Review Article

Chemotherapy for Hepatocellular Carcinoma: Current Evidence and Future Perspectives

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Abstract

Hepatocarcinogenesis is a multistep process, heralded by abnormalities in cell differentiation and proliferation and sustained by an aberrant neoangiogenesis. Understanding the underlying molecular pathogenesis leading to hepatocellular carcinoma is a prerequisite to develop new drugs that will hamper or block the steps of these pathways. As hepatocellular carcinoma has higher arterial vascularization than normal liver, this could be a good target for novel molecular therapies. Introduction of the antiangiogenic drug sorafenib into clinical practice since 2008 has led to new perspectives in the management of this tumor. The importance of this drug lies not only in the modest gain of patients' survival, but in having opened a roadmap towards the development of new molecules and targets. Unfortunately, after the introduction of sorafenib, during the last years, a wide number of clinical trials on antiangiogenic therapies failed in achieving significant results. However, many of these trials are still ongoing and promise to improve overall survival and progression-free survival. A recent clinical trial has proven regorafenib effective in patients showing tumor progression under sorafenib, thus opening new interesting therapeutic perspectives. Many other expectations have been borne from the discovery of the immune checkpoint blockade, already known in other solid malignancies. Furthermore, a potential role in hepatocellular carcinoma therapy may derive from the use of branched-chain amino acids and of nutritional support. This review analyses the biomolecular pathways of hepatocellular carcinoma and the ongoing studies, the actual evidence and the future perspectives concerning drug therapy in this open field.

Introduction

Epidemiology

Hepatocellular carcinoma (HCC) is the sixth most frequent neoplasia (749,000 new cases/year), with a constantly increasing worldwide incidence and about 745,000 deaths/year.[1] It represents the third cause of tumor-related death (692,000 cases/year), and its incidence is higher in males (M/F ratio of 2.4), mainly affecting people in the sixth to seventh decade of life.[2,3] The incidence of HCC is higher in Chinese and black-African populations, representing 85% of all HCC diagnoses worldwide; moreover, younger patients are usually involved, especially for the hepatitis B virus (HBV)-related cirrhosis. Hepatitis C virus (HCV)-related liver disease, on the other hand, is the main cause of chronic liver disease leading to HCC in Europe, the United States and Japan.[2] In general, in developed countries (Europe, the United States and Japan) the incidence is low, with the exception of Southern Europe.[4]

Risk factors

In 90% of cases, HCC is associated with a well-identified risk factor, the most important of which are HBV and HCV chronic hepatitis, alcohol intake and exposure to toxic agents (e.g. aflatoxin B). HBV infection is predominant in Eastern Asia and Africa (60%), while HCV is predominant in Europe, Japan and Northern America (50–60%). According to global incidence,

54% cases of HCC are due to HBV infection and 31% to HCV.[5,6]

HBV is a risk factor for HCC in both cirrhotic livers (annual incidence of 2–6%) and non-cirrhotic ones (annual incidence of 0.5%).[7] In the latter case, HBV directly acts as a carcinogenic factor through its integration in the hepatocyte genome, determining genomic instability.[8,9] Exposure to aflatoxin enhances the possibility of generating HCC, especially in the case of concurrent HBV infection;[10] this is frequent in developing nations, where food conservation is altered by poor lifestyle and climatic conditions.[11]

In the majority of cases, HCC emerges in cirrhotic livers, with an estimated risk of 3–4% patients per year, regardless of etiology;[12] chronic viral infection (HBV, HCV) and alcoholic liver disease are the most well known risk factors. Autoimmune hepatitis, primary biliary cholangitis, inherited metabolic diseases (hemochromatosis, alpha-1-antitrypsin deficiency, and Wilson's disease) are other less frequent risk factors.[13–15] Obesity, non-alcoholic fatty liver disease and diabetes mellitus have been recently associated with advanced liver fibrosis.[16] Current evidence indicates that these factors, and in particular insulin-resistance, are also clearly associated with HCC.[17–19] On the other hand, observational studies have shown that the use of metformin and statins could prevent the risk of HCC,[20] but randomized clinical trials are unavailable on this topic.

Standard-of-care

The therapeutic strategy for HCC is defined by the Barcelona Clinic Liver Cancer (BCLC) classification, which considers the three important variables of tumor staging (size, number of nodules, vascular invasion and/or extrahepatic disease), liver function evaluation (using the Child-Pugh score) and performance status (PS), according to the Eastern Cooperative Oncology Group (ECOG).[21]

In the early stages and according to tumor location and number, liver function and portal hypertension assessment, surgical resection and percutaneous ablation (including radio-frequency ablation (RFA), microwave ablation (MWA) and percutaneous alcohol injection (PEI)) are the main therapeutic strategies. Liver transplantation may be taken into account in patients younger than 70 years old without significant comorbidities and with HCC staged within Milan or slightly enlarged criteria (up to seven, according to the University of California, San Francisco), independent from the degree of liver function failure or portal hypertension.[22–24]

Trans-arterial chemoembolization (TACE) should be offered to patients with intermediate BCLC tumor stage (preserved function, PS = 0, large or multifocal tumors, no vascular invasion or extra-hepatic spread).[21,25,26] Advanced HCC in compensated patients are treated with sorafenib. To date, it represents the only systemic therapy with a documented improvement in overall survival (OS).[27] Even if there are not yet results from prospective randomized clinical trials, Yttrium 90 trans-arterial radio embolization (TARE) is an effective tool, often used in large specialized centers for the treatment of intermediate-advanced HCC with unfavorable predictors of response or non-responders to TACE, such as patients with macroscopic vascular invasion.[28,29] In some circumstances, it has been used as a bridge to liver transplantation.[30]

Curative strategies (resection, PEI, RFA, liver transplantation) are limited to the early stage, cases of which account for less than 40% of the neo-diagnoses of HCC. Palliative treatments (TACE, sorafenib and TARE) are effective but they can only prolong survival.[21]

Advanced HCC: the field of molecular target therapies

Molecule-targeted therapies represent a new promising field in advanced HCC treatment. They are based on the identification of different carcinogenetic mechanisms.

