Chapter 4
Immune Checkpoint Inhibitors for the Treatment of Hepatocellular Carcinoma

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4.1 Immune Checkpoint Molecules and the Immune Response Against Tumors

The adaptive immune response against cancer is a complex process that takes place at different sites. Following capture of cell debris, dendritic cells (DC) uptake and process tumor associated antigens (TAA) inside the tumor, become activated and migrate to the regional lymph nodes, where they present the TAA inside a major histocompatibility complex (MHC) class II molecule to CD4+ T cells [1]. Antigen recognition then stimulates CD4+ T cells to proliferate and produce interferon gamma (IFN-γ) in a process called type 1 T helper cell (Th1) polarization. Th1 polarization occurs in the presence of type I interferon and interleukin 12 (IL-12) released by DC, and is governed by intracellular co-stimulatory signals resulting from CD28 on the CD4+ T cell membrane binding to CD80 and CD86 on the DC surface. Th1 cells license DCs for cross-presentation of TAA to CD8+ T cells, thus assisting in the development of CD8+ cytotoxic T lymphocytes (CTL). Circulating CTL eventually migrate to tumor sites, where they can interact with their cognate MHC class I-TAA complex on the membrane of the tumor cells. The antitumor activity of TAA-specific CD8+ T cells relies on their ability to produce IFN-γ, which inhibits tumor cell growth, and on their cytotoxic activity mediated by the release of granzyme B and perforin, and by the interaction with FAS and TRAIL receptors on tumor cells [2]. In HCC, the relevant role of the Th1 response is supported by clinical findings showing that the expression of Th1 cytokines (IL-1α, IL-1β, IL-2 and IFN-γ) in tumor tissue is associated with good prognosis, whereas

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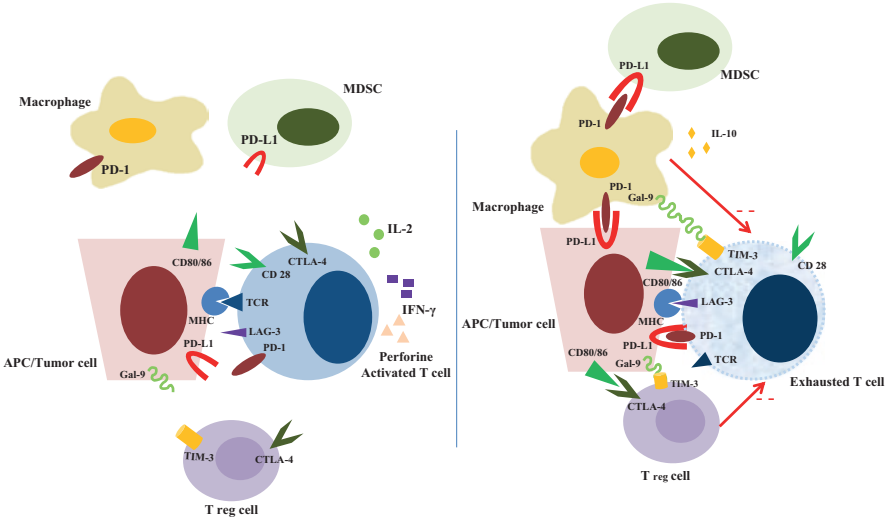

Fig. 4.1 Interplay of the main immune check points in liver cancer. CTLA-4 binds CD80 and CD86, antagonizing the interaction of CD28 with these receptors. PD-1 binds PD-L1 and inhibits CD4+ and CD8+ activation (Tcell exhaustion). LAG-3 synergizes the inhibitory effect of PD-1 on T cell function by binding MHC class I molecules. TIM-3 binds different ligands and inhibits T cell activation and enhances Treg activity

