Review

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Targets for immunotherapy of liver cancer

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Summary

Drug development in hepatocellular carcinoma (HCC) has been characterised by many failures in the past. Despite good rationales and promising phase II data, many phase III trials failed. Immunotherapy represents an alternative treatment approach that has been successful in many different cancer types. As an inflammation induced cancer, HCC represents a very interesting target for immune based approaches. Indeed, early results from clinical trials testing immune checkpoint inhibitors are not only promising, but have already led to evaluation in a phase III setting. Herein, we summarise our current knowledge on the rationale, mechanism of action and clinical data for immune checkpoint blockade in HCC. In addition, we provide an overview of other novel immune based approaches currently under development for the treatment of HCC, such as adoptive cell based and antibody-based approaches.
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Introduction

The story of drug development for hepatocellular carcinoma (HCC) has been disappointing. Many drugs have failed in phase III trials, in the past eight years.[1] Only the RESORCE trial, testing regorafenib in patients progressing on sorafenib, resulted in increased survival.[2] Immune based approaches focused on vaccination strategies, cytokines or non-specific T cell activation have been tested for many years in HCC, mostly with disappointing results.[3,4] However, the era of immune-oncology has changed dramatically with the FDA approval of immune checkpoint inhibitors for the treatment of different cancer types (Table 1). In 2013, the journal *Science* declared cancer immunotherapy as the breakthrough of the year,[5] and in the two last years, the American Society of Clinical Oncology has considered immunotherapy the Advance of the Year. As of today, the FDA has approved six different immune checkpoint inhibitors, sparking great interest in immune based treatment approaches for patients with HCC. Initial results from three published clinical trials using immune checkpoint inhibitors (tremelimumab [anti-CTLA-4] and nivolumab [anti-PD-1]) as well as preliminary results from other ongoing trials published as abstracts, suggest a promising role for immunotherapy in the treatment of HCC (Table 2). One immune checkpoint inhibitor (nivolumab) has already been approved in the US as a second line treatment and is currently being tested against sorafenib in a phase III trial in the first line setting (NCT02576509).

Herein, we describe the rationale and mechanism of action of immune interventions for the treatment of patients with HCC, with particular emphasis on immune checkpoint inhibitors (Fig. 1). We summarise the data currently available and ongoing clinical trials. We discuss future developments and provide an overview of alternative immune based treatment options for HCC.

Checkpoint inhibitors: development and mechanisms of action

Immune checkpoints are a specific subtype of membrane-bound molecules that fine tune the immune response. Different cell types involved in the immune response express immune checkpoints, including B and T cells, natural killer (NK) cells, dendritic cells (DC), tumour-associated macrophages, monocytes, and myeloid-derived suppressor cells (MDSC). The physiological function of these complexes is to prevent continuous T cell effector function upon initial stimulation and engagement of antigen-specific T cells. Thus, most of these molecules display an immunosuppressive activity that prevents uncontrolled T cell responses against infection, limiting collateral tissue damage. The immune checkpoints most studied in human cancer are cytotoxic T lymphocyte protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), lymphocyte activation gene 3 protein (LAG-3), B and T lymphocyte attenuator (BTLA), and T cell immunoglobulin and mucin-domain containing (TIM-3). A comprehensive review of their variety and functions is provided in.[6,7]

CTLA-4 is essential for the activation of $CD4^+$ T cells and the priming phase of the immune response. Expressed on activated T cells, CTLA-4 has great affinity for CD80 and CD86. Thus, it may antagonise the interaction of CD28 with these receptors, resulting in decreased T cell activation upon antigen presentation. Regulatory T cells (Treg) also express CTLA-4 constitutively. Treg are CD4+ T cells that can be characterised by the presence of CD25, CTLA-4, CD62L and FoxP3 molecules in their membrane. Activated by T cell receptor (TCR) engagement, concurrent with IL-10 and TGF-β signalling, Treg inhibit the immune response through various mechanisms including depletion of IL-2 and secretion of immunosuppressive factors, such as TGF-β, IL-10 or adenosine, as well as competition with co-stimulatory CD28

Key point

Over the last decade, identification and increasing knowledge of the role of immune checkpoint molecules has fostered the development of a new class of therapeutic agents.

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Table 1. Immunotherapy agents approved by FDA for the treatment of cancer.

Disease	Class of agent(s)
AIDS-related Kaposi	interferon alpha-2b
Hairy cell leukaemia	interferon alpha-2b
Lymphoma (Hodgkin and non-Hodgkin)	anti-PD-1 mAb & interferon alpha-2b
Merckle cell carcinoma	anti-PD-L1 mAb
Urothelial carcinoma	anti-PD-1 & anti-PD-L1 mAb
Melanoma	anti-CTLA-4 mAb and anti-PD-1 mAb
	interferon alpha-2b and interleukin 2
	oncolytic HSV-1 encoding GM-CSF
Non-small cell lung cancer	anti-PD-1 and anti-PD-L1 mAb
Prostate carcinoma	autologous DC vaccine against PAP
Renal cell carcinoma	anti-PD-1 mAb and interleukin 2
Squamous cell carcinoma of the head and neck	anti-PD-1 mAb
Hepatocellular carcinoma	anti-PD-1 mAb

