Liver immunotolerance and hepatocellular carcinoma: Patho-physiological mechanisms and therapeutic perspectives

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Received 11 May 2017; received in revised form 2 October 2017; accepted 13 October 2017
Available online 13 November 2017

KEYWORDS
Immunotolerance; Immunotherapy; Hepatocellular carcinoma; Immune checkpoint; Anti-PD-1

Abstract  At the moment of the diagnosis of hepatocellular carcinoma (HCC), 70% of patients have only access to palliative treatments, with very few therapeutic options. Liver immunology is very specific, and liver immunotolerance is particularly developed because of the constant and massive influx of antigens. Deregulation of hepatic immunotolerance is implicated in chronic liver diseases development and particularly in liver carcinogenesis. For these reasons, HCC may be an excellent candidate for anticancer immunotherapies such as immune checkpoint inhibitors targeting CTLA-4 and PD-L1/PD-1. Nonetheless, because of the specific immune environment of the liver and the frequent association of HCC with hepatocellular insufficiency, the safety and the efficacy of these new treatments have to be properly studied in this situation. Thus, multiple phase II and III studies are in progress studying immune checkpoint inhibitor monotherapies, combination of different immunotherapies or local strategies such as transarterial chemoembolization combined with immune checkpoint inhibitors. Currently, only the final results of the tremelimumab phase II and the Nivolumab phase I/II study (CheckMate-040) are available. The latter is promising but need to be confirmed by the ongoing phase III studies to confirm the place of immunotherapy in the treatment of HCC. With many new molecular targets and therapeutic combination, immunotherapy represents a new hope in treating HCC patients although serious evaluation is still needed to confirm its interest.

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1. Introduction

Liver cancer is the second cancer-related death worldwide with 750,000 new cases of hepatocellular carcinoma (HCC) per year [1]. When diagnosed, 70% of the patients have only access to a palliative treatment [2,3]. Sorafenib is the only available first-line therapy, with an overall survival (OS) of 10.7 versus 7.9 months with placebo [4], and regorafenib is the first second-line treatment to show an OS benefit [5]. HCC is a very complex disease as more than 160 driver genes were described [6], and the liver constitutes a singular immune environment where immunotolerance is particularly developed. Because tumour initiation and progression are partially due to an escape to immunosurveillance system that fails to detect and destroy cancer cells [7], HCC seems to be a good candidate to immunostimulatory therapies aiming to restore antitumour immunity. These therapies are currently in full swing in HCC with the advent of monoclonal antibodies directed against immune checkpoints.

2. Hepatic immune tolerance and carcinogenesis

Due to intimate relationships with the digestive tract and its systemic filter role, the liver is constantly and massively exposed to many antigens [8]. Consequently, multiple mechanisms of immunotolerance have been developed to prevent autoimmune liver injuries [9].

