



The future is now: beyond first line systemic therapy in hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is becoming a worldwide concern due to its rising incidence. Although for the incipient stages there are curative therapies, the advanced disease represents a major provocation for the clinicians. 2008 marked as an important year for the hepatology community with the administration of sorafenib for late stages of HCC. Six years after this major discovery, the multikinase inhibitor still represents an important pillar, the first line treatment for the advanced liver cancer. Lenvatinib may represent a new promising first line strategy, but it is still unavailable in many countries. The last years represented an explosion in the research of HCC. Beyond the first line treatments there are a plethora of new emerging therapies. By far immunotherapy represents the major revolution in oncology. While adoptive immunotherapy is still at the beginning, immune check-point inhibitors bursted in many clinical trials with very encouraging results. This review summarises the major discoveries in the field of HCC with an emphasis on immunotherapy. It also briefly describes the important aspects of primary liver cancer immunology and the major ongoing clinical trials.

Keywords: Hepatocellular carcinoma (HCC); systemic therapy; advanced; first line; immunotherapy

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Background

Hepatocellular carcinoma (HCC) is a major global health issue, being the 5th most prevalent and the 2nd most deadly cancer. Moreover, the incidence of HCC has shown a growing tendency in the recent years, the rate of newly diagnosed HCC patients increased by 75% from 1990 to 2015 (1). Projections that have been made assume that in the USA, by 2030 HCC will be the third leading cause of cancer, preceding breast, prostate and colorectal cancer (2,3). HCC is most commonly found in the elderly, with its peak incidence at about 70 years old, twice more frequent in men than women (4). Europe belongs to the lower incidence region, however, in the South the incidence is significantly higher (5).

In approximately 90% of cases, the etiology of HCC is established, chronic viral hepatitis, alcohol intake and aflatoxin exposure being the most common causes. In Western world, chronic hepatitis C virus is the main risk factor (1). Even though direct-acting antiviral (DAA) treatment has a high efficacy in the eradication of HCV, which should correlate with a lower risk of tumour development, there are some reports that suggest a higher risk of HCC occurrence and recurrence, with a more aggressive pattern in DAA treated patients. This may be due to changes in immune system because of the rapid decrease in viral load (6). Many epidemiological studies are needed regarding this concern. Mice studies, as well as epidemiological observations demonstrates that HCC

may appear in the settings of non-alcoholic steatohepatitis (NASH) and obesity via liver inflammation and tumorigenesis, interleukin-6 (IL-6) and tumour necrosis factor-alpha (7).

Surveillance examinations is based on abdominal ultrasound (US). US should be performed every 6 months in high-risk groups (cirrhosis, hepatitis B, DAA treated patients) (8). Unfortunately, there is no serological test for an early diagnosis of HCC. Alpha-feto-protein (AFP) which is one of the most used biomarkers, has increased serum levels in flares of HBV, HCV infections or decompensations of underlying liver disease, which means it has low accuracy in detection (9).

There are several staging systems for the classification of HCC in order to assess the prognosis, from which the BCLC system is the most widely used because it correlates the treatment method with the tumour stage and the severity of subsequent liver cirrhosis. Although there are curative treatments for initial stages (transplantation, resection, ablation), many patients are still diagnosed when effective therapy is no longer possible; therefore advanced disease represents the major concern for the Hepatology community (10). Although sorafenib (11), a multi-kinase inhibitor, revealed improved overall survival (OS) and disease-free survival (DFS) in clinical trials and in real-life scenarios, more emerging therapies are now tested with potential benefit. Moreover, immunotherapy is showing a great promise and might stand as a revolutionary therapy.

First line treatment

In order to review what's beyond the first line of treatment in HCC, it's important to remember which are the current recommendations from the guidelines (12).

Sorafenib is the first drug which showed survival benefits in patients with advanced HCC and is considered a major revolution in hepatology (13). Since 2008, when the SHARP (Sorafenib HCC Assessment Randomised Protocol) trial showed an improved overall survival in Child-Pugh A (CP-A) patients with advanced HCC treated with sorafenib, this therapy is considered standard of care for advanced HCC. In addition, the benefits and safety of the administration of sorafenib were strengthened by the phase 4 large GIDEON study (Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and of its treatment with sorafeNib). This prospective, observational study revealed that sorafenib has the same safety profile, drug related

adverse effects and overall survival between CP-A and CP-B patients, [13.6 (12.8–14.7)] *vs.* [5.2 (4.6–6.3)] (14).