Effect of the main immune check points in liver cancer: CTLA-4 is able to bind CD80 and CD86, antagonizing the interaction of CD28 with these receptors, that results into a decreased T cell activation upon APC antigen presentation. PD-1 expressed on T-cells and other immune cells such as macrophages bind PD-L1 expressed on APC, tumor cells and MDSC and inhibits both CD8+ activation and proliferation and CD4+ activation by blocking the TCR signaling, decreasing the secretion of IFN-gamma from T cells (T-cell exhaustion). On tumor associated macrophages, the binding of PD-1 to its ligand leads to an increased secretion of IL-10 that exert inhibitory effect on T-cells. LAG-3 synergizes inhibitory effect of PD-1 on T cell function by binding MHC class I molecules. TIM-3 expressed on T-cells and T-reg cells and tumor associated macrophages. It binds different ligands such as Gal-9 expressed on APC, Tumor cells and macrophpages. The main effect of TIM-3 is the inhibition of T-lymphocytes activation and the enhancement of T-reg cells activity

Th2 cytokines (IL-4, IL-5 and IL-10) are upregulated in advanced HCC with vascular invasion and metastasis [3].

Immune checkpoints are a specific subtype of membrane-bound molecules that provide fine-tuning of the immune response. A comprehensive review of their variety and functions can be obtained in [4, 5] and their key functions are summarized in Fig. 4.1. Immune checkpoints are expressed in different cell types involved in the immune response, including B and T cells, natural killer (NK) cells, DC, tumor associated macrophages (TAM), monocytes, and myeloid-derived suppressor cells (MDSC). Under physiological conditions, most of these molecules display an immunosuppressive activity that prevents T cell overactivation during the immune response against infection and limits 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).

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 and may thus antagonize the interaction of CD28 with these receptors, with resulting decreased T cell activation upon antigen presentation. CTLA-4 is also constitutively expressed on regulatory T cells (Treg). Treg are CD4+ T cells that can be characterized by the presence of CD25, CTLA-4, CD62L and FoxP3 molecules in their membrane. Activated by TCR engagement concurrent with IL-10 and TGF-β signaling, 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 via CTLA-4. Hence, CTL-4 is also required for Treg to exert its suppressive activity on activated Tcells [6]. But the role of CTLA-4 is not restricted to the priming phase. Inside the tumor, CTLA-4 also promotes immunosuppression by inducing Treg activity and differentiation and upregulating IDO and IL-10 in DC [7].

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-1 is expressed in hematopoietic cells, including APC and MDSC, and in different types of parenchymal cells too, while PD-L2 expression is limited to the haematopoietic compartment. PD-L1 is upregulated by various cytokines, particularly IFN-γ. Upon binding to its ligands, PD-1 inhibits CD8+ T cell activation by blocking the TCR signaling, 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 tumor microenvironment, IFN-γ produced by TAA-specific T cells induces PD-1 expression on reactive T lymphocytes and upregulates PD-L1 in APC and tumor cells. PD-1–PD-L1 engagement then blocks TCR signaling and inhibits T cell proliferation and secretion of cytotoxic mediators, in a process called T cell exhaustion [8]. The expression of PD-L1 is enhanced by IFN-γ release under the hypoxic conditions present in most tumors.

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 in animal models coexpress PD-1, and these dual-expressing cells exhibit greater defects in both cell-cycle progression and effector cytokine production IL-2, TNF, and IFN-γ than cells that express PD-1 alone. The TIM-3 pathway may thus cooperate with the PD-1 pathway to promote the development of a severe dysfunctional phenotype in CD8+ T cells in cancer [9].

LAG-3 is a membrane protein that binds MHC class II molecules with high affinity, thus 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 [10, 11]. Finally, BTLA is an immunoglobulin-like molecule expressed by several immune cells including B and T lymphocytes, NK and antigen presenting cells. 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 [12, 13].

4.2 The Relevance of Immune Checkpoint Molecules in the Immunological Background of Liver Cancer

The liver has a unique immunological milieu compared to any other organ of the human body. The interaction between different resident cells, such as Kupffer cells (KC), hepatic stellate cells (HSC), liver sinusoidal endothelial cells (LSEC), and different types of immune cells, such as DC, NK, T or B lymphocytes, contribute to maintain a predominantly immunotolerant microenvironment in the liver. This is probably a protective mechanism aimed to limit the inflammatory response that may result from the continuous exposure of the liver parenchyma to different types of antigens transported from the gut through the portal circulation. Indeed, activation of the cellular immune response inside the liver parenchyma is limited by different mechanisms. Particularly by a high expression of inhibitory membrane molecules such as PD-1 and PDL-1, a low expression of costimulatory molecules such as CD80 and CD86, and a high concentration of immunosuppressive cytokines such as IL-10. While this immunotolerant environment can be considered a protective mechanism under physiological conditions, it may have detrimental consequences when liver cancer arises.

