in the TME and tumor draining lymph nodes. Among the most extensively explored cancer vaccine targets in HCC are: carcinoembryonic antigen, NY-ESO-1, alpha-fetoprotein, glypican-3 (GPC-3), melanoma-associated antigen, and human TERT [100]. While the majority of cancer vaccines have demonstrated enhanced immune responses against the targets, clinical efficacy has been limited [101] (Table 1). Glypican-3, the most studied target for HCC therapy, is overexpressed in 80% of HCC tumors [102]. GPC-3 expression in tumor cells is associated with decreased patient survival [103]. In clinical trials, a peptide vaccine targeting GPC-3 led to disease control [partial response (PR) and stable disease (SD)] for 2 months in 61% of patients (3% PR and 58% SD of which 21% had responses that do not meet criteria for PR), was well tolerated, and patients with higher anti GPC-3-specific lymphocyte frequencies had better OS [104]. In a follow-up phase II single arm trial, this vaccine resulted in a trend toward decreased recurrence rates after surgery in patients with GPC-3 positive tumors compared to selected case–control patients (24% compared to 43% with surgery alone, $p = 0.054$, Table 1) [105] with minimal side effects.

Table 1 Cancer vaccine clinical trials for HCC patients

Trial Identifier	Phase	Vaccine	Route	Target	Patient population	*N*	Status	Results
UMIN-CTR# 000001395	I	Peptide	Intradermal	GPC-3	Advanced disease, HLA-A2 or A24	33	Complete	PR (3%), SD (58%) [104]
UMIN-CTR# 000002614	II	Peptide	Intradermal	GPC-3	Post resection and RFA	41	Complete	Improved 1 year RFS vs surgery only [105]
NCT01522820	I	Fusion protein (with DEC-205)/sirolimus	Intranodal	NY-ESO-1	Post resection and TACE, NY-ESO-1 expressing tumors	30	Ongoing	NA
NCT02133079	I/II	Autologous tumor derived protein	NA	Heat Shock Protein gp96	Post resection	20	Ongoing	NA
NCT02232490	III	HBV-HCV derived antigens	NA	Hepcortespenlisimut-L	Advanced disease	100	Ongoing	NA
NCT02256514	II	HBV-HCV derived antigens	Oral	Hepcortespenlisimut-L	Advanced disease	120	Ongoing	NA
NCT02338778	I/II	DPT, staphylococcus aureus, typhoid and paratyphoid	Intravenous	Multiple vaccines as adjuvants	All	20	Complete	NA
NCT01974661	I	DC	Intratumoral	All TAAs	After TACE ± sorafenib	18	Ongoing	NA
NCT01828762	I	DC	Subcutaneous	Irradiated tumor cells	1ary HCC after TACE	8	Complete	NA
NCT00004604	I	DC	Intravenous	CEA	All CEA expressing solid tumors	24	Complete	NA
NA	II	DC	Intravenous	HepG2 cell line	Advanced disease	35	Complete	PR (4%), SD (24%) [106]
NCT00629759	I	Oncolytic virus JX-549 + Sorafenib	Intratumoral	All TAAs	Non-resectable	14	Complete	Well tolerated, decreased tumor perfusion and necrosis [137]
NCT02509507	I	T-VEC	Intratumoral	All TAAs	Advanced HCC and liver metastases	100	Ongoing	NA

CEA carcinoembryonic antigen; *DPT* diphtheria, pertussis, tetanus; *NA* not available; *RFA* radiofrequency ablation; *RFS* recurrence free survival; *T-VEC* talimogene laherparepvec

Patient-derived DCs present another popular method of cancer vaccine delivery. This treatment consists of isolating patient's DCs and pulsing them with a specific antigen or tumor lysate then re-infusing them. Multiple studies used this vaccination approach to target a variety of antigens. In HCC patients, one trial tested the efficiency of DCs pulsed with tumor lysate in 35 patients with unresectable disease. Most patients had increased IFN-γ and decreased AFP levels following vaccination, suggesting immunological response. Disease control rate according to the International Union Against Cancer Criteria for ≥ 3 months was 28% with no significant toxicities observed (PR in 1 patient and SD in 6) [106]. Targeting tumor antigens using peptide vaccines administered directly or pulsed on DCs is an attractive modality for the treatment of HCC that should be combined with immune checkpoint inhibitors in clinical trials.

Intratumoral in situ vaccination using oncolytic virus with or without immune adjuvant is another approach that leads to the release of tumor antigens. A phase 2 study of the intratumoral vaccinia virus (JX-549) in 30 patients with unresectable HCC showed a 50% disease control rate at 8 weeks and median survival of 14.1 month and resulted in a single-grade 4 toxicity. A GM-CSF producing oncolytic herpes simplex virus (T-VEC) was approved for treatment of unresectable stage IIIB/C and IV melanoma in October 2015. T-VEC is currently in a phase I study for advanced HCC (NCT02509507). There may be promise for extending the benefits of this approach to HCC, alone or in combination with other modalities.