Sorafenib inhibits not only the RAF kinase, but some other tyrosine kinases involved in the development of HCC such as mitogen activated protein (MEK), extracellular signal regulated kinase (ERK), vascular endothelial growth factor receptor 2 (VEGFR2), platelet derived growth factor receptor (PDGFR), the receptor-type tyrosine-protein kinase FLT3, the proto-oncogenes Ret and cKIT (15). Major etiologic factors such as hepatitis B and C viruses overexpress Raf1 kinase thus leading to the activation of the Raf/MEK/ERK pathway (11). Therefore, the administration of sorafenib with consequent inhibition of MEK can further reduce cell proliferation and can induce apoptosis (15). Moreover, sorafenib demonstrates its antiangiogenic properties due to the fact that HCC is a hypervascular tumour with an overexpression of VEGFR (16).

The efficacy of sorafenib assessed by the overall survival (OS) was outlined and confirmed even from the beginning by two phase III trials (11,17) that obtained an OS of 10.7 months as compared with placebo. Moreover, recent studies, analysed the OS under sorafenib treatment according to the BCLC class. Apart from patients with BCLC-C, also the BCLC-B patients who failed to respond or had contraindication to the standard of care, transarterial chemoembolization (TACE) or other ablative therapies (18) had a benefit. The median OS ranged from 7.2 to 15.7 months in BCLC-C (18-21) and from 19.6 to 21.5 months in BCLC-B patients (18-21).

Due to the heterogeneity of the HCC population included in the BCLC-C class, where the recommended treatment option is sorafenib, there is a variable response to this systemic treatment.

In order to predict or evaluate the response to sorafenib in an early phase and therefore the impact of OS, several factors that can impact the treatment outcomes have been analysed. Regarding the tumour related factors, several histological and serological biomarkers have been identified: extra-hepatic spread (EHS), CRIPTO, AFP decrease, ANXA3, PIVKA, FGF2, BCL2, COX2/PGE2 axis (22-29).

Some patient's characteristics and associated comorbidities and its treatment may impact the sorafenib response: type 2 diabetes mellitus (T2DM), systemic inflammatory index (SII), FT4xTSH score, neutrophil to lymphocyte ratio (NLR), platelet level and pre-sarcopenia/sarcopenia (18,19,21,30-35).

Furthermore, there are currently several clinical trials analysing whether the association of sorafenib to

immunotherapy may enhance its effect, with an impact on the overall survival, which will be discussed later in the review.

Lenvatinib is the second first line drug approved in the treatment of HCC, being non-inferior to sorafenib. Lenvatinib is an orally multikinase that inhibits VEGFR 1-3, FGFR 1-2, PDGF- β , KIT and ret kinases (36).

Promising phase II trial, showing 37% response rate (by mRECIST), 7.2 months median time to progression and an acceptable safety profile led to a phase III multicentre, randomized, open-label trial (37). The REFLECT trial included CP-A patients with advanced HCC and ECOG-PS 0/1 and excluded the patients with previous systemic anticancer therapy, main portal vein invasion and tumour spread >50% of the liver volume (38). The primary end-points of the study were met, with a median OS of 13.6 months in the lenvatinib arm versus 12.3 months in the Sorafenib arm (38). Lenvatinib performed better in median progression free survival (7.4 *vs.* 3.7 months) and time to progression (8.9 *vs.* 3.7 months) (39). Therefore, for the treatment of advanced HCC there are two first line treatment options, with similar survival benefits and with a different safety profile as sorafenib is more frequently associated with HFSR as the most important AE, while lenvatinib is associated with a higher rates of hypertension, anorexia and fatigue.

Several other therapeutic agents (sunitinib, brivanib, linifanib and erlotinib) were tested as a first line treatment, showing no benefit in overall survival compared with Sorafenib. In part, the failure of these substances can be attributed according to Baxter *et al.* to the lack of understanding of the critical driver and flaws in the trial design, including significant toxicity due to a lack of understanding of the tyrosine kinase inhibitors (38).