The immune response is relevant to HCC development and behavior, and the detection of a specific immune response against HCC has been associated with less advanced tumors and better prognosis [14]. As a matter of fact, different studies have shown that among HCC patients treated by liver resection or transplantation, a dense lymphocytic infiltration of the tumor carries a better prognosis [15, 16]. The configuration of such infiltrate is also important. Tumor infiltrating Treg correlate with poor outcome in HCC patients after resection [17] while an inverse correlation has been shown between the number of MDSC and patient outcome after RFA ablation [18]. On the other hand, most HCC tumors develop in the setting of cirrhosis due to chronic viral infection. Chronic IFN-γ release resulting from chronic inflammation may also lead to Tcell exhaustion. Increasing evidence suggests that the exhaustion of the immune response may impair the prognosis of HCC. The inability of the tumor-infiltrating CD8+ lymphocytes to produce IFN-γ upon antigen stimulation has been described in human HCC [14]. High expression of PD-1 and PD-L1 in liver cancer tissue has been reported to predict poor prognosis in HCC

patients undergoing liver resection with an increased rate of recurrence after resection; and is associated to more aggressive tumor characteristics [19, 20].

But PD-1/PD-L1 is not the only pathway that has been involved in HCC. An overexpression of LAG-3 in tumor infiltrating CD8+ lymphocytes compared to peripheral lymphocytes was observed in patients with HCC related to HBV infection [21]. In patients with HBV-related HCC, an overexpression of TIM-3 on tumor infiltrating CD4+ and CD8+ T lymphocytes has been reported and found to be associated to replicative senescence of T cells [22]. In an animal homograft model of liver cancer, TIM-3 expression in TAM enhanced tumor growth in vivo [23]. The high concentration of TGF-b produced by liver tumor cells seems to upregulate TIM-3 in TAM and induces an M2 phenotype in these cells. Moreover, TIM-3 is able to promote the alternative activation of TAM in a TGF-beta independent mechanism [23]. The high levels of cytokines, mainly IL-6 and IL-10, produced by M2 TAM may ultimately promote tumor growth. In patients with HCC, the expression of TIM-3 in monocytes and TAM strongly correlated with higher tumor grades and poor survival [23].

As mentioned above, 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 [12, 13]. A high expression of HVEM expression in HCC is associated with reduced lymphocyte infiltration, diminished levels of effector T cell mediators, and worse prognosis after resection [24]. It has recently been shown that in patients with HCC the majority of BTLA+ CD4+ T cells also express PD-1 [25]. This suggests that BTLA may identify a highly dysfunctional CD4+ T cells population within liver cancer. Interestingly, a high concentration of BTLA+ PD1+ CD4+ T cells, but not of BTLA- PD1+ CD4 T cells, was associated with more advanced HCC stages.

4.3 Clinical Experience with the Use of Checkpoint Inhibitors in Hepatocellular Carcinoma

All these preclinical information provides a valid rationale for an immunologic approach to the treatment of HCC based on the interaction with immune checkpoints. Clinical studies have only recently been conducted but the results are more than encouraging. There is no hyperbole in saying that checkpoint inhibitors have revolutionized cancer care. Signals delivered by immune checkpoints plays a major role in the induction and maintenance of tumor immune tolerance. Monoclonal antibodies that block negative signals for T lymphocytes may allow the amplification of the T cell response, avoidance of T cell exhaustion, or elimination of Treg. These compounds have shown a wide spectrum of anticancer activity that resulted in a survival advantage over standard therapies in several cancer types, including melanoma, head and neck squamous carcinoma, non-small cell lung cancer, bladder cancer, renal cell cancer, or Hodgkin's lymphoma [26–31].