DC, dendritic cells; GM-CSF, granulocyte-macrophage colony stimulating factor; HSV-1, herpes simplex type-1 virus; mAb, monoclonal antibody; PAP, prostatic-acid phosphatase.

via CTLA-4. Hence, CTLA-4 is also required for Treg to exert its suppressive activity on activated T cells.[8] But the role of CTLA-4 is not restricted to the priming phase. Inside the tumour, CTLA-4 also promotes immunosuppression by inducing Treg activity and differentiation, and up-regulating IDO and IL-10 in DC.[9]

PD-1 is a key factor in the effector phase of the immune response. It is expressed by activated CD8 + and CD4+ T cells, B cells, NK, Treg, MDSC, monocytes and DC. PD-L1 and PD-L2 are the ligands of PD-1. PD-L1 is expressed in hematopoietic cells, including antigen presenting cells (APC) and MDSC, and in different types of parenchymal cells, while PD-L2 expression is limited to the hematopoietic compartment. Various cytokines up-regulate PD-L1, particularly IFN-γ. Upon binding to its ligands, PD-1 inhibits CD8+ T cell activation by blocking TCR signalling, and inhibits CD4+ activation and proliferation through increased secretion of IL-10. Cancer cells may also express PD-L1 and PD-L2 and use this mechanism to escape from immunosurveillance. Indeed, in a situation of chronic antigen exposure such as the tumour microenvironment, IFN-γ produced by TAA-specific T cells induces PD-1 expression on reactive T lymphocytes and up-regulates PD-L1 in APC and tumour cells. PD-1–PD-L1 engagement then blocks TCR signalling and inhibits T cell proliferation and secretion of cytotoxic mediators, in a process called T cell exhaustion.[10] IFN-γ release enhances the expression of PD-L1 under the hypoxic conditions present in most tumours.

TIM-3 is a transmembrane protein expressed on cells of the innate and adaptive immune system that interacts with several ligands, including phosphatidylserine on the membrane of apoptotic cells, galectin-9 and others. Galectin-9 is a soluble protein produced by cells from many different tissue types (including the liver) that regulates cell differentiation, adhesion and cell death. Evidence indicates that galectin-9 suppresses T cell responses, which supports the concept that TIM-3 acts as an inhibitory receptor for T cells. Furthermore, CD8+ Tim-3+ T cells co-express PD-1 in animal models, and these dual-expressing cells exhibit greater defects in both cell-cycle progression and production of effector cytokines, *e.g.* IL-2, TNF, and IFN-γ, than cells that express PD-1 alone. Thus, the TIM-3 pathway may cooperate with the PD-1 pathway to promote the development of a severe dysfunctional phenotype in CD8 + T cells in cancer.[11]

LAG-3 is a membrane protein that binds MHC class II molecules with high affinity, reducing the co-stimulatory functions of DC. LAG-3 is not expressed on resting T cells but is upregulated upon activation. It is a marker of exhausted T cells and acts synergistically with PD-1 to promote cancer evasion from immunity.[12,13] Finally, BTLA is an immunoglobulin-like molecule expressed by several immune cells including B and T lymphocytes, NK cells and APCs. BTLA is able to inhibit T cell proliferation and cytokine production upon binding to its ligand, herpesvirus entry mediator (HVEM), which can be expressed in HCC.[14,15]

Table 2. Efficacy data from clinical trials of immune checkpoint inhibitors in advanced hepatocellular carcinoma.

Agent, dose	n	BCLC stage	Sorafenib exposure	ORR/DCR	TTP	OS	Refs.
Tremelimumab 30 mg q 3 months	21	3/6/12	Naïve, intolerant or progressed to sorafenib	3/17 (17.6%) PR 13/17 (76.4%) DCR	6.48 months	8.2 months	[16]
Tremelimumab 10 mg q 28 days + ablation	32	-/7/21	Progressed to sorafenib	5/19 (26.3%) PR	7.4 months	12.3 months	[17]
Nivolumab 3 mg/kg q 15 days*	80		Naïve to sorafenib	1/80 (1.2%) CR 17/80 (21.2%) PR 50/80 (62.5%) DCR	Not reported	28.6 months	[23]
Nivolumab 3 mg/kg q 15 days*	182		Intolerant or progressed to sorafenib	7/182 (3.8%) CR 27/182 (14.8%) PR 114/182 (62.6%) DCR	Not reported	15.6 months	[23]

BCLC, Barcelona Clinic Liver Cancer; DCR, disease control rate; ORR, overall response rate; OS, overall survival; TTP, time to progression.
* Dose used in the expansion cohort.