Beyond first line

Inside the guidelines

Regorafenib is the first drug approved as second line treatment in HCC patients with proved survival benefits in patients that did not respond to sorafenib.

Regorafenib is an oral multikinase inhibitor that blocks the activity of several protein kinases involved in:

- ❖ Angiogenesis;
- ❖ Oncogenesis;
- ❖ Apoptosis;

- ❖ Autophagy;
- ❖ MAPK signalling pathway (40,41).

The benefits of regorafenib in HCC patients which progressed while on sorafenib was outlined in the phase III Resource trial, which included BCLC stage B/C patients with a good liver function (CP-A) that had radiological progression during previous sorafenib treatment. The primary endpoint of the study was met as regorafenib improved the median overall survival from 7.8 to 10.6 months, compared with placebo (42).

In an exploratory subgroup analysis (41,43) in patients treated with regorafenib, the survival benefit was similar regardless of the last sorafenib dose (800 ng/day or less). Further it was proved that Regorafenib significantly improved post-progression survival relative to placebo irrespective of the progression pattern during sorafenib therapy.

Analysing the effects of regorafenib, some studies (44) have outlined that it can influence the level of expression of PD-L1 by reducing it and also, it can prevent the engagement of PD-L1 by PD-1⁺ T cells. As a consequence, the T cell-receptor mediated signalling is up-regulated and the immune response to HCC is reactivated.

For the patients who do not respond to sorafenib, regorafenib seems like a good alternative with almost a 3 months survival improvement as a second line treatment. Although among the eligibility criteria there are: a previous tolerance to sorafenib and a preserved liver function (CP-A), some studies have outlined that almost 30.6% of the patients that were previously treated with sorafenib are eligible for the regorafenib treatment (45). That can be partially explained by a study that analyses the liver function during the sorafenib treatment, showing that almost 27.4% of the patients will have a CP class changed into CP-B class at 4 weeks after the beginning of treatment (35).

Therefore, regorafenib represents a good alternative for a minority of the patients that are sorafenib-resistant and further studies need to analyse if its survival benefits can be extended to a larger group of patients.

Beyond guidelines

Multikinase inhibitors

HCC tumour cells are usually characterized by heterogeneous imbalances in molecular mechanisms and signalling pathways that regulate cell proliferation, survival and death, growth factors (epidermal growth factor, EGF) and growth factor receptors (EGF receptor),

angiogenic factors (vascular endothelial growth factor, VEGF; fibroblast growth factor, FGFR; platelet-derived growth factor receptor, PDGFR; inflammatory cells, tumour stromal cells), oncogenes. Among the intracellular signalling pathways, the mitogen-activated protein kinase (MAPK) cascade, PI3K/Akt/mTOR (mammalian target of rapamycin), hepatocyte growth factor (HGF)/c-Met pathway, IGF and its receptor (IGFR), as well as Wnt/beta-catenin pathway have been studied and used for development of targeted HCC treatments (46).

Inhibition of angiogenesis is one of the therapeutic targets in HCC and several therapies targeting VEGF have entered clinical studies:

- ❖ Cabozantinib (Cabometyx[®])—an oral multi-kinase inhibitor targeting MET, RET, AXL, and VEGFR1-3, has improved median OS compared with placebo in patients with advanced HCC who have previously received sorafenib in the global phase III CELESTIAL trial (NCT01908426) (47);
- ❖ Bevacizumab—a humanized monoclonal antibody that targets VEGF, which besides its antiangiogenic effects, might also enhance chemotherapy administration by decreasing the interstitial pressure in the tumour (48);
- ❖ Sunitinib—an oral multikinase inhibitor for the receptor tyrosine kinases (RTKs), such as VEGFR-1 and -2, PDGFR-alpha/beta, c-KIT, FLT3, and RET kinases (49);
- ❖ Brivanib—a dual inhibitor of VEGFR and FGFR, undergoing evaluation in phase III studies (50);
- ❖ ABT-869—an oral inhibitor of VEGFR and PDGFR, with early evidence of efficacy and ongoing Phase III studies (51);
- ❖ AZD2171—a pan-VEGF receptor tyrosine kinase, PDGF receptors and c-Kit inhibitor (52);
- ❖ PTK787 (vatalanib)—targeting all VEGFR tyrosin kinases, with a higher activity on VEGFR-2 (53);
- ❖ Pazopanib (GW786034)—an inhibitor of VEGFR, PDGFR, and c-Kit (54).