(b) *Adoptive cell therapy* is a novel immunotherapy method where patient-derived T or NKT cells are expanded and activated ex vivo then re-infused. Based on the source and the method used for cell activation, adoptive cell transfer could be classified into: (1) TILs, (2) engineered T-cells that are specific for cancer antigens, (3) T-cells that express a chimeric antigen receptor consisting of antibody bound to the T-cell receptor's intracellular domain, and (4) CIKs consisting of $CD3^+$ $CD56^+$ NKT cells activated with a cocktail of anti-CD3 antibodies, IL-2, IL-1α, and IFNγ [107]. Treatment with T-cells specific to AFP is showing promising results in pre-clinical studies and a clinical trial using this approach is currently ongoing [108]. Furthermore, a retrospective study of over 400 case/control HCC patients receiving surgery and CIK or surgery alone showed a significant survival benefit of CIK administration in multivariate analysis [109]. The same group showed, in a randomized controlled study including 200 patients, that CIK treatment significantly prolonged median time to recurrence, but did not significantly prolong OS or DFS [107] compared to standard treatment. In Korea, another randomized controlled trial included 230 patients with the early stage HCC post-complete resection. Patients who received CIK post-op had significantly lower hazard ratio of any death and of cancer-related deaths [110]. Similar results were also found in a systematic review that included 13 randomized phase II and III studies [111]. The adoptive cell transfer therapy is currently under investigation in many solid tumors including HCC and the results of these studies are eagerly awaited (Table 2).

(c) *Immune checkpoint blockade* since 2014, the FDA has approved checkpoint blocking antibodies for patients with melanoma, lung, head and neck, bladder and renal cancers, Hodgkin lymphoma, and multiple myeloma. The indication of immune checkpoint inhibitors is likely to extend beyond these indications. Many clinical trials are currently testing immune checkpoint blockade in HCC (Table 3). The CTLA4 checkpoint inhibitor tremelimumab was studied in 20 HCC patients in the setting of chronic HCV cirrhosis and mostly Child–Pugh class B, at a dose of 15 mg/kg every 90 days [112]. Disease control was achieved in 76% (18% PR, 59% SD, according to RECIST criteria) of the 17 patients assessed for response at 3 months, along with an observed decrease in HCV viral load in most patients. Importantly, no severe immune-mediated adverse events occurred, and steroid rescue was not required. The PD-1 inhibitor nivolumab has been studied in 262 HCC patients in the non-comparative phase 1/2 CheckMate-040 trial. Disease control was achieved in 64% of the 214 patients treated with nivolumab at 3 mg/kg (1% complete response, 18% PR, 45% SD, according to RECIST criteria); follow-up is currently ongoing. Nivolumab demonstrated limited antiviral activity; responses were independent of previous sorafenib treatment, HBV or HCV infection, and tumor cell PD-L1 expression. PD-L1 was expressed on ≥ 1% of tumor cells in 20% of assessed tumors and in 26% (95% confidence interval: 13–44%) of tumors with objective response [113]. A subsequent randomized phase of this study comparing nivolumab to sorafenib is ongoing. CheckMate-459 trial is also ongoing [114]; it is a phase III randomized, multi-centered trial of nivolumab vs sorafenib in patients with Child–Pugh Class A cirrhosis in 726 patients (NCT02576509, Table 3). Another PD-1 blocking therapy, pembrolizumab, is currently being investigated in the second-line setting in HCC (NCT02702401). Biomarkers of immune response to immune checkpoint blockade including PD-L1 expression on tumor cells, lymphocytic infiltration, and mutational load warrant thorough testing in the HCC setting. These biomarkers have the potential to narrow patient selection and, therefore, increase response rates. The outcome of immune