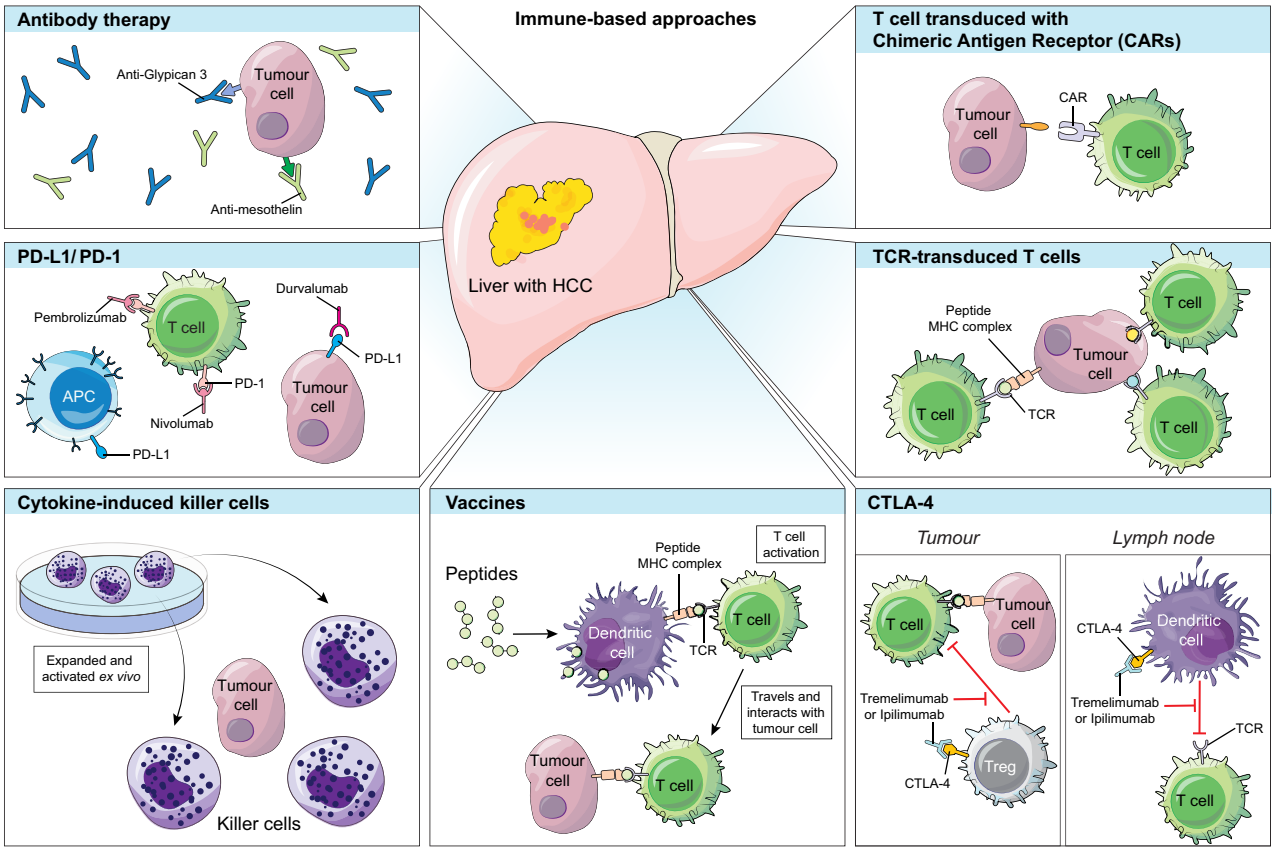

Fig. 1. Immune based approaches in HCC. APC, antigen presenting cell; CTLA-4, cytotoxic T lymphocyte protein 4; HCC, hepatocellular carcinoma; MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TCR, T cell receptor.

Clinical experience with the use of checkpoint inhibitors in hepatocellular carcinoma

In the field of HCC, clinical development has so far focused on CTLA-4 and PD-1/PD-L1 pathways. Among CTLA-4 targeted therapies, tremelimumab (a fully human IgG2 monoclonal antibody) was the first molecule clinically evaluated in HCC. A phase II, non-controlled, multicenter trial targeted the population of patients with HCC and chronic HCV infection who were not eligible for surgery or locoregional therapy.[16] The trial had the dual intention of testing the antitumour and antiviral activity of tremelimumab in a single study. The study was 80% powered to reject the null hypothesis that objective response rate would not exceed 5% at a 0.05 level of significance if the true objective response rate was >25%. Based on a Simon's optimal 2-stage design, three tumour responses among 17 evaluable patients were needed to reject the null hypothesis. Twenty-one patients with advanced disease (57% were at BCLC C stage) were enrolled, most of them (57%) having progressed on previous therapies. Importantly, a significant proportion of patients (42.9%) were in Child-Pugh stage B, indicating some degree of liver dysfunction. Patients received what we now know is a suboptimal dose of 15 mg/kg tremelimumab every 90 days for a maximum of four doses, unless tumour progression or unacceptable toxicities occurred. Despite this suboptimal dosing, three partial responses were observed among 17 evaluable patients and the trial was found to be positive based on the initial assumptions. Stable disease was the best response in 10 additional patients, accounting for a remarkable disease control rate of 76.4%. Importantly, almost half (45%) of these stabilisations lasted longer than six months. Among 11 patients that had alpha-fetoprotein levels higher than 100 ng/ml at baseline, 36% showed a >50% drop following treatment, providing further evidence of antitumour activity. Median time to progression was 6.48 months (95% CI 3.95–9.14 months). Although potentially biased by a long tumour assessment interval, this prolonged time to progression compares favourably with several targeted agents (Table 3). The observed overall survival of 8.2 months (95% CI 4.64–21.34 months) was similar to that observed in patients receiving placebo in second-line trials, but the high proportion of Child B patients in this cohort likely had a significant negative impact on this outcome.