The EGFR signaling pathways are another important target for HCC therapies and two classes of EGFR agents have proved relevant clinical activity:

- ❖ EGFR tyrosin kinase inhibitors, such as erlotinib (with modest activity), gefitinib or lapatinib (with no proven activity as single agents) (55-57);
- ❖ Monoclonal antibodies against EGFR, such as cetuximab, with demonstrated antitumor activity only in combinations (58,59).

mTOR inhibitors (sunitinib, temsirolimus, everolimus) have demonstrated cell growth and tumour vascularity inhibition in several cancers including HCC cell lines, but studies have not shown significant therapeutic activity for the agents alone (60).

Until this year, sorafenib was the only approved systemic treatment for patients with HCC BCLC-C class. Since 2008, when it became available, many other substances were evaluated for being either superior or non-inferior to sorafenib, but without conclusive results. Meanwhile, another TKI, lenvatinib proved to be non-inferior in terms of OS but with a better progression free survival, time to progression and response rate (61).

The first substance to prove her efficacy in the second line therapy was regorafenib with an improvement in OS from 7.8 months on placebo to 10.6 months (HR: 0.63, $P < 0.0001$) (42).

Apart from regorafenib, in the second line therapy, two other substances met their end-point of an improved survival compared to placebo: cabozantinib from 8 months on placebo to 10.2 months (HR: 0.76, $P = 0.005$) (62) and ramucirumab, after a sub-group analysis, in patients with advanced HCC and an AFP > 400 ng/mL from 4.2 months on placebo to 7.8 months (HR: 0.674; $P = 0.006$) (63).

Several other substances have been tested alone or in combination with sorafenib in the first line (sunitinib, linifanib, brivanib, erlotinib) or in the second line treatment (everolimus, brivanib, tivantinib), but none of them met their primary end-points (64-70).

Chemotherapy

Nowadays, systemic chemotherapy is used only occasionally, in the settings of (very) advanced disease, being out shadowed by the use of multikinase inhibitors. Chemotherapy in the treatment of HCC has two main challenges, the frequent presence of cirrhosis that can perturb the drug metabolism and enhance its toxicity and the additional severity of the chemotherapy related complications in a patient already immune-compromised (71).

Doxorubicin is one of the first chemotherapeutic agents used in HCC treatment with 10% objective response rate and inconclusive survival benefits (72). Nevertheless, it is currently one of the most commonly used chemotherapeutic agents in TACE treatment (73).

Another chemotherapeutic agent with promising results is TS-1 that acts on 5FU metabolism, increasing its toxicity on neoplastic cells (74) Although the initial trial, S-CUBE, failed to fulfil its primary end-points, a subgroup analysis

outlined better results in TNM stage III, IVa, IVb, CP-A patients and in those with a low level of tumour markers (74).

Regarding combo treatment schemes, some other chemotherapeutic regimens have shown negative results.

The PIAF regimen, although with higher response rates 20.9% and better OS 8.67 months, the differences in comparison to doxorubicin regimen were not statistically significant (75). Unfortunately, PIAF was also associated with a significant higher rate of myelotoxicity.

When analysed in comparison with Doxorubicin alone, FOLFOX4 showed better results in terms of progression free survival (2.7 *vs.* 1.7 months), better response rate but with no significant difference in OS, which was one of the primary endpoints (76).

Recently, promising results seem to come from hepatic intra-arterial chemotherapy (HIAC). It is considered a more effective method than systemic chemotherapy because it facilitates the drug to directly reach the tumour throughout the hepatic artery (76). In Japan, HIAC is indicated mainly in localized advanced HCC with evidence of vascular invasion (74).

A recent randomized multicentre, prospective study (77) has shown interesting results in the treatment of patients with HCC and portal vein thrombosis either by sorafenib alone versus sorafenib + HIAC. The OS and time to progression were significantly longer in HIAC group than in sorafenib group (14.9 *vs.* 7.2 months). The safety profile was good, with fewer overall side effects and fewer serious adverse events but with a higher rate of grade 3 and 4 toxicities. Although conducted on a small number of patients, this study shows promising results than need to be confirmed in further studies. As it has favourable shrinking tumour effects, lenvatinib plus HIAC could be another direction of research.