In the field of HCC, clinical development has focused on CTLA-4 and PD-1/PD-L1 pathways (Tables 4.1 and 4.2).Tremelimumab is a fully human IgG2

Table 4.1 Trial design and patient characteristics in reported clinical trials using immune checkpoint inhibitors in HCC

Author, year	Study phase	Agent	Dose	Target	N	Etiology	BCLC B/C	Child A	Prior sorafenib
Sangro, 2013 [33]	2	Tremelimumab	15 mg/kg every 90 days	CTLA-4	21 17[a]	HCV	28/57%	57%	24%
Duffy, 2016 [35]	1b/2	Tremelimumab + RFA/TACE	3.5 to 10 mg/kg every4 weeks × 6 doses and then every 3 months	CTLA-4 + tumor ablation[b]	32 19[a]	No infected, HBV and HCV	25/75%	86%	65%
El-Khoueiry A, 2017 [38]	1b/2	Nivolumab	0.1 to 10 mg/kg every 2 weeks (3 mg/kg on expansion phase)	PD-1	262[c]	No infected, HBV and HCV	12/88%	98.4%	69%

[a]Number of patients evaluable for tumor response
[b]5 weeks after 1st dose
[c]48 patients from dose-escalation phase and 214 from dose-expansion phase

Table 4.2 Results reported in prospective clinical trials using immune checkpoint inhibitors in HCC

	Efficacy				Safety			
					Any grade (grade ≥ 3) CTCAE			
Author, Year	ORR	SD	TTP (95% CI), months	OS (95% CI), months	Rash	Pruritus	Diarrhea	ASAT
Sangro, 2013 [33]	17.6%	58.8%	6.48 (3.95–9.14)	8.2 (4.64–21.34)	65% (5%)		30% (5%)	70% (45%)
Duffy, 2016 [35]	26%	63%	7.7 (4.7–19.4)	12.3 (9.3–15.4)	15% (0)	9.3% (0)	6.2% (0)	34% (22%)
El-Khoueiry, 2017 [38]	18%	44%	nr	15 m[a] (9.6–20.2 m)	16.7% (0.7%)	20% (0.3%)	12% (1.1%)	10% (5%)

[a]Dose-escalation phase. Not reported in those-expansion phase

monoclonal antibody that blocks the binding of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). As explained, CTLA-4 at the immune synapse outcompetes the binding of the CD28 co-stimulatory receptor to CD80 and CD86 with much superior avidity. This binding sends an inhibitory signal that serves as a natural brake for T cell activation. Tremelimumab blocks the inhibitory effect of CTLA-4, and therefore enhances T cell activation and proliferation [32]. Among CTLA-4 targeted therapies, tremelimumab was the first molecule to be clinically evaluated in HCC. Our group led a phase II, non-controlled, multicenter trial that targeted the population of patients with HCC and chronic HCV infection who were not eligible for surgery or locoregional therapy [33]. We had the dual intention to test the anti-tumor and antiviral activity of tremelimumab in a single study. The study was 80% powered to reject the null hypothesis that objective response rate did not exceed 5% at a 0.05 level of significance if true objective response rate was >25%. Based on a Simon's optimal 2-stage design 3 tumor responses among 17 evaluable patients were needed to reject the null hypothesis. Twenty-one patients with fairly advanced disease (57% were at BCLC C stage) were enrolled, most of them (57%) having progressed to 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 to a maximum of 4 doses unless tumor progression or unacceptable toxicities occurred. Despite this suboptimal dosing, 3 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%. Quite importantly, almost half (45%) of these stabilizations lasted longer than 6 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 antitumor activity. Median time to progression was 6.48 months (CI 95% 3.95–9.14 months). Although potentially biased by a long tumor assessment interval, this prolonged time to progression compares favorably with several targeted agents as shown in Table 4.3. The observed overall survival of 8.2 months (CI 95% 4.64–21.34 months)

Table 4.3 Systemic agents for the second line treatment of advanced HCC: a perspective to understand the data from immune-oncology agents