Key point

In patients with advanced hepatocellular carcinoma, immune stimulation by means of CTLA-4 blockade with Tremelimumab has provided strong signals of antitumour efficacy in pilot clinical trials.

Table 3. Combination therapies based on PD-1/PD-L1 blockade under study for the treatment of hepatocellular carcinoma.

Anti-PD-1/PD-L1 agent	Combining agent	Mechanism of action	Patients	Population	NCT number
Combinations with other immunotherapies					
Nivolumab	Ipilimumab	anti-CTLA-4	620*	HCC	01658878
Durvalumab	Tremelimumab	anti-CTLA-4	144	HCC	02519348
Nivolumab	Pexavec	GMCSF-armed oncolytic virus	30	HCC	03071094
Combinations with antiangiogenics					
Durvalumab	Ramucirumab	anti-VEGFR2 mAb	114	HCC and other histologies	02572687
Pembrolizumab	Lenvatinib	TKI	30	HCC	03006926
Pembrolizumab	Nintedanib	TKI	18	HCC and other histologies	02856425
SHR-1210	Apatinib	TKI	30	HCC and other histologies	02942329
PDR001	Sorafenib	TKI	50	HCC	02988440
Combinations with other targeted agents					
Nivolumab	Galunisertib	TGF-beta inhibitor	75	HCC	02423343
Nivolumab	CC-122	Pleiotropic pathway modifier	50	HCC	02859324
Pembrolizumab	XL888	Hsp90 inhibitor	50	HCC and other histologies	03095781
PDR001	INC280	c-met inhibitor	108	HCC	02474537
PDR001	FGF401	FGFR4 inhibitor	238	HCC	02325739
Combinations with locoregional therapies					
Nivolumab	TACE	Ischaemia	14	HCC	03143270
Nivolumab	Y90	Beta radiation	40	HCC	03033446
Nivolumab	Y90	Beta radiation	35	HCC	02837029
Pembrolizumab	Y90	Beta radiation	30	HCC	03099564

CTLA-4, cytotoxic T lymphocyte protein 4; GM-CSF, granulocyte-macrophage colony stimulating factor; HCC, hepatocellular carcinoma; mAb, monoclonal antibody; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TACE, transarterial chemoembolisation TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.
* Includes nivolumab monotherapy.

Regarding safety, tremelimumab was well tolerated, with few patients experiencing grade 3 disabling adverse events, even in the presence of liver dysfunction among patients in the Child-Pugh B class. No patient received systemic steroids and there were no treatment-related deaths. An itching skin rash was the most frequent adverse event (65%), which was successfully managed with topical agents and oral antihistamine drugs. Diarrhoea occurred in 30% of patients, but only reached grade 3 in one patient. A remarkable rise in serum transaminases was observed after the first dose in more than half of the patients, grade 3 or higher in 45% of cases, but with no other signs of liver dysfunction. This effect on transaminases was transient, did not recur in the following cycles, and was not related to the antitumour or antiviral responses, or with changes in circulating cytokines.

Following the same path, a second trial tested a very appealing hypothesis, namely whether antigenic stimulation caused by incomplete tumour ablation using percutaneous radiofrequency (RFA) or transarterial chemoembolisation (TACE) could safely enhance the effects of tremelimumab.[17] The rationale for this combination is based on the fact that RFA or TACE could induce immunogenic tumour cell death, which in turn could stimulate a peripheral systemic immune response, potentially amplified by immune checkpoint blockade. In a phase I/II trial, increasing doses of tremelimumab were followed by subtotal tumour ablation, tumour response was then evaluated in those lesions not targeted by RFA, cryoablation or TACE procedures. This was a pilot study with no specific sample size assumptions. It enrolled 32 patients mostly with advanced HCC (75% at BCLC C stage), 78% having progressed on previous therapies. Therefore, patient characteristics were quite similar to the previous study, except that liver function was preserved in the vast majority of patients, with only 14% of patients in Child-Pugh class B. Viral hepatitis was the cause of liver cirrhosis in most patients (75%). Enrolled patients were treated with an optimal dose of tremelimumab at two dose levels (3.5 and 10 mg/kg IV) given every four weeks for a total of six doses, followed by 3-monthly infusions until off-treatment criteria were met. The radiological intervention (TACE for BCLC B and thermal ablation for BCLC C patients) was performed five weeks after the first dose of tremelimumab. Nineteen patients were evaluable for response because they had measurable lesions that were not targeted by RFA or TACE. Of these patients, partial response was recorded in five patients (26%), and stable disease in 12 patients (63%), accounting for a disease control rate of 89%. Again, almost half (45%) of the stabilisations lasted longer than six months and median time to progression was 7.4 months (95%CI 4.7–9.4 months). Given the small number of patients in both tremelimumab trials, the small differences in response rates and time to progression seem of little relevance, but they indicate a consistent antitumour effect. The better overall survival of 12.3 months (95% CI 9.3–15.4 months) in the combination trial could be explained by the good liver function, but a true enhancing effect of prior ablation cannot be ruled out. Regarding safety, one relevant observation was that there was no clear trend in adverse events across the different dose cohorts. The most

common clinical toxicity was pruritus, although less frequent than in the previous trial (9%), and predominantly grade 1. Less frequent side effects were diarrhoea (6%), autoimmune pneumonitis (3%) and angioedema (3%). Again, the most frequent laboratory alteration was hypertransaminasemia, which occurred in 34% of patients and was grade 3 or 4 in 21% of them.