Classical molecular targets in HCC

Molecular mechanisms involved in the pathogenesis of HCC are represented by altered intracellular signal transmission as well as angiogenetic and growth factors. Accordingly, specific targets were established for the treatment of HCC. Currently established treatments are represented by tyrosine kinase inhibitors and monoclonal antibodies.

The most evaluated molecular intracellular pathways, to date, are the following (Fig. 1):

- **Ras/Raf/MEK/ERK (MAPK) pathway**, characterized by the phosphorylation of four major kinases: Ras, Raf, mitogen-activated protein extracellular kinase (MEK), and extracellular signal-regulated kinase (ERK). This pathway is the most frequently hyper-activated in HCC (about 50% of early stage cases and the majority of advanced ones).[31]
- **Wnt/catenin pathway**, characterized by the Wnt protein binding to its ligand, which results in the accumulation and activation of β-catenin in cells that in turn is rapidly transferred to the nucleus, where it regulates transcriptional mechanisms. Around 50% of all patients with HCCs have activation of the WNT signaling pathway.[32,33]
- **Phosphoinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway**, activated in 30–50% of HCCs.[34] It is localized downstream of several receptor tyrosine kinases (e.g. ERK), controlling cellular replication, apoptosis and cell motility, and involved in invasiveness and metastasis.[35,36] PI3K is partially controlled by the tumor suppressor phosphatase and tensin homolog (PTEN) protein, that is frequently mutated in HCC.[37] The overstimulation of this pathway leads to the inactivation of some apoptotic mechanisms, determining the hyper-activation of mTOR, an enzyme serving a pivotal role in cellular proliferation and angiogenesis.[38]

Several growth factors (and their receptors) involved in hepatic carcinogenesis have been identified. HCC is a highly vascularized tumor and both vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang-2) are key elements for tumor emergence and proliferation; in particular, VEGF promotes the growth, migration and morphogenesis of endothelial cells, and increases vascular permeability.[39,40] A vascular targeting approach is crucial in HCC treatment; several studies have demonstrated that high-density vascularization is a predictor of poor response to treatment and shorter disease-free period after hepatic resection.[41,42] Platelet derived growth factor (PDGF) determines the involvement of pericytes and smooth muscle cells around the new vascular shoots.[43] These factors are strictly related to metastatic potential of tumor cells; for this reason, their inhibitors are a promising therapeutic agent.[44]

Epidermal growth factor (EGF) is frequently overexpressed in HCC;[45,46] other involved factors are fibroblast growth factor (FGF), whose signal are involved in Ras/Raf/MAPK
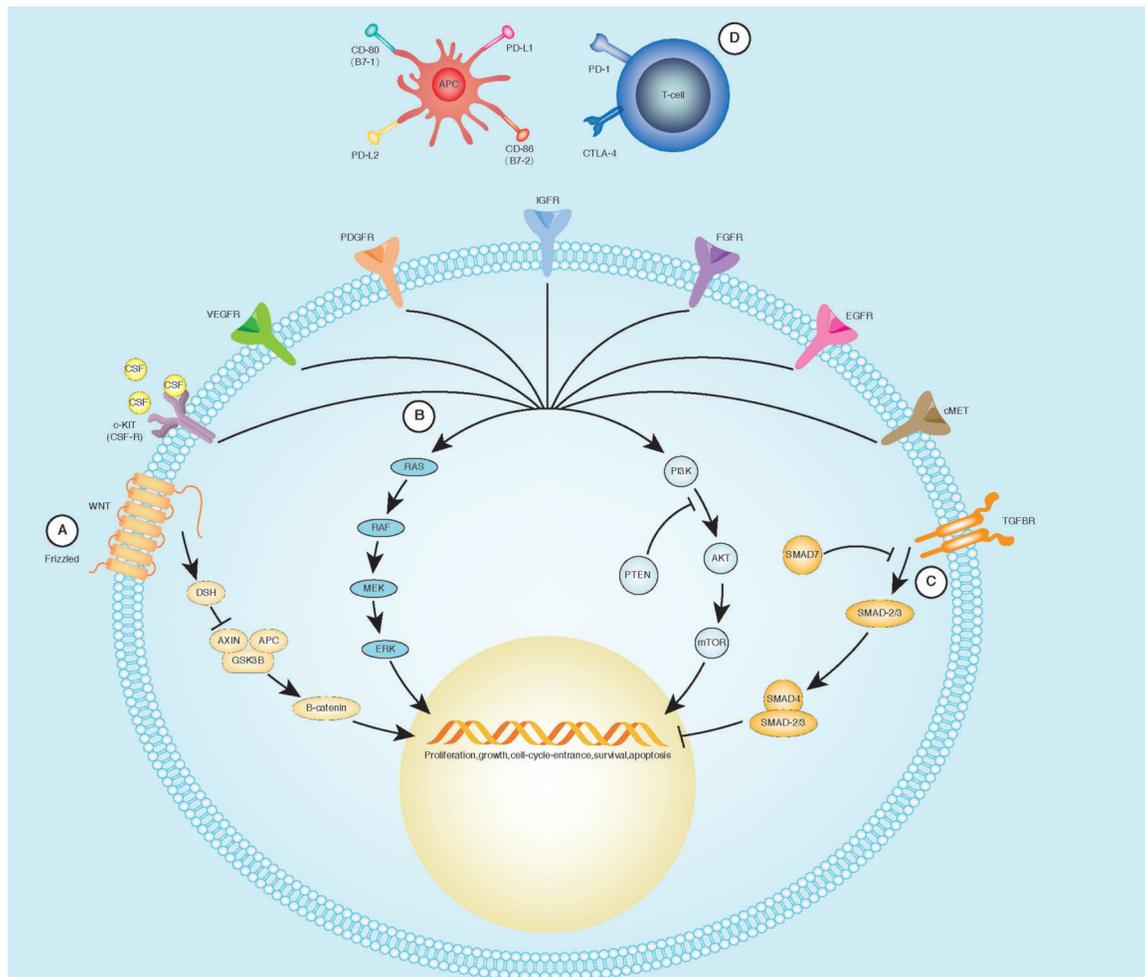
Fig. 1. Main molecular pathways in HCC pathogenesis. (**A**) The activation of Frizzled (WNT receptor) determines the recruitment of disheveled (DSH), preventing the destruction of β-catenin through the dissolution of a molecular complex composed by Axin, adenomatosis polyposis coli (*APC*), and glycogen synthase kinase 3β (GDK3B). (**B**) Growth factors bind their specific receptors, leading to their dimerization and activation of the tyrosine-kinase. From this point, it is possible to recruit two different groups of molecules: MAPK and PI3K pathways, culminating with modifications in cell-cycle regulation, protein synthesis, and gene transcription. (**C**) Stimulation of TGF-βR recruits the SMAD complex, leading to a negative regulation of the cellular cycle. (**D**) CTLA-4 and its ligands: CD-80/CD-86 transmit an inhibitory signal in the antigen presenting cells (APCs), while CD-28 represents an activating signal. Programmed cell death protein-1 (PD-1), through its binding to PD-L1 (B7-1) and PD-L2 (B7-2), down-regulates the immune system and promotes self-tolerance. Adapted from Harding *et al*.[121]