2.1. Antigen-presenting system

In the liver, classic antigen-presenting cells (APCs) such as resident dendritic cells (DCs) are present, as well as liver-specific APCs such as hepatic stellate cells (HSCs), Kupffer cells (KCs) and more particularly liver sinusoidal endothelial cells (LSECs) [10]. These immune cells present innate immune function thanks to pattern recognition receptors (PRRs), which recognise specific bacterial antigens such as pathogen-associated molecular patterns and host antigens called damaged-associated molecular patterns [11]. PRRs can trigger interleukin (IL)-6-mediated pro-inflammatory responses while they can also decrease tumour necrosis factor (TNF) production by KCs [12] or promote tolerogenic signals through IL-10 secreted by KCs [13] and TGF-β secreted by both KC and LSEC [11] for protective purposes [9,14]. Moreover, as the name implies, these cells have antigen presenting functions. They express major histocompatibility complex (MHC) class-2 molecules, which will bind the T-cell receptor (TCR) [15], leading to CD4+ T-cell activation (also called T helper [Th]-cells) [10]. Besides, APC expresses CD80 (B7-1) and CD86 (B7-2) ligands, which will activate CD4+ T-cells, through their CD28 receptor [15] (Fig. 1). CD4+ T-cell activation induces CD8+ T-cells (also called cytotoxic T [Tc]-cells) production by antigen cross-presentation [16]. Then, APCs such as LSECs will induce T-cell differentiation in memory T-cells to allow a strong and fast CD8+ T-cells reaction during further antigen exposure [17]. Nonetheless, KC and LSEC, through TGF-β secretion, will also induce CD4+ T-cell differentiation in regulatory T-cells or Tregs (CD4+, FOXP3+ and CD25+), which have an immunosuppressive role [18]. Interactions and mechanisms regulating the imbalance between CD4+ T-cells and Tregs pathways are still insufficiently characterised. Moreover, LSEC role in antigen-presenting is controversial as they also seem to be a key element of liver immunotolerance. They indeed promote immunosuppressive cytokines production by KC as IL-10 and prostaglandin-E2, which decrease MHC class-2 molecules and CD80-CD86 expression by LSEC, impairing their APC activity [19].

Some of these tolerogenic mechanisms are also involved in the remarkable immune tolerance, which can be developed in case of liver allograft, leading sometimes to the ending of immunosuppressive drugs [20,21]. Among these mechanisms, the local attenuation of alloreactive T-cell responses and the production of Treg seem essential [22,23]. Indeed, in a mouse model naturally failing to reject allograft, it was shown that the pharmaceutical inhibition of Tregs with an anti-CD25 therapy induced rejection and was associated with an increase of IL-10 and IL-2 production by graft-infiltrating T cells [24].

2.2. Immune checkpoints

Key factors called immune checkpoints have been described in immunotolerance mechanisms, whose deregulation is involved in chronic diseases pathogenesis and carcinogenesis.

Cytotoxic T-lymphocyte–associated protein 4 receptor (CTLA-4) is constitutively expressed on Tregs, activated T-cells and can also be expressed on naïve T-cells [25]. CTLA-4 has an immunosuppressive role by increasing T-cell differentiation in Tregs and by binding CD80-CD86 ligands on APC with a higher affinity than CD28 [26–28]. This will competitively reduce the CD28-mediated CD4+ T-cell activation and directly induce an intracellular immunosuppressive signals counteracting TCR stimulatory signalling [15,29,30] (Fig. 2A).

Programmed death receptor 1 (PD-1) and its ligands PD-L1/L2 (programmed cell death ligand 1/2) constitute also major factors of immunotolerance. PD-1 is expressed on CD4+ and CD8+ T-cells as well as B-cells and natural killer T-cells [16]. PD-L1 is expressed by KC [31], HSC [32], LSEC [17] and even hepatocytes [33]. PD-1 activation affects antigen-presenting function of LSEC, impairs T-cell activation through an increase of IL-10 secretion by resident DC, KC [16] and monocytes [34], and promotes T-cell differentiation in Tregs [35] (Fig. 2A).

Lymphocyte-activation gene 3 (LAG-3) is a CD4-like molecule, which decreases APC activity by binding their
MHC class-2 molecules [36]. Similar to PD-1, LAG-3 is expressed on Tregs and regulates T-cell interactions [37,38]. They both have a synergistic effect leading to T-cell exhaustion [39].

T-cell immunoglobulin and mucin domain-3 (TIM-3) is a transmembranous protein expressed by cells from both innate and acquired immunity. TIM-3 promotes Tregs activity [40] and induces T-cell exhaustion [41]. Galectin-9, its ligand, is mainly expressed on KC [42].

2.3. Tumour progression and liver immunotolerance

Under physiologic conditions, systems promoting immunotolerance have a protective role [8]. In chronic liver diseases, their deregulation leads to an exacerbation of immunotolerant signals allowing hepatic injuries progression and HCC emergence.