Tremelimumab's encouraging antitumour activity in advanced HCC and its good safety profile in patients with cirrhosis of viral aetiology, provided a strong reason to test other checkpoint inhibitors.[18] The PD-L1/PD-1 pathway provides another mechanism of tumour-induced immune tolerance. PD-1 expression on effector phase CD8 + T cells is increased in patients with HCC compared to cirrhotic patients or healthy controls.[19] Indeed, patients with HCC and higher numbers of tumour infiltrating and circulating PD-1+CD8+ T cells showed earlier and more frequent disease progression after hepatic resection. PD-L1 is also highly expressed on peritumoural stromal cells (Kupffer cells, LSEC, and monocytes) as well as cancer cells, promoting a PD-L1/PD-1 pathway-driven inhibition of antitumour T cell responses.[20,21] Thus, there is a strong rationale supporting the use of PD-1 and PD-L1 blocking antibodies against HCC. Building on the experience with tremelimumab, a clinical trial has assessed the safety and clinical benefit of nivolumab (a fully human IgG4 monoclonal antibody targeting PD-1) as a first or second-line treatment in patients with advanced HCC across different aetiologies (HCV infection, HBV infection, non-viral cirrhosis).[22]

The target population of the CheckMate 040 trial included patients with intermediate or advanced HCC and preserved liver function (Child-Pugh A) that were candidates for systemic therapy and had progressed or were intolerant to sorafenib or had refused this drug. Firstly, a dose-escalation cohort of 48 patients received doses ranging from 0.3 mg/kg to 10 mg/kg every two weeks with the primary endpoint of establishing the safety and tolerability of nivolumab in patients with HCC. Afterwards, the 3 mg/kg dose level was chosen for an expansion cohort of 214 patients in whom the primary endpoint was efficacy, evaluated as objective response rate using RECIST 1.1 criteria. Patients in this expansion cohort were divided into four specific groups:uninfected patients who had progressed on sorafenib, uninfected patients naïve or intolerant to sorafenib, patients with HCV infection and patients with HBV infection. In both cohorts, patients infected with HBV had to be on effective antiviral therapy (circulating viral DNA <100 UI/ml).

Contrary to the tremelimumab trials, this study recruited patients from Europe, Asia and America. Most were at the advanced BCLC stage C (88%), had extrahepatic metastases (68%), and had received prior systemic therapy (76%), mainly sorafenib. Treatment was by and large well tolerated. Adverse events were observed at similar rates across dose levels and a maximal tolerated dose was not reached. The most frequent symptomatic adverse events in the large expansion cohort treated with 3 mg/kg were usually mild and included rash (23%), pruritus (21%) and diarrhoea (13%). Grade 3 or higher treatment-related symptomatic adverse events occurred in less than 2% of patients. Hypertransaminasemia was the most frequent laboratory alteration (20%), reaching grade 3 or higher in only 5% of patients. Regarding aetiologies, the rates of symptomatic treatment-related AEs were comparable in the uninfected and HCV- or HBV-infected cohorts. Overall, frequencies of grade 3/4 treatment-related AEs and treatment-related serious AEs were 20% and 7%, respectively, while no treatment-related deaths occurred. Immune related hepatitisrequiring steroid therapy occurred very rarely. Only 3% of patients discontinued nivolumab because of treatment-related adverse events and no treatment-related deaths were reported.

Convincing signs of efficacy were reported. In the escalation and expansion cohorts, objective tumour responses occurred in 15% and 20% of patients, respectively. They were meaningful, durable responses that lasted for a median of 17 months. An additional 45% of patients had stable disease that was frequently durable too, lasting more than six months in most cases. The majority of objective responses occurred during the first three months of treatment. It has to be stressed that response rates were similar across different aetiologies, and both in sorafenib-naïve and sorafenib-exposed patients. These signs of efficacy were consistent with the more recently reported median overall survival of 28.6 months (95% CI 16.6–NE) in the population naïve to sorafenib, and 15.6 months (95% CI 13.2–18.9) in the population exposed to sorafenib (90% progressors).[23] This median survival was observed irrespective of prior sorafenib treatment, and compares well with any other phase II or III clinical trial of targeted agents including regorafenib, the first agent shown to prolong survival following sorafenib in a selected group of sorafenib-tolerant patients. Indeed, these results support nivolumab as a viable second-line therapy following sorafenib (Fig. 2).

Key point

Nivolumab, an agent that stimulates the immune response through PD-1 blockade, has shown unequivocal signs of efficacy in a large phase II trial that recruited mostly patients refractory or intolerant to the standard of care sorafenib.