Still, up until now, there is no registered clinical trial that proves a survival benefit of systemic chemotherapy in the treatment of HCC.

Although the promising results of multi-kinase inhibitors and immune checkpoint inhibitors in the treatment of advanced HCC have taken the frontline, there seems to be a glimpse of hope for the systemic therapy that comes from some observational studies of metronomic capecitabine (MC).

The metronomic regimes which are currently becoming popular in oncology, rely in the chronic administration of chemotherapeutic agents, in a continuous manner, with the aim of optimizing the antiangiogenic properties of the drug with the reduction of the gastrointestinal and bone marrow toxicities (78,79).

Metronomic capecitabine was analysed in few observational studies, mainly in a second line setting, demonstrating in all of them a good efficacy at the cost of a low rate of adverse events in comparison with best supportive care BSC (78-81).

As second line therapy in patients unresponsive or intolerant to sorafenib, a grey area in the treatment of HCC, the treatment with MC showed superior progression free survival and median overall survival rates compared to best supportive care (79,80). Furthermore, in patients with moderate compromised liver function (CP-B) not eligible for sorafenib, there was a 42% reduction in the death risk for patients on MC compared to those receiving just BSC (78).

However, future prospective randomised clinical trials should analyse the efficacy of MC in the treatment of advanced HCC.

Immunotherapy—the revolution against cancer

Liver immunology

The liver has a specific, dual blood supply. 75% of the blood enters the liver through the portal vein bringing many microbial antigens from the gut, also known as microbial associated molecular patterns (MAMPs). One such antigen is lipopolysaccharide (LPS), an endotoxin from the Gram-negative bacteria (82), that interacts with hepatic non-parenchymal cells such as liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (h-SCs), Kupffer cells, dendritic cells (DCs), and lymphocytes capable of inducing immunotolerance. Some of the most important mechanisms of inducing immunotolerance are: decrease of the costimulatory immune receptors B7-1, B7-2 versus up-regulation of programmed cell death protein 1 (PD-1) receptor and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) immuno-checkpoint inhibitors of different immune cells (83,84).

Chronic inflammation of various causes (HBV, HCV, AI, NASH or alcohol) may lead to cirrhosis and liver cancer. The tumoral microenvironment promotes T cells deregulation and an increase of the immune checkpoint inhibitors expression (85,86). Probably this is one of the most studied mechanisms in the last years in cancer biology and stands as a cornerstone of the immunotherapy. Also, represents one of the hallmarks of cancer. In addition, forkhead box P3 (FOXP3)⁺ T-regulatory lymphocytes (Treg), a subset of CD4⁺ T cells found in the tumour microenvironment are specialized in the suppression of the host immune