Trial	Agent	n	Patient profile				Overall survival (months)		Time to progression (months)	
			Child B	ECOG >0	EHD	MVI	Median	95%CI	Median	95%CI
Randomized trials with targeted agents										
BRISK-PS	Brivanib	263	7%	43%	65%	31%	9.4	nr	4.2	nr
	Placebo	132	9%	39%	64%	18%	8.2	nr	2.7	nr
Tivantinb 2 L	Tivantinib	71	4%	42%	69%	60%	6.6	4–6-9.0	1.6	1.4–2.8
	Placebo	36	3%	42%	78%	64%	6.2	3.8–9-4	1.4	1.4–1.5
EVOLVE-1	Everolimus	362	2%	40%	74%	32%	7.6	6.7–8.4	3.0	2.8–4.0
	Placebo	184	1%	43%	73%	33%	7.4	6.3–8.7	2.6	1.5–2.8
REACH-1	Ramucirumab	283	2%	44%	73%	29%	9.2	8.1–10.6	3.5	2.8–4.5
	Placebo	282	2%	46%	71%	28%	7.6	6.0–9.3	2.6	1.6–2.8
RESORCE	Regorafenib	379	1%	35%	70%	29%	10.6	9.1–12.1	3.2	2.9–4.2
	Placebo	194	3%	33%	76%	28%	7.8	6.3–8.8	1.5	1.4–1.6
Single arm trials with immune checkpoint inhibitors										
	Tremelimumab	21	43	28%	9%	28%	8.2	4.6–21.3	6.5	3.9–9.1
	Tremelimumab + ablation	32	14	75%	45%	nr	12.3	9.3–15.4	7.7	4.7–19.4
CheckMate040	Nivolumab	262	2%	nr	76%	8%	15.0	9.6–20.2	nr	nr

Refs.: BRISK-PS [52], Tivantinib 2L [53], EVOLVE [54], REACH-1 [55], RESORCE [56], Tremelimumab [33, 35], CheckMate-040 [38]

was not much different from what could be observed in patients receiving placebo in second-line trials but the high proportion of Child B patients in this cohort likely had a significant impact in this outcome.

A significant antiviral effect was also observed, with a decrease in median 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). The progressive course of this decline in viral load was observed in most patients followed for at least 3 months, and three patients had a transient complete viral response during follow-up. 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-g-producing cells against viral antigen) versus 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 antitumoral effect was not associated to this antiviral effect or to patient characteristics including systemic inflammatory signals such as C reactive protein. The lack of repeated tumor biopsies precludes any interpretation of the mechanism behind the antitumor activity while the expansion in circulating Treg following tremelimumab therapy was in line with observations in other tumor types [34].

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 topic agents and oral antihistamine drugs. Diarrhea was observed in 30% of patients but reached grade 3 in only one patient. A remarkable rise in serum transaminases was observed after the first dose in more than half of the patients, being 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 antitumor or antiviral responses, or with changes in circulating cytokines.

Following the same path, a second trial tested a very appealing hypothesis i.e. whether an antigenic stimulation provided by means of incomplete tumor ablation using percutaneous radiofrequency (RFA) or transarterial chemoembolization (TACE) could safely enhance the effects of tremelimumab [35]. The rationale for this combination is based on the fact that RFA or TACE could induce immunogenic tumor cell death and this in turn could stimulate a peripheral systemic immune response that may be further amplified by immune checkpoint blockade. In a phase I/II trial increasing doses of tremelimumab were given followed by subtotal tumor ablation and tumor response was evaluated in those lesions not targeted by RFA, cryoablation or TACE procedures. This was a pilot study with no specific sample size assumptions. Thirty-two patients with mostly advanced HCC (75% at BCLC C stage) were enrolled, 78% having progressed to previous therapies. Patient characteristics were therefore 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. Most patients (75%) had viral hepatitis as cause of liver cirrhosis.

Enrolled patients were treated this time with an optimal dose of tremelimumab at two dose levels (3.5 and 10 mg/kg IV) given every 4 weeks for a total of 6 doses, followed by 3-monthly infusions until off-treatment criteria were met. The interventional radiologic procedure (TACE for BCLC B and thermal ablation for BCLC C patients) was performed 5 weeks after 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 5 patients (26%), and stable disease in 12 patients (63%), accounting for a disease control rate of 89%. Again, almost half (45%) of the stabilizations lasted longer than 6 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 provide a signal of the consistency of the antitumor effect. The better overall survival of 12.3 months (95% CI 9.3–15.4 months) in the combination trial could be explained on the basis of the good liver function but a true enhancing effect of prior ablation may not 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 was predominantly grade 1. Less frequent side effects were diarrhea (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. The antiviral activity was also confirmed in this trial. The HCV viral load of 14 quantifiable patients decreased after 3 months in 12 patients, with a median HCV viral load decrease from 1275×10^3 UI/ml to 351×10^3 UI/ml.