pathway and tyrosine protein kinase Met (c-MET), the tyrosine kinase receptor for hepatocyte growth factor (HGF).[47] The HGF-cMET axis has been implicated in tumor cell migration, invasion, proliferation, and angiogenesis. High c-Met and HGF expression correlates with early recurrence of HCC after hepatectomy and shorter survival in HCC.[48]

All these agents can become a target for HCC treatment, alone or combined with each other or to other strategies (loco-regional treatment or resection), as previous and recent trials have demonstrated.

Role of sorafenib

Traditional chemotherapy agents and hormonal therapies have been tested for HCC, but they did not improve the prognosis of these patients. An important frontier was reached with the introduction of sorafenib, a multikinase inhibitor, in the treatment of advanced HCC. This drug opened the era of molecular targeted therapy for HCC and

represents the current first-line pharmacological treatment for advanced HCC.

Sorafenib is a tyrosine kinase inhibitor, targeting Raf serine/threonine kinases, vascular endothelial growth factor receptors 1–3 (VEGFR1–3), platelet-derived growth factor receptor (PDGFR)-b, tyrosine-protein kinase Kit (c-Kit), fms-like tyrosine inase-3 (FLT-3) and p38 tyrosine kinases.[49] Its use in advanced HCC was approved in 2008, on the basis of the positive results of two multicenter, phase 3 studies (the so-called SHARP and Asia-Pacific trials).[27,50]

SHARP was a phase 3 randomized placebo-controlled study involving 602 patients with advanced HCC (299 receiving sorafenib and 303 receiving placebo). An improvement in OS was demonstrated in the sorafenib arm: 10.7 versus 7.9 months (hazard ratio [HR] in the sorafenib group, 0.69; 95% confidence interval [CI]: 0.55 to 0.87; $p < 0.001$).[27] A similar result was obtained by the Asia-Pacific trial (6.5 versus 4.2 months for sorafenib and placebo respectively, HR 0.68 [95% CI: 0.50−0.93]; $p = 0.014$). Sorafenib-related

major adverse effects are diarrhea, hand-foot skin reaction (HFSR), fatigue and weight loss. In the SHARP trial, the overall incidence of treatment-related adverse events (AEs) was 80%, of which most were described as grade 1 or 2 in severity. Some grade 3 AEs, such as diarrhea (8% vs 2% in the placebo group, $p < 0.001$) and HSFR (8% vs <1%, $p < 0.001$), occurred more frequently in the sorafenib group. The trial was prematurely stopped at second interim analysis for a significant survival benefit in the sorafenib arm.

For better understanding of efficacy and safety in real practice, a prospective multicenter observational study was conducted in six Italian referral hospitals.[51] That study enrolled 296 patients affected by HCC in BCLC C stage (222, 75%) or BCLC B stage (74, 25%), who were unfit for or failed to respond to loco-regional treatments. Median OS was 10.5 months in the overall cohort, with results showing 8.4 months in the BCLC C patients versus 20.6 months in the BCLC B patients ($p < 0.0001$). The overall incidence of treatment-related AEs was higher than in the SHARP study (91%), with a greater percentage of grade 3 and 4 AEs, such as fatigue (25%) and arterial hypertension (7%). Discontinuation, dose reduction and interruption were greater than in the SHARP study. Surprisingly, a dose reduction for AEs was associated with a better OS: 21.6 months in the patients receiving a half-dose of sorafenib (95% CI: 13.6–29.6) compared to 9.6 months (95% CI: 6.9–12.3) for the remaining patients, who remained at full dose.