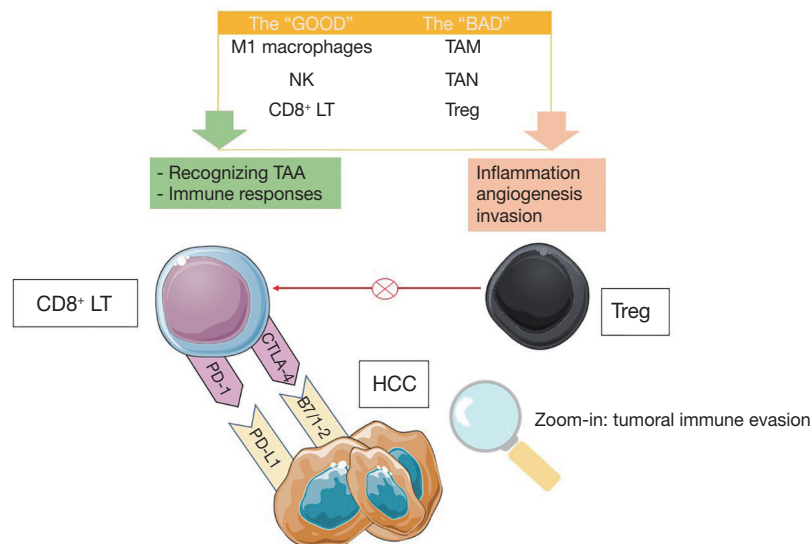


Figure 1 The mechanisms of immunotolerance in HCC. The main actors involved in the processes of silencing the “good” immune cells are the regulatory lymphocytes and myeloid derived cells which are also inducing inflammation, angiogenesis and invasion. Tregs are responsible for inhibiting CD8⁺ cells by activating the immune checkpoints CTLA-4 and PD-1/PD-L1. HCC tumour cells express the ligand of PD-1 to further inhibit the immune cells. M1, macrophages; NK, natural killer; LT, T lymphocyte; TAM, tumor associated macrophages; TAN, tumour associated neutrophils; Treg, regulatory T cells; TAA, tumour associated antigens; CTLA4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1. Parts of the illustration were downloaded from Servier Medical Art (<https://smart.servier.com/>).

system, thus promoting tumour development (87). The main mechanisms involved in tumoral immune evasion and the main actors involved in liver cancer are illustrated in *Figure 1*.

The suppressive function of FOXP3⁺ Tregs may be related to target cells killing, modulation of target cell signalling via cell-cell contact, and immunosuppressive cytokines secretion (IL-10, IL-35, and TGF-β) (88).

In order to combat the tumour-specific immune response oncologists revealed three possible mechanisms that could be also specifically applied to liver cancer:

- ❖ Adoptive immunotherapy—immune cells that destroy cancer cells;
- ❖ Indirect immunological therapies—immune checkpoint blockade, cancer vaccines used to increase immune system activity;
- ❖ Indirect non-immunological strategies—antigen-encoding mRNA strategy in HCC, oncolytic viruses.

Each type of approach will be described, in order to understand HCC’s complex biology and to highlight the most important discoveries in liver cancer.

Adoptive immunotherapy

The principle of adoptive immunotherapy is simple. Scientists take away from patients’ immune cells such as NK or T lymphocytes and after growing them in the laboratory gives them back to the patient in order to fight back cancer cells.

Natural killer cells destroy cancer cells or virus-infected cells, being known as key effector cells in cancer immune-surveillance and early viral immunity. Unfortunately, the cytotoxic effect of NK cells is diminished in patients with advanced HCC (89). Furthermore, it was demonstrated that radiofrequency ablation (RFA) can reactivate the NK’s (90). Following these assumptions, two clinical trials are ongoing, combining autologous NK’s reinfusion with resection and transplant. The results are expected in the near future.

The main objective of T cell engineering is to generate tumour-targeted T cells through genetic transfer of antigen specific receptors. Thus, T cells armed with chimeric (artificial) antigen receptors (CAR-T cells) are able to target and destroy cancer cells. CAR-T cell revolutionised haematology, with promising results in acute myeloid leukaemia, lymphoid leukaemia and lymphomas (91-93).

There are 7 ongoing clinical trials regarding engineered T cells in HCC. Although the experience of CAR-T in solid tumours is scarce, scientists hope to obtain promising results.

Indirect immunological therapies

Indirect immunological strategies in liver cancer are consisted of vaccines and immune check-point inhibitors.

HBV vaccination led to the decrease of HCC incidence, therefore it could be considered a prophylactic vaccine for HCC. However, a therapeutic vaccine for HCC as in prostate cancer is still awaited (94). The presence of dendritic cells (DCs) in the tumoral microenvironment of tumours has been associated with a good prognosis. In addition, it was shown that DC infiltration in HCC lesions has been associated with a better prognosis in resected patients (95). Therefore, many clinical trials bursted trying to find the best DC-immunotherapy approach. One DC vaccine pulsed with autologous tumour lysate reported that 12.9% of advanced HCC patients had partial response and 54.8% had stable disease (96). Other studies revealed tumour recurrence after combining radiotherapy or TACE with DC vaccine (97,98).

Immune check-points, found on many type of immune cells, prevent T cell overactivation against different antigens and thus limiting self tissue damage by are physiologically induced immunosuppression (99). Liver tumour cells use cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death-1 (PD-1) inhibitory pathways to silence the host's immune cells activity in order to evade and proliferate, which are shown on *Figure 1*.