Table 2 Adoptive cell transfer clinical trials for HCC patients

Trial identifier	Phase	Cell type	Target	Patient population	*N*	Status	Results
NCT00699816	III	T-cells	All TAAs	Stage I or II after curative resection	230	Complete	NA
NCT00769106	III	CIK	All TAAs	After radical resection	200	Complete	Prolonged median time to recurrence [107]
NA	III	CIK	None	After radical resection	230	Complete	Prolonged OS and CSS [110]
NCT01147380	I	NK cells, IL-2 stimulated	All TAAs	After liver transplant	18	Complete	0% adverse events
NCT01462903	I	TILs, IL-2 stimulated	All TAAs	Metastatic	20	Ongoing	NA
NCT01758679	IV	CIK	I-131	After resection	120	Ongoing	NA
NCT01801852	I	NKT cells	All TAAs	Metastatic	300	Ongoing	NA
NCT01821482	II	DC and CIK	All TAAs	After resection or TACE	100	Ongoing	NA
NCT01897610	II	T-cells and/or Sorafenib	All TAAs	Stage III or IV	40	Ongoing	NA
NCT02008929	II	NK	All TAAs	After resection	5	Ongoing	NA
NCT02026362	I/II	DC and T-cells	17 total TAAs	After resection or RFA	100	Ongoing	NA
NCT02425735	I/II	DC-CIK and/or T cell	I-131	After resection	40	Complete	NA
NCT02487017	II	DC and/or CIK	All TAAs	After TACE	60	Ongoing	NA
NCT02568748	III	CIK	All TAAs	After TACE	20	Ongoing	NA
NCT02632006	I/II	T cell	PD-1	Advanced	40	Ongoing	NA
NCT02632188	I/II	T cell	Multiple TAAs	After resection	60	Ongoing	NA
NCT02638857	I/II	T cell	Multiple TAAs	After TACE	60	Ongoing	NA
NCT02662348	I	T cell	CD3 + HER-2	All, HER-2 expressing tumors	6	Ongoing	NA
NCT02678013	III	T cell	All TAAs	After RFA	210	Ongoing	NA
NCT02709070	III	T cell	All TAAs	After resection	210	Ongoing	NA
NCT02715362	I/II	T cell	GPC-3	Unresectable GPC-3 expressing HCC	30	Ongoing	NA
NCT02723942	I/II	T cell	GPC-3	Advanced GPC-3 expressing HCC	60	Ongoing	NA

CSS cancer-specific survival, *NA* not available

checkpoint inhibitors in HCC is promising and is likely to gain more traction as the final results of these studies are revealed.

(d) *TGF-β receptor inhibitor* a novel immunotherapeutic modality constituted of a small molecule galunisertib (LY2157299) which inhibits TGF-β receptor signaling is being investigated in the treatment of HCC patients. As discussed earlier, TGF-β signaling has immunological role in promoting HCC progression. Interim analysis from 109 HCC patients who progressed on sorafenib and treated with galunisertib showed decrease in AFP levels by > 20% after treatment in 24% of patients. Patients who had a decrease in AFP had a longer median OS (21 vs 7 months, $p = 0.0006$) [115].

(e) *Combination therapies* several locoregional therapies have been shown to increase antigen presentation and elicit significant immune responses in HCC. Increased infiltration of dendritic cells and activated T-cells has been demonstrated in patients treated with radiofrequency ablation [116, 117]. Similar immune stimulation has been documented in patients treated with TACE [54]. These ablative and locoregional therapies may function as autologous cancer vaccines by abruptly exposing cancer antigens to the immune system. In some instances, shrinkage of distant untreated tumors (abscopal effect) was observed [118, 119]. A recent study of HCC patients (Child–Pugh class A/B7 cirrhosis) treated with tremelimumab followed by TACE or radiofrequency ablation showed a 23% partial response rate in patients evaluable for response outside of locally treated lesions [120]. While no complete responses were reported, there was a tolerable adverse event profile, with pruritus occurring most commonly. Interestingly, responders had increased CD4/T-reg and CD8/T-reg ratios and a marked decrease in HCV viral load.

A recent study combined cyclophosphamide which suppresses T-reg activity [121] with low-dose hepatic radiation (3.5 Gy over 3 days) and adjuvant intratumoral injection of

Table 3 Immune checkpoint inhibitors and combination therapy clinical trials for HCC patients

Trial identifier	Phase	Target	Drug	Other treatments	Patient population	*N*	Status	Results
NCT01008358	II	CTLA4	Tremelimumab	None	Chronic HCV + unresectable	20	Completed	NA
NCT02595866	I	PD-1	Pembrolizumab	None	Unresectable	39	Ongoing	NA
NCT01853618	I	CTLA4	Tremelimumab	TACE or RFA	Advanced	100	Ongoing	PR: 23% [120]
NCT02239900	I/II	CTLA4	Ipilimumab	SBRT	Unresectable	120	Ongoing	NA
NCT02576509	III	PD-1	Nivolumab	Sorafenib	Advanced	726	Ongoing	NA
NCT02702401	III	PD-1	Pembrolizumab	Best supportive care	Resistant to sorafenib	408	Ongoing	NA
NCT01658878	I	PD-1 CTLA4	Nivolumab and/or ipilimumab	Sorafenib	Advanced	91	Ongoing	ORR: 9%. And 6mo OS rate: 69% [138]
NCT02821754	I/II	CTLA4 PD-L1	Durvalumab and/or tremelimumab	TACE or RFA	Resistant to sorafenib and chemotherapy	90	Ongoing	NA
NCT02519348	I/II	CTLA4 PD-L1	Tremelimumab and/or durvalumab	None	Unresectable	144	Ongoing	NA
NCT02572687	I	PD-L1 VEGF	Ramucirumab and durvalumab	None	Resistant to sorafenib	114	Ongoing	NA
NCT02795429	I/II	cMET PD-1	Capmatinib and/or PDR001	None	Advanced	108	Ongoing	NA
NCT02562755	III	Oncolytic virus	Pexa Vec and sorafenib	Sorafenib	Advanced	600	Ongoing	NA