Chronic liver inflammation is promoting Tregs activity [43] as well as IL-10 and TGF-β secretion by KC and LSEC [11,13,44]. This is resulting in T-cell exhaustion and antigen-presenting inhibition. Besides, immune checkpoints as PD-1/PD-L1 [31,33] and CTLA-4 [45] are upregulated leading to a defective immunosurveillance with an insufficient neoplastic cell recognition. Chronic liver diseases also impair LSEC functions, normally involved in liver regeneration, through the capillarization process. While differentiated LSECs promote HSC quiescence, capillarised LSECs are permissive for HSC activation leading to liver fibrosis [46]. The inflammation during chronic liver diseases also generate a pro-inflammatory environment with the induction of KC recruiting and the secretion of multiple growth factors such as epidermal growth factor, insulin-like growth factor, vascular endothelial growth factor (VEGF) and platelet-derived growth factor [9,16].

In HCC, inflammation is important as well, with specific interactions between cancer cells, classic and tumour-associated immune cells [44,47]. We observe a marked inhibition of antigen-presenting systems, a CD4+ T-cell decrease and an intensified Tregs production, particularly in tumour-infiltrating lymphocytes (TILs) [48]. Tumour-associated macrophage proliferation promotes tumour initiation and growth [49], and a wealth of immunosuppressive cytokines are also secreted as interleukins (IL-4, IL-5, IL-8 and IL-10), TNF and interferon gamma (IFN-γ) [16,50]. Immune checkpoints are deregulated, with an overexpression of CTLA-4 by CD8+ T-cells, Tregs and dendritic cells, leading to a reduced T-cell production and an increase of their apoptosis [51]. In this context, T-cell activity is also inhibited due to PD-L1 overexpression by KC, LSEC, peri-tumour monocytes and tumour cells themselves [52,53]. Finally, CD4+ and CD8+ TILs orchestrate TIM-3 and promote galectine-9 production by tumour-infiltrating KC through IFN-γ secretion, reinforcing T-cell inhibition [42] (Fig. 1).

3. Immunotherapy: which therapeutic targets?

As previously mentioned, HCC is a very complex disease with multiple signalling pathways involved in its carcinogenesis and varying according to HCC aetiology [6]. It has a poor prognosis and few therapeutic options,
as most of traditional anticancer chemotherapy and targeted therapies failed to improve its survival [54]. These last years, numerous therapeutic strategies, aiming at restoring a competent immunity against tumour cells, have been developed with heterogeneous results. Classic techniques of immunotherapy such as antitumour vaccines or adoptive cell transfer methods have been tested and present various levels of antitumour efficacy [16].

The true novelty is coming from the development of monoclonal antibodies blocking immune checkpoints such as anti-CTLA-4 or anti-PD-1 [25].

3.1. Vaccines

Tumour-associated antigens (TAAs) are potential targets to develop antitumour vaccines [55] such as alpha-fetoprotein (AFP) [56,57], the telomerase reverse transcriptase [58] or glypican-3 [59]. These vaccines can either be directly synthesised from RNA sequences, peptides or proteins, or after maturation of dendritic cells exposed to diverse cytokines or ligands binding their toll-like receptor. In a recent study assessing 27 TAAs related to HCC, recognition of at least 1 TAAs by T-cells with a CD8+ T-cell production was observed in 77.4% of patients [60]. In another study, the presence of TAA-specific CD8+ T-cell responses was associated with a better progression-free survival (PFS) [61]. The feasibility and safety of this therapeutic approach were demonstrated in many early-phase clinical trials, but none of them showed any significant antitumour effect or improved OS in HCC.