Both CTLA-4 and PD-1 binding share the negative effects on T-cell activity. However, the signalling mechanisms and location of the immune inhibition differ. CTLA-4 inhibits the immune priming phase by silencing the effector T cells and by recruiting more Treg cells to further support the suppression of T-cells (100).

PD-1 works on the effector phase and inhibits the function of T cells in the periphery following extended or high levels of tumoral antigen exposure (101).

All the preclinical information discussed earlier provides a valid rationale for an immunologic approach to the treatment of HCC based on the interaction with immune checkpoints. The most important checkpoint inhibitors in HCC clinical trials are outlined in the *Table 1*.

Clinical studies have only recently been conducted and the results are very promising. The CTLA-4 antibody

(ipilimumab) became the first check-point inhibitor approved for systemic treatment of cancer patients and it was used successfully in non-resectable melanoma (102). Since then it was tested on a variety of cancers, including HCC. Regarding liver cancer, the first CTLA-4 inhibitor chosen was tremelimumab. A phase I clinical trial from Spain tested tremelimumab alone on patients with advanced HCC in a phase I trial. The treatment was well tolerated with 17.6% and 58.8% of partial response (PR) and stable disease, respectively. In addition, it decreased the viral load of HCV infected patients (103). Patients received a suboptimal dose of 15 mg/kg tremelimumab every 90 days to a maximum of 4 doses till tumour progression or toxicities occurred. Despite the suboptimal dosing, 3/17 partial responses were observed and therefore the trial was found to be positive. Stable disease was the best response in 10 patients and the median time to progression was 6.48 months (95% CI, 3.95–9.14 months), not different compared to other second line trials in HCC. Authors also attest the possibility that maybe the final results were influenced due to the high proportion of Child B patients.

In order to enhance tremelimumab's antineoplastic effect, other scientists thought to associate it with percutaneous radiofrequency (RFA) or TACE (104). The hypothesis stands on the fact that after performing RFA or TACE on a HCC tumor, the cell death will determine a strong immunogenic response that will be further amplified by CTLA-4 blockade. Thus, the patients were treated with an optimal dose of tremelimumab at two dose levels (3.5 and 10 mg/kg IV) every 4 weeks, a total of 6 doses, followed by 3-monthly infusions until off-treatment criteria were met. 5 weeks after the first dose of the anti-immune checkpoint was performed the interventional procedure. The better OS of 12.3 months (95% CI, 9.3–15.4 months) in the combination trial could be explained by better liver function, but also by the enhanced immunologic effect of prior ablation.

Based on these findings on tremelimumab (good antitumor activity in advanced HCC and good safety profile in cirrhotic patients), other immune checkpoint inhibitors were tested. Therefore PD-1/PD-L1 pathway provides another mechanistic approach. In addition, PD1-1/PD-L1 checkpoint inhibitors revolutionised lung cancer and gave a new hope for these patients (105).

Nivolumab, a fully human molecular antibody anti-PD-1 has been tested in patients with intermediate or advanced HCC and preserved liver function (CP-A) that were candidates to systemic therapy and had progressed or were

Table 1 Checkpoint inhibitors currently under investigation in hepatocellular carcinoma clinical trials

Phase	NCT number	Agents	Target
Check-point inhibitors as monotherapy			
1B/2	01658878	Nivolumab	PD-1
3	02702414	Pembrolizumab	PD-1
3	02576509	Nivolumab vs. Sorafenib	PD-1
3	02702401	Pembrolizumab vs. BSC	PD-1
Combination of check-point inhibitors			
1B/2	01658878	Nivolumab + Ipilimumab	PD-1 + CTLA-4
1B/2	03071094	Nivolumab + PexaVac	PD-1
1B/2	02519348	Tremelimumab + Durvalumab vs. Durvalumab vs. Tremelimumab	PD-L1 + CTLA-4
Association of check-point inhibitors with other antineoplastic agents/therapies			
2	03439891	Nivolumab + Sorafenib	PD-1 + multikinase
1	03299946	Nivolumab + Cabozantinib	PD-1 + multikinase
1	03418922	Nivolumab + Lenvatinib	PD-1 + multikinase
1B/2	02859324	Nivolumab + CC-122	PD-1 & pleiotropic pathway modifier
1B/2	02423343	Nivolumab + Galunisertib	PD-1 & TGFb
1	03382886	Nivolumab + Bevacizumab	PD-1 + VEGF
1B/2	03033446 02837029	Nivolumab + Y90 radioembolization	PD-1+ radiation
1	01853618	Tremelimumab + TACE	CTLA-4 + chemoembolization
		Tremelimumab + RFA	CTLA-4 + ablation
1A/B	02572687	Durvalumab + Ramucirumab	PD-1 + VEGFR2
1B	02856425	Pembrolizumab + Nintedanib	PD-1 + multikinase
1	03006926	Pembrolizumab + Lenvatinib	PD-1 + multikinase
1B	02988440	PDR001 + Sorafenib	PD-1 + multikinase
1B/2	02795429	PDR001 vs. PDR001 + Capmatinib	PD-1 + c-met

PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; Y90, yttrium 90; TACE, transarterial chemoembolization; RFA, radiofrequency ablation.

intolerant to sorafenib or had refused this drug. The trial, also known as the CheckMate 040 study (106), showed a median response duration was 17 months. Very impressive, response was ongoing beyond 24 months in 1 patient who stopped treatment with a complete response. Following the extraordinary results obtained against melanoma when combining immune checkpoint inhibitors, the next objective of the 1b phase of the Checkmate 040 trial is the dual blockade of PD-L1 and the CTLA-4 where different doses of ipilimumab and nivolumab are tested. The results

will be available this year in November. Furthermore, by following the promising results of this trial, the hepatology community hopes that the administration immune checkpoints will be approved in Europe.

Pembrolizumab is another PD-1 antibody currently under investigation in HCC with promising results. It is also investigated in earlier phase combination therapy with lenvatinib or regorafenib and other immunotherapeutics as well as in combination with locoregional therapies. The results of the trials will be revealed in the recent future.

High levels of PD-1 and PD-L1 in tumor tissue is associated with negative prognostic in patients undergoing liver resection with consequent increased rate of recurrence after surgery (107). Regarding this concern, many studies are focusing on combining of immune check point inhibitors with other therapies: ablation, TACE, anti-angiogenic therapies. From the immunological point view, vascular endothelial growth factor (VEGF) inhibits dendritic cell maturation and T-cell activation. VEGF promotes the expression of PD-1 and CTLA-4 by inducing CD8⁺ T cells' exhaustion (108). Paradoxically, when given high doses of anti-VEGF therapies the induced hypoxia produces an enhancement of the check point molecules. To this respect, Huang *et al.* stated that a careful titration of anti-VEGF therapy with VEGF blockage but without excessive pruning of tumour vasculature may enhance immunotherapy efficacy. The clinical trials outgoing were shown in the table earlier (109).

Immune check-point inhibitors represent a hope for primary liver cancer patients.

Indirect non-immunological strategies

Mouse models of HCC revealed another antitumor strategy: vaccines with mRNA. DCs which are cultivated and electroporated with mRNA are restituted into the tumor, with important results (110). Oncolytic viruses can induce tumor cells lysis during viral replication and also being able to reveal tumor antigens (111). A randomized phase II trial tested the feasibility of two doses of JX-594 (Pexa-Vec), an oncolytic and immunotherapeutic vaccine virus in 30 HCC patients, revealing a higher OS in the high-dose arm versus the low-dose arm (14.1 and 6.7 months, respectively) (112). Currently, a phase III study investigates the administration of this virus followed by sorafenib versus sorafenib alone. In addition, another trial is associating the combination of Pexa-Vec and nivolumab.

Conclusions

Sorafenib and lenvatinib are the first line approved systemic therapies for advanced primary liver cancer, while regorafenib is the second line stated in the guidelines. Emerging therapies are on their way with important and promising results. Immune strategies such as adoptive immunotherapy and immune check-point inhibitors are the most studied in clinical trials. A possibility of obtaining efficient results stands in the combination of

the immunotherapies with other antitumoral agents like multikinase inhibitors. The objectives of clinical trials will not be only to obtain an increased survival but also to determine the exact doses in order to have less toxicities. Furthermore, the future stands in to the hands of precision and translational medicine, to engineer one individual's immune cells and tumoral antigens in order to destroy targeted cancer cells.

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