This trial was enriched with important correlative studies. The amount of peripheral blood CD3, CD4, CD8, CD38 and HLA-DR positive cells was analyzed after every cycle by multicolor flow cytometry. Tumor biopsies were obtained from some patients immediately before ablation (after 2 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. Such increase was especially intense and sustained for CD8 + T cells. Immune cell tumor infiltration was observed in all 12 patients in whom post-tremelimumab tumor samples could be evaluated. Among those 6 patients with paired tumor 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 a higher post-tremelimumab CD3+ and CD8+ infiltration compared to 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 to be confirmed.

The encouraging signs of antitumor activity of tremelimumab in advanced HCC and its good safety profile in cirrhotic patients of viral etiology, provided a strong

reason to test other checkpoints inhibitors [36]. The PD-L1/PD-1 pathway provides another mechanism of tumor-induced immune tolerance. PD-1 expression on effector phase CD8 + T cells is increased in HCC patients compared to cirrhotic patients or healthy controls [19]. And indeed, HCC patients with higher numbers of tumor 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 peritumoral stromal cells (Kupffer cells, LSEC, and monocytes) as well as cancer cells, promoting a PD-L1/PD-1 pathway-driven inhibition of antitumor T cell responses [20, 37]. Thus, a strong rationale supports the use of PD-1 and PD-L1 blocking antibodies against HCC. Building on the experience with tremelimumab, we helped develop the first clinical trial to assess 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 etiologies (HCV infection, HBV infection, non-viral cirrhosis) [38].

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 to systemic therapy and had progressed or were intolerant to sorafenib or had refused this drug. First, a dose-escalation cohort of 48 patients received doses that ranged from 0.3 mg/kg to 10 mg/kg every 2 weeks with the primary endpoint of establishing the safety and tolerability of nivolumab in HCC patients. 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 in four specific groups of uninfected patients progressing to sorafenib, uninfected patients naïve or intolerant to sorafenib, patients with HCV infection and patients with HBV infection. In both cohorts, HBV-infected patients had to be on effective antiviral therapy (circulating viral DNA < 100 UI/ml) [38].

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 rash (23%), pruritus (21%) and diarrhea (13%), that were usually mild. Grade 3 or higher treatment-related symptomatic adverse events occurred in less than 2% of patients. Hypertransaminasemia was the most frequent laboratory alteration (20%) reached grade 3 or higher in only 5% of patients. Regarding etiologies, 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 overall were 20% and 7%, respectively, while no treatment-related deaths occurred. Immune related hepatitis needing steroid therapy occurred very rarely. Only 3% of patients discontinued nivolumab due to treatment-related adverse events and no treatment-related deaths were reported.

Convincing signs of efficacy were reported. In the escalation and expansion cohorts, objective tumor responses were reported in 15% and 20% of patients, respectively. And 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 6 months in most cases. The majority of objective responses occurred during the first 3 months of treatment. It has to be stressed that response rates were similar across different etiologies, and both in sorafenib-naive and sorafenib-exposed patients. This signs of efficacy were consistent with the 9-month survival rate of 70% reported in the large expansion cohort and the median overall survival of 15 months (95% CI 9.6–20.2 months) reported in the dose-escalation cohort with a longer follow-up. This median survival was observed irrespective of prior sorafenib treatment, and compares well with any other phase 2 or 3 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 (Table 4.3).

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

4.4 Ongoing Studies and Potential Combinations

Building upon this experience, clinical development around immune checkpoints in HCC has thriven. A summary of ongoing studies is provided in Table 4.2. Some of them are designed to help define the place of specific agents in the treatment paradigm for HCC. This group includes two pivotal phase 3 trials comparing nivolumab vs. sorafenib as first-line systemic therapy for advanced HCC, and pembrolizumab vs. best supportive care as second-line therapy for patients that progress or are intolerant to sorafenib. Some others are designed to expand the potential of immunotherapy based on the interaction with checkpoint molecules in several ways. The potential rationale according to treatment platforms is illustrated in Fig. 4.2.