3.2. Cytokines

Interferon alpha was the first cytokine used for its immunostimulating properties in viral hepatitis C, but several studies showed disappointing results concerning its antitumour efficacy [62,63]. In adjuvant setting, conflicting results were published but IFN therapy does not prevent HCC recurrence except in pure HCV patient by reduction of late recurrence due to treatment of the underlying liver disease [64]. Besides, because systemic immunostimulation is usually bad tolerated, safety and efficacy of cytokine intra-tumour injection have been tested. Interleukin (IL)-12 either by direct injection of an adenovirus containing IL-12 coding sequence [65] or by transfection of infected dendritic cells with these viruses [66] was evaluated, showing a good tolerance, but no antitumour efficacy. Also, galunisertib (LY2157299 monohydrate), a TFG-β receptor inhibitor, seemed to improve survival with an OS of 36 weeks in a phase II trial intermediate analysis on 109 patients. OS was 93.1 weeks in patients considered as AFP responders (AFP decrease of 20%) versus 29.6 weeks in AFP non-responders [67]. This small drug is also tested in non-

Fig. 2. (A) CTLA-4 and PD-1 immune checkpoint signalling in hepatocellular carcinoma. CD80/86 and PD-L1/L2 ligands, present at the surface of hepatic antigen-presenting cells are respectively binding CTLA-4 and PD-1 immune checkpoints on T-cells, resulting in an inhibitory signalling with an increased T-cell differentiation Tregs, a competitive inhibition of CD28 T-cell activatory signalling, and an increased T-cell exhaustion and apoptosis. (B) Biological effects of pharmaceutical immune checkpoint blockade in hepatocellular carcinoma. Anti-CTLA-4 immune checkpoint inhibitors are competitively binding CTLA-4 receptor on T-cells, resulting in the resaturation of the antigen presenting system, the depletion of Treg and the activation of T-cells through CD28 signalling. PD-1 blockade by anti-PD-1 and anti-PDL-1 antibodies also induces a T-cell activatory signalling by resaturing their cytotoxicity and stimulating the production of pro-inflammatory cytokines. Abbreviations: MHC, major histocompatibility complex; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death receptor; PD-L1/2, programmed cell death ligand 1/2; TCR, T-cell receptor.
randomised phase II trials, in association with sorafenib or ramucirumab (NCT01246986), and in combination with nivolumab (Table 1), or stereotactic body radiotherapy (SBRT) (NCT02906397).

3.3. Adoptive cell transfer

Adoptive cell transfer (ACT) is based on the autologous infusion of *ex vivo* expanded TILs or cytokine-induced killer cells (CIKs), as well as genetically modified T-cells (GMTEs) [68].

Autologous TILs transfer requires isolation of TILs from fresh tumour sample. They are *ex vivo* amplified and activated in contact with anti-CD3 antibodies and cytokines such as IL-2 and re-infused in patients after lymphodepletion [69]. In a randomised trial including 150 curatively resected patients, adjuvant autologous TILs transfer improved recurrence-free survival (RFS) and recurrence frequency [70]. Another non-randomised study testing a postoperative association of autologous TILs combined with a DC vaccine versus curative surgery alone, showed promising results with improved RFS (24.5 versus 12.6 months, *p* = 0.011) and OS (97.7 versus 41.0 months, *p* = 0.029) [71].

CIKs correspond to peripheral blood mononuclear cells of patients, *ex vivo* incubated with cytokines and anti-CD3 antibodies before being re-infused as well [72]. CIKs can be largely produced and recognise a broad spectrum of TAAs. They correspond to killer cells expressing natural killer group 2 member D (NKG2D) receptor such as natural killer (NK) cells, γδ+ T-cells and activated CD8+ T-cells. NKG2D ligands MIC-A and MIC-B are expressed on tumour cells, and their intensity is proportionally related with antitumour immune response [73]. CIKs showed interesting results in a retrospective study with improved OS and PFS in patients previously treated with transcatheter chemoembolisation (TACE) or radiofrequency ablation (RFA) [74]. Combination of CIKs with these locoregional techniques also seems beneficial with a decrease of recurrence frequency [75]. Recently, a phase III trial using adjuvant IL-2 induced CIKs after liver resection showed a significantly better RFS (44.0 versus 30.0 months, *p* = 0.010) [76].