The multivariate analysis confirmed that "full dose" treatment was an independent predictor of mortality (HR: 1.8, 95% CI: 1.4–2.4). Dose reduction for AEs is common in real-life practice and may improve the treatment tolerability, following adjusting of the drug dosage to the patient;[52] however, in a propensity score matching study, no differences in terms of OS and progression-free survival (PFS) have been reported between initial "half-dose" and "standard-dose" treatments.[53] Indeed, a better outcome may be more related to an increased incidence of AEs leading to dose reduction, than to the dose itself. This result is better understood in the light of other observational studies showing that early AEs (such as diarrhea or HFSR) are positive predictive factors for clinical response to sorafenib therapy.[54,55]

Given the hypothesis that loco-regional treatments may increase the production of angiogenic factors, particularly VEGF and thus enhancing angiogenesis and metastasis,[56] sorafenib was then evaluated as adjuvant therapy in combination with curative (resection, ablation) or loco-regional palliative treatments (such as TACE). The STORM trial phase 3 study explored the efficacy of sorafenib as adjuvant treatment for preventing HCC recurrence after surgical resection or ablation, but it failed to demonstrate a better efficacy in terms of recurrence-free survival.[57] On the other hand, the phase 3 Sorafenib or Placebo in Combination with Transarterial Chemoembolization (known as the SPACE trial), failed to demonstrate a longer time to progression (TTP) in the sorafenib arm (compared to placebo) after doxorubicin-eluting beads TACE.[58]

To date, new treatments are clearly needed as alternatives to sorafenib or for administration after sorafenib failure.

Other first-line promises

Despite a vast number of trials studying new possible therapies for advanced HCC, almost all phase 3 trials failed to show better outcomes than sorafenib in the first-line setting (Table 1);[59–62] consequently, no other systemic treatments are approved for advanced HCC. Only two drugs are still being tested in phase 3 trials compared in first-line to sorafenib in advanced HCC. The first one is lenvatinib, an antiangiogenic small molecule, and the second one is nivolumab, an immune check-point inhibitor (see below).

Lenvatinib

Lenvatinib is an oral multikinase inhibitor targeting VEGF1–3, fibroblast growth factor receptors 1–4 (FGFR1–4), RET, c-kit, stem cell growth factor receptor (SCGFR) and PDGFRα. It has antiangiogenic and direct antitumor activity.[63] According to results of two large randomized trails (phase 3 and 2)[64,65] it has been recently approved by the Food and Drug Administration (FDA) for the treatment of adult patients with radioactive iodine refractory thyroid carcinoma and for adult patients with advanced renal cell carcinoma who have been previously treated with VEGF inhibitor. In the latter case, it is combined with everolimus. The most common side effects are those of other antiangiogenic drugs: hypertension, fatigue, proteinuria, nausea, decreased weight, abdominal pain, and HFSR.

For advanced HCC, a phase 2, single-arm, open-label multicenter study was conducted.[66] Forty-six patients were enrolled at sites across Japan and Korea; the primary endpoint, median TTP, was 7.4 months (95% CI: 5.5–9.4). Seventeen patients (37%) reported partial response (PR) and 19 patients (41%) had stable disease (SD) according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[67] The median OS was 18.7 months (95% CI: 12.7–25.1). Moving forward based on these positive results, a multicenter, randomized, open-label, sorafenib controlled, non-inferiority phase 3 trial was planned and performed in patients affected with unresectable HCC.[68] That trial is ongoing but no longer recruiting, and therefore it is possible that results will be available in the near future.

Second-line therapies

Among second-line attempts to overcome sorafenib failure, many drugs have themselves failed (Table 2). In particular, brivanib, a selective dual inhibitor of VEGFR and FGFR tyrosine kinases, did not show in the phase 3 BRISK-PS study[69] a significant gain in survival compared to placebo (9.4 vs 8.2 months, HR: 0.89; 95.8% CI: 0.69 to 1.15; $p = 0.3307$), despite a longer TTP (4.2 vs 2.7 months; HR: 0.56; 95% CI: 0.42 to 0.76; $p < 0.001$) and a greater overall response rate (ORR) (10% vs 2%, Odds ratio: 5.72) according to mRECIST criteria.[70] In the same way, everolimus, an mTOR inhibitor, and erlotinib, an EGFR inhibitor, did not demonstrate the ability to prolong OS compared to placebo in patients progressing during sorafenib treatment or who were intolerant to the drug.[62,71]

A peculiar interest is still present for ramucirumab. It is an intravenous recombinant immunoglobulin (Ig)G1 monoclonal antibody directed against VEGFR-2.[72] Efficacy and safety in the HCC setting have been largely tested in a phase 3, randomized, placebo-controlled, double-blind, multicenter trial (known as REACH).[73] Eligible patients were adult patients with advanced HCC who had previously stopped sorafenib due to progression or intolerance. Five-hundred-and-sixty-five patients were enrolled, of whom 283 were assigned to the ramucirumab group and 282 were assigned to the