The activity of PD-1/PD-L1 inhibition not only in HCC but also across tumor types makes it a sound backbone for combinatorial strategies. The simultaneous blockade of different checkpoints may produce synergistic effects and has shown impressive results in patients with melanoma [39]. Dual blockade of PD-L1 and the non-redundant CTLA-4 is attempted in a phase 1b cohort of the Checkmate 040 where different doses of ipilimumab (a CTLA-4 blocking IgG1 monoclonal antibody) and nivolumab are tested [38]. Another trial with a 1b/2 design is testing the

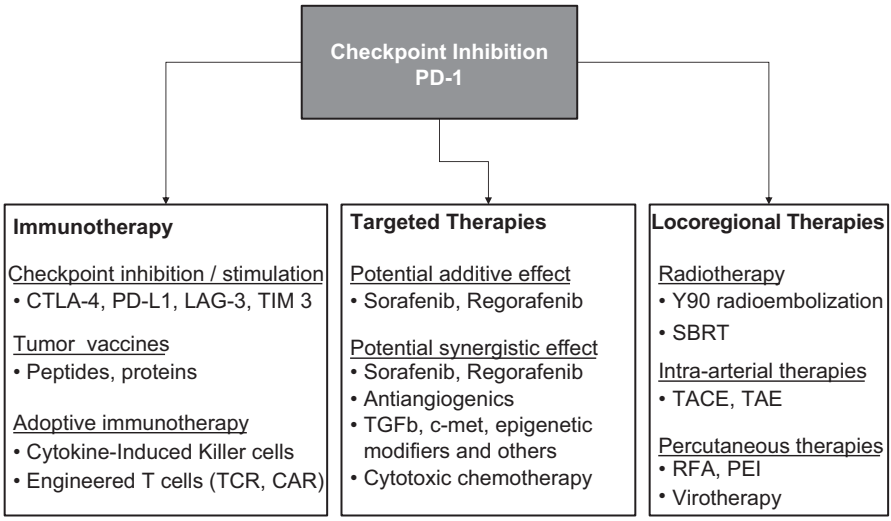

Fig. 4.2 Potential combinations for therapeutic development in immunotherapy of HCC (*TCR* T cell receptor, *CAR* chimeric antigen receptor, *TACE* transarterial chemoembolization, *TAE* transarterial (bland) embolization, *RFA* radiofrequency ablation, *PEI* percutaneous ethanol injection)

combination of durvalumab (a PD-L1 blocking monoclonal antibody) and tremelimumab compared to each agent as monotherapy. Durvalumab has shown a good safety profile in a small cohort of HCC patients treated in a basket trial [40].

Stimulation of co-stimulatory molecules such as CD137 (4-1BB), CD134 (OX40), glucocorticoid-induced tumor necrosis factor receptor (GITR) or CD40 may potentiate the effector functions activated T cells and NK, constrain the suppressive activity of Treg, and enhance antibody-dependant cellular cytotoxicity. Combining PD-1/PD-L1 inhibition with stimulation of these co-stimulatory molecules would simultaneously release the brakes and press the gas pedal of the immune response. As a matter of fact, this strategy has proven effective in HCC models [41] and deserves clinical testing.

Inhibition of oncogenic pathways may have an effect on the antitumor immune response. For instance, BRAF inhibition in melanoma may increase the expression of melanoma differentiation antigens and HLA molecules on tumor cells, induction of PD-1 expression, and inhibition of suppressive cytokines as IL-10 [42]. Even more importantly, BRAF inhibition may increase CD8+ T cell tumor infiltration, a potential hallmark for immuno-oncology agents effectiveness. Regarding antiangiogenic drugs, VEGF modulates antitumor immunity through different mechanisms including the expansion of suppressive cell subtypes such as Treg and MDSC, inhibition of DC maturation, or suppression of T cell responses [43]. Again in melanoma patients, combination of ipilimumab and the VEGFR antagonist bevacizumab produced intense tumor infiltration by CD8+ T cells and dendritic macrophages as well as high numbers of peripheral memory T cells [44]. Little is known about the specific immune effect of sorafenib. Studies performed in animal HCC models