GMTEs are engineered either by grafting a cloned TCR on T-cells or a chimeric antigen receptor (CAR) with an optimised affinity for TAAs [77]. In the first technique, after *in vitro* T-cell exposition to TAAs, the most reactive lymphocytes are selected, and their TCR α/β chains are transferred in patients through a retroviral vector [78]. The limit of this technique is that antigen recognition is HLA-dependent, and therefore, it is specific to each patient. The use of CAR-modified T-cells avoids this limit [79] but needs to be improved as their half-life is very limited. GMTE showed promising results in melanoma and synovial carcinoma [80], without confirmation of its efficacy in other cancers.

3.4. Immune checkpoint inhibitors

3.4.1. Anti-CTLA-4

Ipilimumab, a fully human monoclonal antibody (IgG1), was the first studied anti-CTLA-4 [81]. The latter proved its antitumour efficacy in a phase III trial in advanced melanomas with a significantly improved OS in bitherapy with gp10. Nonetheless, safety was problematical with 10–15% of grade III–IV adverse events (AEs) as colitis, pneumonitis, thyroiditis, hepatitis and 14 drug-related deaths [82]. Ipilimumab is also being tested in advanced HCC in combination with nivolumab, an anti-PD-1, in a prospective non-randomised phase II/I study (Table 1).

Tremelimumab, a fully human IgG2 monoclonal antibody blocking CTLA-4 (Fig. 2B), showed interesting results in a phase II trial including 21 patients with advanced HCC, where partial response and disease control rate were, respectively, 17.6% and 76.4% [83]. This immune checkpoint inhibitor was also tested in addition to locoregional therapies (TACE, RFA and cryoablation) in a pilot study recently published [84]. Abscopal effect was observed in some cases, without increasing severe AEs occurrence, demonstrating the feasibility of these combinations of immunotherapy with these techniques in HCC (Table 1). However, tremelimumab development has been considerably slowed down by a negative phase III trial in metastatic melanoma [85]. Associations with anti-PD-1 such as durvalumab are still undergoing evaluation (Table 1).