Table 1. First-line phase 3 trials on advanced HCC

Year of publication	Trial name	Study design	Number of patients	Drug	Main target of experimental drug	Primary endpoints	Secondary endpoints	Status	Outcome
2008	Sorafenib in advanced hepatocellular carcinoma (SHARP)	Phase 3, randomized, placebo-controlled, double-blind, multicenter	602 (299 vs 303)	Sorafenib vs placebo	Raf-1, B-Raf, VEGFR1, 2, 3. PDGFRβ, c-KIT	OS, TTSP	TTP, DCR and safety	Completed	Reached
2009	Effcacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase 3 randomized, double-blind, placebo-controlled trial (Asia-Pacific)	Phase 3, randomized, placebo-controlled, double-blind, multicenter	226 (150 vs 76)	Sorafenib vs placebo	Raf-1, B-Raf, VEGFR1, 2, 3. PDGFRβ, c-KIT	OS	TTP, TTSP, DCR and safety	Completed	Reached
2013	Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase 3 trial (SUN 1170)	Phase 3, randomized, sorafenib-controlled, open-label, multicenter	1,074 (530 vs 544)	Sunitinib vs sorafenib	VEGFR, PDGFR, KIT, RET, Flt-3	OS	PFS, TTP and safety	Completed	Failed
2013	Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase 3 BRISK-FL study (BRISK-FL)	Phase 3, randomized, sorafenib-controlled, double-blind, multicenter	1,155 (578 vs 577)	Brivanib vs sorafenib	FGFR, VEGFR	OS	TTP, ORR, DCR and safety	Completed	Failed
2015	A phase 3, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma (SEARCH)	Phase 3, randomized, sorafenib + placebo-controlled, double-blind, multicenter	720 (362 vs 358)	Sorafenib + erlotinib vs sorafenib + placebo	EGFR	OS	TTP, DCR, ORR and safety	Completed	Failed

(continued)

Table 1. *(continued)*

Year of publication	Trial name	Study design	Number of patients	Drug	Main target of experimental drug	Primary endpoints	Secondary endpoints	Status	Outcome
2015	Linifanib versus sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase 3 trial	Phase 3, randomized, sorafenib-controlled, open-label, multicenter	1,035 (514 vs 521)	Linifanib vs sorafenib	VEGFR, PDGFR	OS	TTP, PFS, ORR and safety	Completed	Failed
Not yet published	A multicenter, open-label, phase 3 trial to compare the efficacy and safety of lenvatinib (E7080) versus sorafenib in first-line treatment of subjects with unresectable hepatocellular carcinoma (NCT01761266)	Phase 3, randomized, sorafenib-controlled, open-label, multicenter	954 (estimated)	Lenvatinib vs sorafenib	VEGFR, PDGFR, FGFR, RET, SCFR	OS	PFS, TTP, ORR, and safety	Ongoing, not recruiting	Ongoing
Not yet published	A study of nivolumab compared to sorafenib as a primary treatment in patients with advanced hepatocellular carcinoma (CheckMate-459; NCT02576509)	Phase 3, randomized, sorafenib-controlled, open-label, multicenter	726 (estimated)	Nivolumab vs sorafenib	PD-1	OS, ORR	PFS, PD-L1 expression	Ongoing, recruiting	Ongoing

Abbreviations: VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet-derived growth factor receptor; EGFR, epidermal growth factor receptor; SCFR, stem cell factor receptor; PD-1, programmed death-1; OS, overall survival; TTSP, time to symptomatic progression; TTP, time to (radiologic) progression; DCR, disease control rate; PFS, progression-free survival; ORR, objective response rate; PDL-1, programmed death ligand-1. Flt-3, Fms-like tyrosine kinase-3; FGFR, fibroblast growth

Table 2. Second-line (after sorafenib) phase 3 trials on advanced HCC

Year of publication	Trial name	Study design	Number of patients	Reason for sorafenib discontinuation	Drug	Main target of experimental drug	Main inclusion criterion	Primary endpoints	Secondary endpoints	Status	Outcome
2013	Brivanib in patients with advanced hepatocellular carcinoma who were intolerant to sorafenib or for whom sorafenib failed: results from the randomized phase 3 BRISK-PS Study)	Phase 3, randomized, placebo-controlled, double-blind, multicenter	395 (263 vs 132)	Progression or intolerance	Brivanib vs placebo	FGFR, VEGFR		OS	TTP, ORR, DCR and safety	Completed	Failed
2014	Effect of everolimus on survival in advanced hepatocellular carcinoma after failure of sorafenib, the EVOLVE-1 randomized clinical trial	Phase 3, randomized, placebo-controlled, double-blind, multicenter	546 (362 vs 184)	Progression or intolerance	Everolimus vs placebo	mTOR		OS	TTP, QoL and safety	Completed	Failed
2015	Ramucirumab versus placebo as second-line treatment in patients with advanced hepatocellular carcinoma following first-line therapy with sorafenib (REACH): a randomized, double-blind, multicenter, phase 3 trial	Phase 3, randomized, placebo-controlled, double-blind, multicenter	565 (283 vs 282)	Progression or intolerance	Ramucirumab vs placebo	VEGFR 2		OS	PFS, TTP, ORR, DCR and safety	Completed	Failed

(continued)

Table 2. *(continued)*

Year of publication	Trial name	Study design	Number of patients	Reason for sorafenib discontinuation	Drug	Main target of experimental drug	Main inclusion criterion	Primary endpoints	Secondary endpoints	Status	Outcome
2016	Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE); a randomized, double-blind, placebo-controlled, phase 3 trial	Phase 3, randomized, placebo-controlled, double-blind, multicenter	573 (379 vs 194)	Progression	Regorafenib vs placebo	VEGFR, PDGFR, BRAF, FGFR, KIT, RET		OS	TTP, ORR, QoL and safety	Completed	Reached
Not yet published	A phase 3, randomized, double-blind study of tivantinib (ARQ 197) in subjects with MET diagnostic-high inoperable hepatocellular carcinoma treated with one prior systemic therapy (METIV-HCC; NCT01755767)	Phase 3, randomized, placebo-controlled, double-blind, multicenter	368	Progression or intolerance	Tivantinib (ARQ 197) vs placebo	c-MET	High MET on IHC	OS	PFS and safety	Ongoing, not recruiting	
Not yet published	Randomized, double-blind, placebo-controlled, phase 3 study of ramucirumab and best supportive care (BSC) versus placebo and BSC as second-line treatment in patients with hepatocellular carcinoma and elevated baseline alpha-fetoprotein (AFP) following first-line therapy with sorafenib (REACH-2; NCT02435433)	Phase 3, randomized, placebo-controlled, double-blind, multicenter	399 (estimated)	Progression or intolerance	Ramucirumab vs placebo	VEGFR 2	α-FP > 400 ng/mL	OS	PFS, TTP, CR, PR, ORR, QoL and safety	Ongoing, recruiting	