showed that sorafenib-induced hypoxia may inhibit the immune response by increasing intratumoral expression of PD-L1 and enhancing the recruitment of Treg and M2 macrophages [45]. In this study the combination of an anti-PD1 antibody and sorafenib was not more effective than sorafenib alone. A different study suggested that sorafenib increases the local recruitment of tumor-associated neutrophils and ultimately populates the tumor stroma with macrophages and Treg, thus promoting an immunosuppressive environment [46]. In this study, depletion of tumor-associated neutrophils combined with sorafenib led to a stronger anti-tumoral activity compared to sorafenib alone. In the clinical setting, the combination of sorafenib and PD-1 blockade will be tested.

The release or expression of tumor antigens and the immune-adjuvant like effect of tumor irradiation is the basis for the well-known phenomenon of the abscopal effect [47, 48]. In a sense, radiotherapy may act as a "local tumor vaccine". In animal models, a variety of synergistic effects occur when radiation therapy is combined with CTLA-4 blockade including diversification of the TCR repertoire of tumor infiltrating lymphocytes and modeling of the repertoire of expanded T cell clones [49]. The potential synergy of this combination has been also suggested in advanced melanoma patients [50]. In HCC, selective internal radiation therapy or radioembolization is increasingly used as a locoregional therapy for different stages. Clinical trials trying to exploit this potential synergy are underway (Table 4.4). The ability of other forms of locoregional treatment of HCC such as TACE or RFA to

Table 4.4 Ongoing clinical trials testing immuno-oncology agents in HCC

Phase	Population	Agents	Target	NCT number
IO agents as monotherapy				
1b/2	1L and 2L	Nivolumab	PD-1	01658878
2	2L	Pembrolizumab	PD-1	02702414
3	1L	Nivolumab vs. Sorafenib	PD-1	02576509
3	2L	Pembrolizumab vs. best supportive care	PD-1	02702401
IO agents in combination with other IO agents				
1b/2	2L	Nivolumab + Ipilimumab	PD-1 & CTLA-4	01658878
1b/2	1L and 2L	Tremelimumab + Durvalumab vs. Durvalumab vs. Tremelimumab	PD-L1 & CTLA-4	02519348
IO agents in combination with non-IO agents				
1b/2		PDR001 vs PDR001 + Capmatinib	PD-1 & c-met	02795429
1b/2		Nivolumab + CC-122	PD-1 & pleiotropic pathway modifier	02859324
1b/2		Nivolumab + Galunisertib	PD-1 &TGFb	02423343
1a/b		Durvalumab + Ramucirumab	CTLA4 & VEGFR2	02572687
1b		Pembrolizumab + Nintendanib	PD-1 &multikinase	02856425
1		Pembrolizumab + Lenvatinib	PD-1 &multikinase	03006926
1b		PDR001 + Sorafenib	PD-1 &multikinase	02988440
1b/2		Nivolumab + Y90 radioembolization	PD-1 & radiation	03033446 02837029

favor immune responses is much less established. Nevertheless, ongoing clinical trials are taking advantage of the information about the combination of subtotal TACE/RFA cited above. Intratumoral injection of the vaccinia oncolytic virus Pexavec was able to produce distant responses but failed to prove effective in prolonging survival of patients with advanced HCC [51].

Natural interaction between tumor and host defines the amount and specificity of pre-existent tumor reactive T cells. If the number of T cell clones primed by tumor-associated antigens is low (as it could be particularly for tumors with a low mutational load), the tumor immune infiltrate may not be intense enough to benefit from the immune stimulation of checkpoint inhibitors and the efficacy of checkpoint inhibitors would be reduced or abolished. Effective tumor vaccines may overcome this problem. Co-administration of tumor-associated neoantigens and a strong immune adjuvant is the basis of the HEPAVAC project and clinical trial (http://www.hepavac.eu/).

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

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