Results from correlative studies

The identification of prognostic markers, which will help to identify patients, who will benefit from treatment with immune checkpoint inhibitors is important. Different experimental studies have already been conducted to better understand which HCC patients respond to treatment with checkpoint inhibitors and how this occurs. One must consider that distinct from other patients

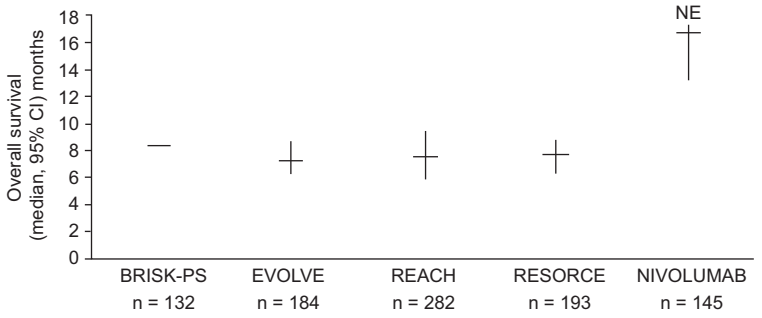

Fig. 2. Survival following nivolumab among patients that progressed or are intolerant to sorafenib. The survival reported in placebo-treated arms in large clinical trials addressing the second-line advanced HCC population is presented for comparison.[2,22,50–52]

with cancer, the majority of patients with HCC also suffer from chronic viral hepatitis.

Tremelimumab also has a significant antiviral effect. In the tremelimumab alone trial, a decrease in median HCV viral load from 3.78×10^5 IU/ml at day 0 to 3.02×10^4 IU/ml at day 120 (n = 11, p = 0.011), and 1.69×10^3 IU/ml at day 210 (n = 6, p = 0.017) was observed.[16] The progressive course of this decline in viral load was observed in most patients followed for at least three months, and three patients had a transient complete viral response during follow-up. The antiviral activity was confirmed in the tremelimumab plus ablation trial in which the HCV viral load of 14 quantifiable patients decreased after three months in 12 patients, with a median HCV viral load decrease from $1,275 \times 10^3$ UI/ml to 351×10^3 UI/ml.[17] In the first trial, the immunological origin of this viral response was supported by the fact that it was observed in 75% of patients with an immune response (defined as a >5-fold increase at any time in the sum of IFN-γ-producing cells against viral antigen) *vs.* 20% of patients with no immune response. Patients with an early decrease in IL-6 had a higher chance of having a viral response (100%) than those with increased values at that time (43%). The anti-tumoural effect was not associated with this antiviral effect or to patient characteristics including systemic inflammatory signals such as C reactive protein. The lack of repeated tumour biopsies precludes any interpretation of the mechanism behind the antitumour activity while the expansion in circulating Treg following tremelimumab therapy mirrored observations in other tumour types.[24]

The second tremelimumab trial was enriched with important correlative studies. Peripheral blood CD3, CD4, CD8, CD38 and HLA-DR positive cells were counted after every cycle by multicolor flow cytometry. Tumour biopsies were obtained from some patients immediately before ablation (after two doses of tremelimumab). The number of cytotoxic T cells (CD3 and CD8 positive) was measured by immunohistochemistry in these samples and compared to archival samples obtained prior to enrollment. Interestingly, the number of peripheral activated CD4+ and CD8+ T cells increased after tremelimumab treatment. Such increases were especially intense and sustained for CD8+ T cells. Immune cell tumour infiltration was observed in all 12 patients in whom post-tremelimumab tumour samples could be evaluated. Among these six patients with paired tumour samples, an increase in both CD3+ and CD8+ cells was observed, although the differences were not statistically significant, likely because of the small number of cases. Patients with objective remissions in non-ablated lesions had higher post-tremelimumab CD3+ and CD8+ infiltration than non-responders. Unfortunately, the effect of ablation on T cell infiltration could not be evaluated and in the absence of a remarkable difference in patient outcomes, the synergy between TACE/RFA and CTLA-4 blockade remains an appealing hypothesis, requiring confirmation.

A comprehensive biomarker analysis has not yet been reported for the CheckMate 040 trial. Expression of PD-L1 prior to nivolumab was studied in fresh or archival tumour specimens. The rate was remarkably low. Even with a cut-off for positivity of 1% of tumour cells exhibiting membrane PD-L1 staining of any intensity, only 20% of 174 evaluable patients had PD-L1 positive tumours. Objective remissions occurred in 26% of PD-L1 positive patients and 19% of PD-L1 negative patients, making this marker unsuitable for patient selection. The more relevant rate of PD-L1 expression in tumour stromal cells and its association with response to nivolumab have not been reported yet.

Key point

PD-1 or PD-L1 blockade now serves as the backbone of a number of combination regimes that are being tested as first or second line therapies in phase II and phase III trials.

Combination therapies

While clinical trials evaluating the safety and efficacy of anti-CTLA-4 and anti-PD-1/PD-L1 are ongoing, different investigators have already initiated trials evaluating the combination of immune checkpoint inhibitors with other drugs or the combination of anti-CTLA-4 with anti-PD-1 or PD-L1. Based on promising data in melanoma, these combinations are mostly being tested in the absence of any preclinical data. Combination therapies include combinations of different checkpoint inhibitors, as well as combinations with oncolytic viruses, small molecules and ablative therapies. A summary of ongoing combination studies is presented (Table 3). These combinations may be based on the potential additive effect of a therapy with a treatment benefit that has been proven (TACE or sorafenib) or is being investigated (ramucirumab, cabozantinib). However, they should preferably be based on exploiting synergistic effects or avoiding primary resistance.