(continued)

Table 2. *(continued)*

Year of publication	Trial name	Study design	Number of patients	Reason for sorafenib discontinuation	Drug	Main target of experimental drug	Main inclusion criterion	Primary endpoints	Secondary endpoints	Status	Outcome
Not yet published	Phase 3 randomized, double-blind, controlled study of cabozantinib (XL184) versus placebo in subjects with hepatocellular carcinoma who have received prior sorafenib (CELESTIAL; NCT01908426)	Phase 3, randomized, placebo-controlled, double-blind, multicenter	760 (estimated)	Progression or intolerance	Cabozantinib vs placebo	c-MET, VEGFR, RET		OS	PFS	Ongoing, recruiting	

Abbreviations: FGFR, fibroblast growth factor receptor; VEGFR, vascular endothelial growth factor receptor; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; OS, overall survival; TTP, time to (radiologic) progression; ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; IHC, immunohistochemistry.

placebo group. The median OS gain in the ramucirumab group did not reach statistical significance versus the placebo arm (9.2 vs 7.6 months, HR: 0.87, 95% CI: 0.72–1.05; $p = 0.14$). The safety profile was manageable; the most frequent treatment-emergent serious AE (grade 3 or more) was malignant neoplasm progression (6% in the ramucirumab group vs 4% in the placebo group). In a subgroup analysis of patients with Child A functional status and baseline α-fetoprotein (αFP) levels of 400 ng/mL or more, patients who received ramucirumab did achieve significantly longer OS compared with those who received placebo (for Child A5 patients, HR was 0.61, 95% CI: 0.43–0.87; $p = 0.01$).[74] This result provided the clinical basis for planning another phase 3 trial (titled as REACH-2), recruiting patients who progressed or are intolerant to sorafenib with a baseline αFP of 400 ng/mL or more. The trial is now ongoing and recruiting patients.[75] The estimated study completion date is April 2018.

Recently, positive results from a large phase 3 study on regorafenib have been published.[76] Regorafenib is an oral multikinase inhibitor, pharmacologically similar but more potent than sorafenib, that blocks kinases involved in angiogenesis (VEGFR1–3 and TIE2), oncogenesis (c-kit, Ret and wild-type and V600-mutated BRAF) and the tumor microenvironment (PDGFR and FGFR).[77] It is actually approved as monotherapy for the treatment of refractory metastatic colorectal cancer and gastrointestinal stromal tumor.[78] In the RESORCE study,[76] a randomized, double-blind, placebo-controlled, phase 3 trial, 567 patients affected by HCC were randomized to receive regorafenib ($n = 374$) or placebo ($n = 193$) after sorafenib failure for progression (patients stopping sorafenib for AEs were not included). Regorafenib was demonstrated to be effective in improving OS as second-line therapy (10.6 vs 7.8 months in the placebo arm; HR 0.63, 95% CI: 0.50–0.79; $p < 0.0001$). Regorafenib also reached the secondary endpoints of PFS (HR 0.46, 95% CI: 0.37–0.56; $p < 0.0001$) and TTP (HR: 0.44, 95% CI: 0.36–0.55; $p < 0.0001$). The safety profile appears similar to that of the other antiangiogenic drugs, with the most common grade 3 or 4 AEs being hypertension (15% in the regorafenib group vs 5% in the placebo group), HFSR (13% vs 1%), fatigue (9% vs 5%) and diarrhea (3% vs 0%). Regorafenib is hence a new tool in the systemic therapy for HCC after failure of sorafenib therapy. Nevertheless, it has not been tested in patients stopping sorafenib for AEs, in whom it could be scarcely tolerated.

Another new promising tool in second-line chemotherapy after sorafenib is tivantinib. Tivantinib is an oral selective inhibitor of c-MET.[79] Positive results have been obtained from a randomized phase 2 trial, comparing tivantinib versus placebo in second-line treatment (after sorafenib failure or intolerance) in patients affected by HCC.[80] The trial enrolled 107 patients, including 71 patients who received tivantinib (38 at 360 mg twice-daily and 33 at 240 mg twice-daily) and 36 patients who received placebo. A slightly significant improvement in TTP was obtained in the tivantinib arm for the overall population; however, better outcomes were reached in terms of OS (7.2 vs 3.8 months of the placebo arm; HR: 0.38, 95% CI: 0.18–0.81; $p = 0.01$) and TTP (2.7 vs 1.4 months respectively; HR: 0.43, 95% CI: 0.19–0.97; $p = 0.03$) in a subset of patients whose tumor tissues had an overexpression of MET at immunohistochemistry (MET-high patients). The most common grade 3 or worse AEs in the tivantinib group were neutropenia (14% vs none in the placebo group) and anemia (11% vs 0% in the placebo

group). Four deaths due to tivantinib-related neutropenia were reported. Grade 3 or more neutropenia was more common in the 360 mg twice-daily dose group than in 240 mg twice-daily group (21% vs 6% respectively). Given the survival benefit in the MET-high population, two large randomized, double-blind, placebo-controlled phase 3 studies have been designed to enroll MET-high inoperable HCC patients in Europe, America, Australia (known as the METIV-HCC)[81] and Asia (known as the JET-HCC).[82] The first trial is ongoing and not recruiting participants any longer, but the latter is still recruiting. The daily dosage in the tivantinib arm is 120 mg twice-daily (total daily dose of 240 mg). The results will soon be available.