3.4.2. Anti-PD-1/anti-PD-L1

Nivolumab is a fully human IgG4 anti-PD-1 antibody (Fig. 2B), which improved PFS and OS in two phase III trials in metastatic melanoma either as a monotherapy [86] or in association with ipilimumab [87]. In advanced HCC, nivolumab is currently being evaluated in the CheckMate-040 clinical trial comprising an open-label and non-controlled phase I/II dose escalation and expansion study assessing nivolumab alone, and nivolumab combined with ipilimumab in patients with or without chronic viral hepatitis, as well as a randomised open-label study comparing nivolumab with sorafenib in patients who are naive to systemic therapy (Table 1). The analysis of nivolumab-treated patients from the phase I/II dose escalation and expansion study showed an objective tumour response (OTR) and a stable disease in, respectively, 20% and 45% of the 214 patients from the expansion cohort. Grade III–IV AEs were observed in 19% of cases with serious AEs (pemphigoid, adrenal insufficiency and liver disorder) in 4% of patients without difference between the presence or not of HCV or HBV infection [88]. A phase-III study (CheckMate-459), comparing nivolumab to sorafenib in advanced HCC has been initiated (Table 1). Finally, nivolumab is also undergoing phase I/II trials in association with galunisertib as previously said, or CC-122, a
<table>
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<tr>
<th>Drug</th>
<th>Trial (ClinicalTrials.gov identifier, year)</th>
<th>Phase</th>
<th>Study design</th>
<th>Population (number of patients, inclusion/exclusion criteria)</th>
<th>Objectives</th>
<th>Results (final results, intermediate analyses)</th>
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<td><strong>Anti-CTLA-4</strong></td>
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<td>Tremelimumab</td>
<td>NCT01008358 2009–2012 [83]</td>
<td>II</td>
<td>Prospective</td>
<td>N = 21</td>
<td>PO: TR (by RECIST) SO: changes in HCV viral load</td>
<td>RR: 3 PR, 10 SD/17 assessable patients</td>
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<td>Single arm</td>
<td>Advanced HCC, Child Pugh A-B</td>
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<td>TTP = 6.48 mo. (95 CI = 3.95–9.14)</td>
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<td>Non-Randomised Open label</td>
<td>Inclusion of HCV-induced cirrhosis</td>
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<td>OS = 8.2 mo. (95 CI = 4.6–21.3)</td>
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<td>HCV viral load significant decrease</td>
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<td>Enhanced specific anti-HCV immune response</td>
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<td><strong>Anti-PD-1 and anti-PD-L1</strong></td>
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<td>I/II</td>
<td>Prospective</td>
<td>N = 1014</td>
<td>PO: DLT, safety SO: TR, OTC, OS, PFS, PK</td>
<td>Intermediate analysis on 21 patients</td>
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<td>Durvalumab (anti-PD-L1)</td>
<td>NCT01693562 2012–2018</td>
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<td>Multiple solid tumours, Progression on/refusal of intolerance to first-line treatment</td>
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<td>patients with advanced HCC (ESMO 2014) [89]:</td>
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<td>RR = 0%, OTC at 12 weeks = 21%</td>
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<td>Nivolumab (anti-PD-1)</td>
<td>NCT02576509 (Checkmate-459) 2015–2019</td>
<td>III</td>
<td>Prospective</td>
<td>N = 726</td>
<td>PO: OS, TTP SO: RR, PFS, relation between PD-L1 expression and anti-PD-1 efficacy, life quality</td>
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<td>Randomised Open-label</td>
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<td>Pembrolizumab (anti-PD-1)</td>
<td>NCT02658019 2016–2019</td>
<td>II</td>
<td>Prospective</td>
<td>N = 28</td>
<td>PO: TCR, safety SO: RR, DOR, PFS, toxicity, biomarkers</td>
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<td>HCV treated &lt; 60 days, HBV treated &lt; 3 months excluded</td>
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<td>NCT02702414 2016–2017</td>
<td>II</td>
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<td>PO: RR SO: DOR, OTC, TTP, PFS, OS</td>
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<td>Single arm</td>
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<td>Open label</td>
<td>Non-treated or successfully treated (&lt;4 weeks) HCV, No other systemic treatment except sorafenib</td>
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NCT02702401  2016–2019  III  Prospective Randomised Double-blind Two arms:  (A) Pembro + BSC (B) Placebo + BSC  N = 408  - Advanced HCC, BCLC B/C  - Recused for locoregional strategies  - Child A  - Non-treated or successfully treated (>4 weeks) HCV  - No other systemic treatment except sorafenib  PO: PFS, OS  SO: ORR, DCR, TTP, DOR  RR, TCR, TTP, DOR  N/A

Combined therapies
Tremelimumab + locoregional therapy  NCT01853618  2013–2016 I Prospective, non-randomised Five arms: Tremelimumab +:  (A) TACE or RFA (BCLC C)  (B) TACE (BCLC B)  (C) SBRT (advanced HCC, BCLC C)  (D) CA (advanced HCC, BCLC C)  (E) RFA (iCCA) or SBRT or CA  N = 100  - Advanced or metastatic HCC  - iCCA  PO: Safety and feasibility of Trem + locoregional therapies combination in HCC  SO:  - RR, TTP, OS in HCC  - Safety, feasibility and efficacy of combinations in iCCA  Analysis in 32 RFA- or TACE-treated patients [84]: 6- and 12-month PFS = 57.1% and 33.1%  TTP = 7.4 mo (95% CI 4.7–19.4)  OS = 12.3 mo (95% CI 9.3–15.4)