cMET tyrosine-protein kinase MET, *ORR* overall response rate, *RFA* radiofrequency ablation, *SBRT* stereotactic body radiation therapy

poly-ICLC (a Toll-like receptor-3 agonist) along with arterial embolization [122]. This study included 25 liver cancer patients and showed a mean survival of 26 months, two patients were down-staged and proceeded to transplantation, and one patient was alive at 87 months. This locoregional therapy modality may also be used in the future for tumor immune-embolization by locally injecting antibodies against specific immune targets with the goal of inducing a localized and systemic immune response.

Tyrosine-kinase inhibitors such as sorafenib have immunomodulatory effects including reducing T-regs [123] and inhibiting MDSC [124]. Sorafenib is currently investigated in combination with anti-PD-1 (PDR001) in HCC. The result of this study may reveal a synergistic effect of these two agents; however, the toxicity profile of combining these two modalities needs to be clearly determined.

Many immune checkpoint inhibitors are currently investigated in combination in HCC based on the promising outcomes of single agents. Anti-CTLA4 (tremelimumab) and anti-PD-L1 (durvalumab) are currently being evaluated in combination with TACE or radiofrequency ablation and compared to single immune checkpoint inhibitor in patients with unresectable HCC with or without HBV or HCV who progressed on sorafenib [125]. Combination of anti-CTLA4 (ipilimumab) and anti-PD-1 (nivolumab) is also currently being investigated in clinical trials (Table 3). The orally administered anti-TGF-β (galunisertib) in combination with nivolumab are ongoing supported by pre-clinical data that showed silencing the TGF-β pathway markedly increases sensitivity to anti-CTLA4 and anti-PD-1 antibodies [126] (Table 3). The role for combination therapies in HCC remains to be determined. These combinations should be investigated based on solid rationale for synergy and should be compared to single checkpoint blockade to determine the relative risk/benefit of additional treatment.

Immune targets not yet explored in HCC patients

4-1BB (CD137) is a member of the TNF receptor superfamily. It is expressed on T- and NK-cell membranes, where its ligation inhibits apoptosis and enhances proliferation and effector functions [127]. 4-1BB is expressed on lymphocytes from tumor margins of HCC patients [128]. The therapeutic use of antibodies agonist to CD137 showed promising results in HCC animal models [129, 130] and is currently investigated in clinical trials in various malignancies.

CD134 or OX40 is a TNF receptor that has a co-stimulatory function when expressed on T-cells. Targeting OX40 along other immune-related molecules increased CD8 and CD4 T-cell activation in vitro [131] and increased survival

in a mouse model bearing HCC [132]. Interestingly, in HCV-induced HCC, OX40 was observed to have an immune inhibitory function when expressed on T-regs [133, 134], suggesting that the effectiveness of targeting this molecule will be partially dependent on the ratio of effector/regulatory T-cells in the tumor microenvironment. OX40 targeting antibodies are currently investigated in clinical trials [135, 136]. The role and therapeutic use of OX40 and other TNF receptors have yet to be explored in the HCC setting.

Summary and future direction

Emerging data described in this article provide evidence to support the clinical investigations of novel immunotherapies in HCC. The final results of the ongoing trials including CheckMate-040 trial is crucial for further combination immunotherapy development in HCC based on efficacy and safety profile. Future research should explore biomarkers for response to immunotherapy in HCC beyond PD-L1 expression mechanisms of resistance to immunotherapy (adaptive immune resistance due to increase suppressor receptors), novel target antigens (neo-antigens), and the concept of locoregional immunoembolization in combination with immune checkpoint inhibitors. Future clinical trials should be designed to study these elements. This can be mostly achieved by incorporating pre- and post-treatment biopsies and by encouraging trials for combinations of therapies based on scientific rationale. Indeed, this is the beginning of a new era for HCC treatment that is likely to expand in the near future.

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Compliance with ethical standards

Conflict of interest Osama Rahma receives research support from Merck and is a speaker for activities supported by educational grants from Bristol-Meyers Squibb and Merck. Craig Slingluff Jr. received material from Merck for an ongoing clinical trial. All other authors have nothing to report.

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