Another MET inhibitor is cabozantinib. Its action comprises the inhibition of numerous tyrosine kinase receptors as well, such as VEGFR2, KIT, RET, FLT3 and Tie-2.[83] Cabozantinib was tested in the HCC setting, after a first antiangiogenic therapy failure, in a phase 2 study.[84] The treatment showed effectiveness in reaching a PFS of 4.2 months with an overall disease control rate (DCR) at week 12 of 68%. To date, a phase 3, placebo-controlled trial is ongoing in patients with HCC progressing during sorafenib therapy or showing intolerance to the drug.[85] The study is estimated to enroll 760 patients and it could offer results in the next months.

Immune checkpoint inhibitors in HCC: a look into the future

Moving forward, a great interest has spread in anticancer therapy on the so-called "immune check-point inhibitors". The liver is a "tolerogenic" organ, expecting to receive a large amount of antigens absorbed from the gut. This tolerance is mediated by immunosuppressive cell populations, such as T regulatory cells (Tregs), myeloid-derived suppressor cells and antigen presenting cells (APCs). Among these, there are Kuppfer cells, macrophages and modulatory dendritic cells.[86] Notably, the high number of these immunomodulatory cells in HCC tissue is correlated with disease progression and worse prognosis in HCC patients.[87] Some inhibitory molecules, called "immune checkpoints", have been recognized in the physiologic maintenance of tolerance. The most cited are "programmed cell Death protein 1" (PD-1) and "cytotoxic T lymphocyte antigen-4" (CTLA-4).

PD-1 is a cell surface receptor of the Ig superfamily, expressed on T cells, B cells, and natural killer cells. PD-1 binds two ligands, PD-L1 and PD-L2, expressed on APCs. The binding of PD-L1 to PD-1 transmits an inhibitory signal which reduces the proliferation of CD8+ T cells[88] and cytokine release.[89] PD-1 also mediates immunotolerance through the differentiation and proliferation of Tregs.[90] Clinical studies have shown that chronic viral infections may up-regulate the PD-1/PD-L1 pathway, leading to CD8+ T cell exhaustion and anergy.[91] This suggests a role of sustained inflammation in the genesis of cancer, including HCC. In fact, a CD8+ T cell response exhaustion is a common finding in HCC and in both chronic HBV and HCV infection.[92]

CTLA-4 is a trans-membrane receptor expressed on CD4+ and CD8+ activated cells. After binding with its ligands, CD80 (also called B7–1) or CD86 (B7–2), both expressed on APCs, it transmits into the lymphocyte an inhibitory signal, contributing to the homeostatic regulation of the immune response.[93] In the HCC setting, the aberrant expression of these pathways is responsible for the tumor's evasive mechanism from the immune system.[94] For this reason, these two immune check-point molecules are now available for clinical evaluation.

PD1- PD-L1 Blockade

Nivolumab

Nivolumab is a genetically engineered, fully human monoclonal antibody (IgG4) directed against the negative immunoregulatory human cell surface receptor PD-1. Nivolumab binds to and blocks the activation of PD-1, inhibiting its link to PD-L1 and/or PD-L2. This results in the activation of T cells and cell-mediated immune responses.[95] Its action is employed in raising immune response against tumor cells or pathogens in several solid tumors.

Nivolumab is currently approved and in use in patients with metastatic melanoma, squamous non–small-cell lung cancer, advanced renal cell carcinoma, Hodgkin lymphoma and advanced metastatic squamous cell carcinoma of the head and neck.[96] Nivolumab has been tested in a phase 1/2 trial[97] in 47 subjects with advanced HCC (*n* = 24 uninfected; *n* = 12 HCV-positive; *n* = 11 HBV-positive) not amenable to curative resection. The 68% of subjects had a history of prior sorafenib exposure. The ORR (partial response+complete response) was 19% (8/42) with two subjects experiencing complete responses. The most common (frequency of ≥ 15%) treatment-related AEs were increased serum levels of ALT, AST, lipase, amylase, and skin rash with 4% (2/47) of subjects withdrawn because of an AE. With the aim of confirming and evaluating efficacy and safety, there is now an ongoing randomized, open-label phase 3 trial, comparing sorafenib versus nivolumab as first-line therapy in advanced HCC (known as CheckMate-459).[98] An estimated 726 patients affected by advanced HCC are randomized 1:1 to receive nivolumab or sorafenib until disease progression or unacceptable toxicity. CheckMate-459 started in November 2015, and the estimated primary completion date is May 2017. Primary objectives are OS and TTP. Secondary objectives include ORR, PFS and evaluation of the relationship between PD-L1 expression and efficacy.

Durvalumab

Durvalumab (MEDI4736) is a human IgG1 monoclonal antibody directed against human PD-L1. The first study in humans has been a phase 1, multicenter, open-label study, conducted in adult subjects with advanced solid tumors refractory to standard therapies or for which no standard therapies exist.[99] In this cohort, a total of 21 HCC patients were included. Patients received durvalumab every 2 weeks for a median of 6 doses. The safety profile was acceptable, with only 2 subjects (10%) experiencing grade 3 or higher treatment-related AEs. Regarding efficacy, 21% of patients demonstrated a prolonged SD (≥3 months) and there were no ORs. A phase 2 study to evaluate safety, antitumor activity and pharmacology of durvalumab in monotherapy or combination with tremelimumab, an anti CTLA-4 inhibitor (see above), is currently recruiting participants.[100]

Pembrolizumab

Pembrolizumab is a highly selective, humanized monoclonal Ig against PD-1. In a recent case report, it showed a surprising efficacy in a patient with a HCC extrahepatic

mass non-responsive to sorafenib therapy, in absence of significant AEs.[101] To date, an open-label, single-center, single-arm phase 2 trial is ongoing to evaluate efficacy and safety of pembrolizumab in advanced HCC patients who progressed or are intolerant to a first-line of antiangiogenic therapy.[102]