Hence, understanding the mechanisms of resistance to anti-PD-1/PD-L1 therapies is important for the development of combination therapies (Fig. 3). It has been proposed that mechanisms promoting primary or acquired resistance are

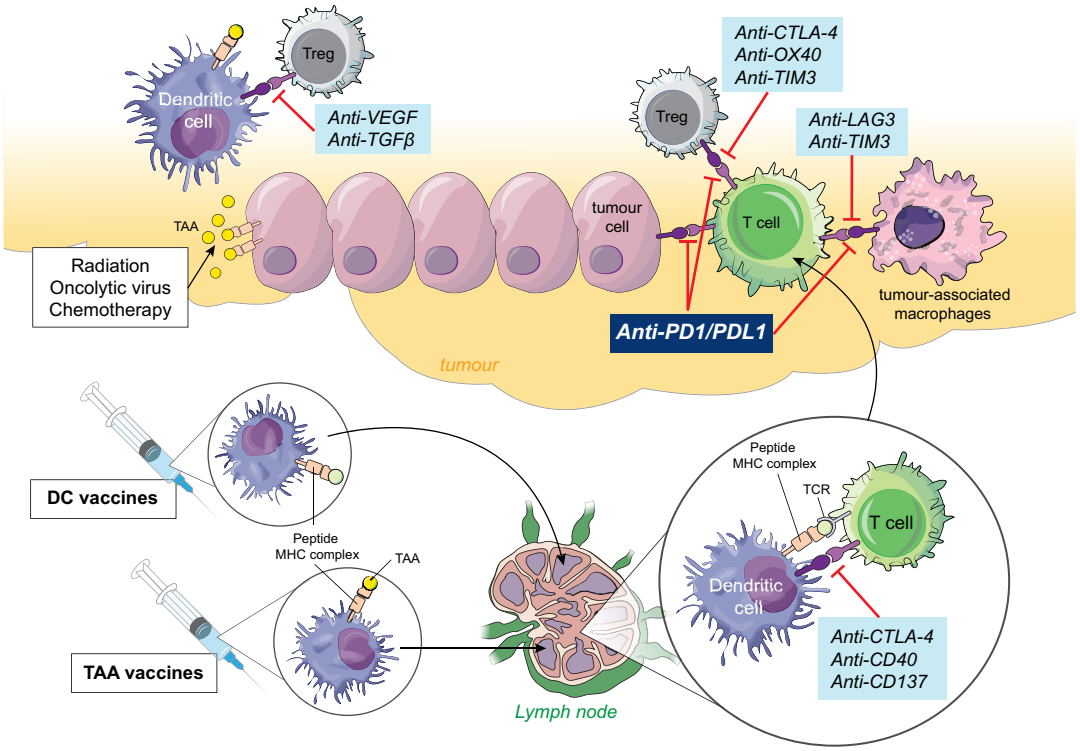

Fig. 3. Strategies to increase the efficacy of anti-PD-1/PD-L1 blockade based on mechanism of action. CTLA-4, cytotoxic T lymphocyte protein 4; DC, dendritic cells; LAG-3, lymphocyte activation gene 3; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TAA, tumour-associated antigen; TAM, tumour associated macrophage; TIM-3, T cell immunoglobulin and mucin-domain containing; TGFβ, transforming growth factor β; VEGF, vascular endothelial growth factor.

largely conserved, and must affect either tumour immunogenicity, antigen presentation and generation of effector T cells, contact of antigen and PD-L1 by tumour-specific T cells, the efficacy of tumour cell killing, or the induction of immunological memory.[25] Although it may seem early to discuss HCC-specific resistance mechanisms to immune checkpoint blockade one should note that primary and adaptive resistance mechanisms have been observed and described in melanoma patients[26]. One may expect to see similar mechanisms occurring in patients with HCC as those described in melanoma, where tumours have been found to mutate and become invisible to tumour-specific CTL responses by MHC downregulation or modulation of the immediate tumour microenvironment. Interestingly, we observed that viral load increased in patients at the time when tumours started to progress in our study using anti-CTLA-4, potentially leading to treatment failure. This suggests that it is not only tumour-specific T cell responses that become weak over time.

Tumours with a low mutation rate (very likely associated with fewer neoantigens), such as pancreatic and prostate, are poorly immunogenic and effectively resistant to anti-PD-1 agents.[27] Hence, sensitivity to anti-PD-1 therapy would likely be enhanced by therapies that may contribute to the release of tumour antigens, including radiotherapy, virotherapy and some chemotherapies.[28] Conversely, autologous cancer vaccination strategies that prime adaptive immune responses with TAAs can enhance sensitivity to anti-PD-1 therapy.[29] Neoantigen vaccination approaches[30] may work even better, although they are currently limited by MHC restriction.