Tremelimumab ± durvalumab  NCT02519348  2015–2018 II Prospective, randomised, open label Three arms:  (A) Durva alone  (B) Trem + Durva  (C) Trem alone  N = 144 patients  - Non-resectable HCC ± Hepatitis B or C  - Progression on, intolerance to, or refusal of sorafenib  - Immunotherapy-naive  PO: Toxicity, SAE  SO: RR, DOR, OS, PD-L1 expression  N/A

Nivolumab ± ipilimumab (anti-CTLA-4)  NCT01658878  2012–2018 I/II Prospective, non-randomised Four substudies:  (A) Nivo alone (dose escalation phase, expansion phase)  (B) Nivo versus sorafenib (randomised)  (C) Nivo + ipilimumab (D) Nivo alone (patients Child B)  N = 620 patients  - Advanced HCC  - Hepatitis B or C  - Progression on/refusal of intolerance to first-line treatment  Study A: Four cohorts:  (1) HBV/HCV-, naive of/intolerant to sorafenib  PO: safety, RR  SO: RC, TCR, DOR, TTP, PFS, OS, biomarkers, PK  Phase I/II escalation dose and expansion study:  Dose-escalation study (48 patients):  - TR = 15%, TTP = 3.4 mo, OS = 15.0 mo (95% CI 9.6–20.2)  - Safety: 25% of grade 3–4 AE (6% of serious AE).  (continued on next page)

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<th>Drug</th>
<th>Trial (ClinicalTrials.gov identifier, year)</th>
<th>Phase</th>
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<th>Objectives</th>
<th>Results (final results, intermediate analyses)</th>
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<tr>
<td>Nivolumab + galunisertib (TGF-β-R1 inhibitor)</td>
<td>NCT02423343 2015–2019</td>
<td>Ib/II</td>
<td>Prospective Single arm</td>
<td>N = 100 patients</td>
<td>- Progression on, intolerance to sorafenib and/or non-accessible to TACE</td>
<td>- Safety: 19% of grade III–IV AE (4% of serious AE)</td>
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<td>- Phase Ib: resistant advanced HCC (in any line of therapy)</td>
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<td>- Phase II: resistant or relapsing HCC with AFP ≥ 200 ng/mL</td>
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<td>Nivolumab + CC-122 (pleiotropic pathway modifier)</td>
<td>NCT02859324 2016–2021</td>
<td>I/II</td>
<td>Prospective Single arm</td>
<td>N = 50 patients</td>
<td>- Progression on/refusal of intolerance to 2 locoregional therapies or naive of systemic therapy</td>
<td>- Safety: 19% of grade III–IV AE (4% of serious AE)</td>
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<td>- Phase I: resistant advanced HCC (in any line of therapy)</td>
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<td>Pembrolizumab + autologous TILs infusion</td>
<td>NCT01174121 2010–2019</td>
<td>II</td>
<td>Prospective Single arm</td>
<td>N = 290 patients</td>
<td>- Metastatic HCC previously treated by sorafenib</td>
<td>- Safety: 19% of grade III–IV AE (4% of serious AE)</td>
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pleiotropic pathway modifier (PTM ®), a new class of molecules supposed to be active on both immune and angiogenic pathways (Table 1).

Pembrolizumab, is a humanised anti-PD-1 antibody (Fig. 2B), being assessed in monotherapy in phase II and III trials (Table 1). This PD-1 inhibitor is also studied in association with autologous infusion of TILs as previously said (Table 1) and in two recently initiated phase-I studies, either in association with Yttrium-90 radioembolization (NCT03099564) or in bitherapy with lenvatinib, a kinase inhibitor, blocking VEGF-R2 (NCT03006926).

Durvalumab is a modified human IgG1 anti-PD-L1 antibody (Fig. 2B), which showed a poor efficacy in an intermediate analysis on 21 patients with advanced HCC, with 0% of OTR and AEs in 62% of patients (grade ≥ III in 10% of cases) [89]. Durvalumab is also assessed in a randomised phase II trial in association with tremelimumab and in a phase I/II study associated with tremelimumab and a locoregional therapy (Table 1).