CTLA-4 Blockade

Ipilimumab

Ipilimumab is a fully humanized anti-CTLA-4 IgG1 antibody, approved for treating unresectable or metastatic melanoma in 2011.[103] In the HCC setting, ipilimumab is currently being tested for safety and efficacy (ORR) combined to nivolumab in patients who are naïve to systemic therapy, using a cohort of a phase 1/2 trial.[104]

Tremelimumab

Tremelimumab is a human IgG2 monoclonal antibody specific for human CTLA-4. Tremelimumab blocks the inhibitory effect of CTLA-4, and therefore enhances T cell activation. In a pivotal study, conducted on a small number of patients affected by HCV cirrhosis-related HCC, it showed an acceptable safety profile, both anti-viral and anti-tumor activities (PR: 17.6%, SD: 58.8%).[105] A recent study by Duffy *et al.*[100] demonstrated that tremelimumab in combination with tumor ablation leads to the accumulation of intratumoral CD8+ T cells.[106] A phase 2 trial testing tremelimumab combined with durvalumab or in monotherapy on patients affected by unresectable HCC with or without HBV or HCV infection, is ongoing and enrolling patients.

The role of branched-chain amino acids (BCAAs) in HCC setting

An important reason for the poor prognosis of patients with HCC is the liver function failure, caused by underlying cirrhosis.[21,25] This condition leads to protein-energy malnutrition, due to poor appetite and disorders in protein synthesis, and it is associated with high morbidity and mortality.[107] Furthermore, patients with liver cancer often have increased protein catabolism; a significant proportion of HCC patients are malnourished or at risk of malnutrition, and this represents a negative prognostic value in the survival outcome.[108]

The BCAAs valine, leucine and isoleucine are essential amino acids with aliphatic branched side chains. Besides constituting proteins, they are a source of glutamate, which detoxifies ammonia through glutamine synthesis in skeletal muscle.[109] This property is usually exploited in treating hepatic encephalopathy, as recommended by international guidelines.[110] The BCAA reduction is an important hallmark of liver cirrhosis. Several studies demonstrate that BCAA supplementation improves nutritional status, prognosis and quality of life in these patients.[111,112]

BCAAs were also found to inhibit hepatocarcinogenesis by different means, such as the amelioration of insulin resistance and hyperinsulinemia,[113] the improvement of immune function and the reduction of oxidative stress.[114] In addition, BCAAs may enhance the sensitivity to chemotherapy by reducing the population of cancer stem cells, which may differentiate into cancer cells, through activation of the mTOR complex 1.[115] Moreover, a lot of studies have shown the usefulness of nutritional supplementation with BCAAs in patients with HCC undergoing interventions such as surgery, RFA or TACE.[116–118]

Takeda *et al.*[119] performed a retrospective cohort study exploring the effect of BCAA therapy (952 mg of L-isoleucine, 1904 mg of L-leucine and 1144 mg of L-valine given three times daily after meals) in patients with unresectable HCC treated with sorafenib. The study showed that OS and the median administration period of sorafenib in the BCAA group were significantly longer than those in the control group (p = 0.020 and p = 0.004 respectively). Moreover, after 3 months, albumin levels in the control group decreased significantly compared with pretreatment value (p = 0.009), whereas in the BCAA group there was not a significant decrease from baseline (p = 0.76); this suggests a role for BCAA in the maintenance of albumin levels during sorafenib therapy.

A recent meta-analysis,[120] including 11 interventional studies (surgical or non-surgical procedures) on HCC found that BCAA-supplemented groups had better outcomes in terms of survival, albumin levels and lower risk of liver decompensation. In particular, 3-year mortality was statistically lower in the BCAA groups than in the control groups (37% vs 44%, p = 0.012), whereas the 1-year survival gain did not reach statistical significance (18% vs 21%, p = 0.214). The survival benefit was more evident in Child B patients, confirming a possible role of BCAA in the maintenance of metabolic homeostasis in moderately decompensated liver cirrhosis.

Thus, oral BCAA supplementation is probably useful for maintaining hepatic functional reserve in HCC patients. BCAA use could avoid early discontinuance of therapy and improve OS in patients with HCC treated with sorafenib, provided that a correct dosage is administered.

Conclusions

After the revolution brought about by sorafenib introduction into clinical practice, patients with advanced HCC have been included in a large number of clinical trials, many of which have yielded non-significant results. In the first-line setting, we are waiting for the result of a lenvatinib phase 3 trial. In second-line field, several antiangiogenic drugs have failed to demonstrate a gain of efficacy in terms of survival. The recent positive results achieved with regorafenib have opened new possibilities in the treatment of patients who progressed on sorafenib. Considerable efforts are being invested in the so-called "immune check-point blockade", that is considered the new frontier of the treatment of many solid tumors. A phase 3 trial is already testing nivolumab compared to sorafenib as first-line in HCC patients. BCAAs and nutritional support will almost certainly play a role in HCC treatment, given their effect in the maintenance of a good nutritional and functional status and the possibility of an intrinsic antineoplastic activity. Maybe in the next future, it will be possible to combine conventional HCC treatments with other targeted-molecules and immune check-point inhibitors, in order to obtain a more effective control of the disease and an improvement of OS; this could be especially obtained in those patients who have progressed during sorafenib or who are intolerant to the drug.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceived the topic (ER), prepared the manuscript (ER, LC), generated the schematic diagrams (ER, LC, IS, MC), contributed to nutritional section (MCM, MC, IS), edited the manuscript (MP, AG). All the authors made significant contributions to this review article.

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