Antigen presentation and T cell stimulation can be enhanced in several ways. Promoting IFNγ release in the tumour microenvironment by intratumoural delivery of oncolytic virus or RNA adjuvants[31] may increase the expression of class I MHC, which is required for T cell antitumour responses and usually downregulated by tumours. Since cytokines such as VEGF and TGF-β play a key role in the suppression of DC function in the tumour microenvironment, agents that neutralise their actions could work synergistically with anti-PD-1/PD-L1 therapy.[32] Agonistic monoclonal antibodies that target immunostimulatory molecules such as CD40 or CD137 may also improve the effector functions of DC, and their combination with anti-PD-1 agents were synergistic in models of melanoma and other tumours.[33,34] Finally, oncolytic viruses may enhance the activity of DC.

PD-L1 is not the only immunosuppressive factor in the tumour microenvironment. Treg stands out among the immunosuppressive cells of the tumour niche. Elevated Foxp3+/CD8+ cell ratios are commonly associated with resistance to anti-PD-1 therapy.[35] Anti-CTLA-4 agents deplete tumour-associated Treg via an FcγR-dependant mechanism in preclinical models, and enhance

the efficacy of tumour-specific T cell-mediated antitumour immunity.[36] The combination of anti-CTLA-4 and anti-PD-1 therapy is highly synergistic in experimental melanoma and results in the highest rates of objective remissions in patients with advanced melanoma (58% *vs.* 19% for anti-CTLA-4 alone and 44% for anti-PD-1 alone).[37] This comes at the cost of increased toxicity, with 36% of patients with melanoma having to discontinue the combination compared to 7% of the patients receiving nivolumab monotherapy. Regarding liver toxicity, the proportion of patients with increased ALT was 3.8% for nivolumab, 3.9% for ipilimumab and 17.6% for the combination.[38] The results of the combination of these two checkpoint inhibitors in HCC are expected soon. Anti-OX40 monoclonal antibodies may also be relevant at selectively depleting tumour-associated Treg,[39] and the combination with anti-PD-1 is synergistic in animal models of cancer resistant to anti-PD-1 therapy.[40] In combination with anti-PD-1/PD-L1 therapy, anti-TIM3 agents may help deplete Treg,[41] and avoid T cell exhaustion. Indeed, anti-TIM3 or anti-LAG-3 in combination with anti-PD-1 have demonstrated synergistic effects in several preclinical models.

Finally, studies comparing immune cell infiltrates within tumours before and after treatment with anti-PD-1 therapy showed that patients that responded poorly to therapy contained significantly fewer tumour-associated effector memory T cells than responsive patients.[42] Strategies that may expand this subset of T cells and protect them from exhaustion would result in promising combination therapies.

Key point

Monoclonal antibodies targeting CTLA-4 and PD-1 or PD-L1 can effectively help overcome the mechanisms of immune evasion in a wide spectrum of human cancers.

Key point

In the past, attempts to enhance antitumour immune responses in HCC by vaccination strategies or with cytokine-induced killer cells have been too weak to produce significant and consistent clinical benefit.

Cell-based therapies

Different types of cell-based therapies are being tested for the treatment of patients with HCC. Most experience exists for the treatment with cytokine-induced killer cells (CIK). CIK are characterised by the coexpression of CD3 and CD56. They can be generated by expanding human peripheral blood mononuclear cells in the presence of interferon-γ (IFN-γ, anti-CD3 and IL-2). Lee *et al.* conducted a randomised controlled trial in 230 patients with HCC in the adjuvant setting (post-surgical resection, RFA and ethanol injection). They were able to demonstrate an increase in PFS from 30 to 44 months upon treatment with CIK.[43] A few much smaller studies tested the use of dendritic cells as a potential cancer vaccine in HCC. While no definitive conclusions on the clinical efficacy of this approach can be drawn, these types of treatment appear, generally, to be safe.[44,45] More recently, predominantly based on studies in haematological malignancies, adoptive T cell therapies using genetically engineered T cells have garnered much interest. Two different approaches are currently being developed for patients with HCC. Autologous T cells are either being transduced with a chimeric antigen receptor (CAR) or TCR. In either case, T cells recognise specific antigens expressed on tumours but not on healthy tissue. CARs enable highly specific targeting of antigen in an MHC-independent fashion. CARs are formed from a combination of antibody-derived or ligand- derived domains and TCR domains. In contrast, TCR transduced T cells, which also recognise a specific antigenic peptide, are MHC restricted.[46] Glypican 3 is a target frequently used for antigen-specific responses in HCC.[47] Preclinical data using CAR T cells against Glypican 3 have been published[48] and two clinical trials using Glypican 3 targeting CAR T cell approaches have either been started or are about to be launched (NCT02723942 NCT02932956). A few investigators are testing AFP directed therapies.[49] However, AFP can also be expressed on healthy tissue, so it is not clear how tumour-specific such therapy will be.

In summary, the field of cancer immunotherapy for HCC has never been as exciting as it is now. Results from the first large randomised phase III trial are expected to be published in 2018. A number of different combination therapies are being evaluated and novel cell-based therapies will hopefully be effective in this difficult to treat disease.

Conflict of interest

Bruno Sangro has received consulting and/or lecture fees from Adaptimmune, Astra Zeneca, Bayer Healthcare, Bristol-Myers-Squibb, Medimmune and Onxeo.

Please refer to the accompanying ICMJE disclosure forms for further details.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhep.2017.09.007.

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Author names in bold designate shared co-first authorship

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