3.4.3. Perspectives and limitations

Immune checkpoint blockade represents a turning point in cancer treatment and is booming since few years. However, while some patients present spectacular responses, some others do not respond at all, and recently, hyper-progressors were even described under immune checkpoint inhibitors with dramatic evolution [90,91]. We have very few information on predictive markers of response to these therapies, and we need to be able to prevent events such as hyperprogressive disease. PD-L1 dosage has been studied as a predictive factor of anti-PD-1 efficacy in several cancers with interesting results [92]. In metastatic melanomas, nivolumab alone seems sufficient in PD-L1-positive tumours while a bitherapy with ipilimumab is required in PD-L1-negative tumours [87]. In advanced HCC, PD-L1 overexpression has already been shown as a marker of poor prognosis [53], but it does not seem to be a predictive marker of response to anti-PD-1 therapy in the recent phase I/II trial [88]. There is therefore an urgent need of an in-depth characterization of different tumours’ immunophenotypes. By analysing tumours from several trials testing anti-PD-1 in melanomas, 4 different immunophenotypic tumour subtypes were defined [93]. This classification based on PD-L1 expression level and the presence of TILs aims to guide the choice of the most optimal immunotherapies in patients, and rationalise new combinations. Its applicability to other cancers remains uncertain. Also, measuring the mutational load of cancers may be a way to identify the ‘good-responders’ to immunotherapies as it was shown in melanoma [94]. However, this strategy is not relevant in HCC due to a very low tumour mutational load. Its median number of mutations is around 39 per tumour as compared with 1000 mutations per tumour in highly mutated cancers such as colorectal cancers with microsatellite instability [95]. Thus, future studies assessing immunotherapy in HCC have to include a precise immunomonitoring to define immune signatures in tumours and tumour microenvironments and rigorously characterise the different escape pathways activated during checkpoint blockade. This work is essential to identify the ‘responders’ and ‘non-responders’ among patients and help clinicians to personalise the therapeutic strategy and design new relevant combinations.

Indeed, prevention of tumour resistance will probably pass through the use of immunotherapies combinations. Many new associations are currently tested with interesting synergistic effects [96], but their safety remains uncertain and concerning. Indeed, although most of immune-related adverse events (IRAEs) are usually reversible under steroid therapy [92,97,98], several cases of severe toxicity as early lethal myocarditis have been reported under nivolumab-ipilimumab bitherapy [99]. Besides, as immunotherapy may induce long-term responses, patients with significantly improved survivals can develop delayed IRAEs. Long-term safety of immune checkpoint inhibitors is poorly described, yet its characterization is essential to balance their expected benefits with their long-term risks, especially in particular situations such as complex combinations, and adjuvant schemas. Moreover, as the vast majority of HCC arise in a context of hepatocellular deficiency, future phase I trials should include cirrhotic patients to properly assess immunotherapy safety in this specific situation.

4. Conclusion

Liver is a singular immune environment where multiple immunotolerance mechanisms have developed aiming to protect it from a constant and massive antigen exposure. These mechanisms are deregulated in various chronic liver diseases and are responsible of a defective immunosurveillance, leading to HCC emergence. The restoration of a competent antitumour immunity with a more balanced immunotolerance is probably a key factor in the response of HCC to immunotherapy. Encouraging results have been obtained in early-phase clinical trials, particularly with anti-PD-1 therapies. Thanks to the abscopal effect, their combination with locoregional antitumour strategies is probably the best strategy to treat advanced HCC. Nonetheless, a careful analysis of tumour and surrounding liver immune-phenotyping is needed to identify performant predictive biomarkers of response, and a long-term follow-up is essential to better characterise immune checkpoint inhibitors’ long-term safety.

Conflict of interest statement

Gaël S Roth does not declare any conflict of interest. Thomas Decaens declares a conflict of interest with Bristol-Myers Squibb France for consulting activities.


