Innovative molecular targeted agents in hepatocellular carcinoma : new gladiators on the arena

Innovative molecular targeted agents in hepatocellular carcinoma: New gladiators on the arena

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ABSTRACT

Treatment of advanced hepatocellular carcinoma remains a challenge, with discouraging results in terms of survival. Following the approval of the multikinase inhibitor sorafenib, a large number of molecular targeted agents have been tested, but many have failed to demonstrate significant efficacy in clinical trials. However, the deeper knowledge in HCC pathogenesis achieved through the years has enabled us to explore new targetable pathways as well as biomarkers that could lead to treatment personalization. In this review, we provide a comprehensive update of the most recent data regarding new drugs under investigation -some like regorafenib, very close to its approval- and new possible targets for future treatments.

Key words: Hepatocellular carcinoma, targeted therapies, biomarkers

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for 70-85% of primary hepatic neoplasms, being the most frequently observed primary tumor of the liver and one of the most common malignancies in the world. Annually, HCC is diagnosed in more than half a million people worldwide and is the second most common cause of death from cancer, estimated to be responsible for nearly 746,000 deaths in 2012 (9.1% of the total). In Europe, 71,000 new cases were diagnosed in 2012, making this cancer the fifth most common in men and the seventh in women[1,2].

HCC is generated predominantly due to risk factors that include hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, alcohol abuse, obesity and type 2 diabetes, with geographic variations[3]. About 75% of HCCs are closely associated with chronic inflammation, caused by viral hepatitis in which

continuous inflammation and hepatocyte regeneration occur[4]. These long-term processes may include the accumulation of genetic and/or epigenetic changes, alteration of the liver tumor microenvironment, and the generation of liver cancer stem cells (CSCs)[5].

HCC is characterized by being very aggressive, with a very high death rate after the onset of symptoms, most commonly jaundice and / or ascites. The fact that a screening program has not been accepted and that in most cases HCC is detected only in the symptomatic phase means a late diagnosis. In advanced stage the untreated patient has an average life expectancy of less than one month, when available treatments are limited and ineffective[6].

The most effective and potentially curative treatment of HCC is liver transplantation, although this is only possible in a small percentage of patients in whom tumors are detected in an early stage before there is multifocal or vascular involvement. Surgical resection may also prolong survival in a fraction of patients, but the number of candidates for this technique is quite low[7].

Ablative techniques may be used in selected patients with marginal resectable tumors. One treatment considered as standard therapy for unresectable hepatocarcinoma is transarterial embolization (TAE) or transarterial chemoembolization (TACE). TACE is appropriate for patients with reasonable hepatic function. Careful selection of patients to avoid toxicity, in particular hepatic failure and / or necrosis in administration, is very important[8]. A new, less toxic possibility is the intraarterial administration of yttrium-90 impregnated into glass or resin microspheres[9].

For patients with advanced disease, embolization is not possible and standard treatment for years has been the systemic administration of doxorubicin, based on single-arm clinical trials with variable response rates. The systemic administration of doxorubicin has been evaluated in more than 1000 patients with response rates of 10%, without being reflected in overall survival (OS)[10]. There have also been studies with combinations of cytostatic PIAF (cisplatin, interferon, doxorubicin, 5-fluorouracil) versus doxorubicin in monotherapy, with response rates of 8.67% and 6.83 respectively, with no significant differences between the two groups[11].

Based on the results obtained in clinical trials, sorafenib was the first systemic drug to increase survival in patients with advanced HCC. This drug is an oral multikinase inhibitor of vascular endothelial growth factor (VEGF), platelet derived growth factor receptor (PDGFR) and Rapidly Accelerated Fibrosarcoma (RAF)[12]. Llovet et al. published in 2008 the results of the randomized, double-blind, controlled, phase 3 multicentre study evaluating the survival effects of sorafenib (400 mg / 12h) versus placebo in 602 Child-Pugh A cirrhotic patients with advanced HCC[13].

The study was interrupted prematurely after the second preliminary analysis due to clear survival differences between the two groups. The OS of patients receiving sorafenib was 10.7 months compared with 7.9 months in the placebo

group (relative risk in the sorafenib group 0.69, 95% CI 0.55-0.87; P <0.001). This improvement in survival was also observed in patients with poor prognosis (macroscopic vascular invasion and extrahepatic dissemination). A prolonged time to radiological progression was also found in patients receiving sorafenib (5.5 vs. 2.8 months, p <0.001). Patients treated with sorafenib had diarrhea, weight loss, skin reactions, and hypophosphatemia more frequently compared to the placebo group.

The authors concluded that in patients with advanced HCC, administration of sorafenib prolongs the survival and the time of radiological progression. They also suggest that this drug should be evaluated as adjuvant treatment of other loco regional therapies. The 2.7 months increase in OS is considered important given the lack of therapeutic options in this group of patients.

The results of the trial demonstrated for the first time that molecular targeted therapies are effective in patients with HCC. Sorafenib was the first molecule to demonstrate clinical efficacy in patients with advanced HCC, but there are currently other new drugs under development such as MET or VEGF inhibitors. Here, the aim of this review is to summarize the main targets that are currently under study in advanced HCC (table 1).

c-MET pathway

Hepatocyte growth factor (HGF)/c-MET pathway has a crucial role in the remodeling and proliferation of hepatocytes[14]. There are preclinical studies both in vitro and in vivo that show that c-Met pathway is strongly involved in angiogenesis and proliferation in HCC, so its inhibition could have therapeutic benefit[15]. Furthermore HGF, the c-MET ligand, would be increased in the plasma of patients with HCC and would be related to negative prognostic factors such as bigger tumor sizes, higher tumor grade and rate of metastases and worse OS[16]. To this, we must add studies in cell lines suggesting that overexpression of c-MET acts as a predictive factor of response to c-MET inhibitors, which points to c-MET as a potential biomarker[17]. C-MET or its ligand HGF inhibition is effective by the use of several selective or pan-tyrosine kinase inhibitors and monoclonal antibodies. There are currently several c-MET inhibitors under development, some of which are targeted at a selected population using biomarkers[18].

Tivantinib (ARQ197)

This is an oral, selective small molecule MET inhibitor. In its Phase Ib[19] study, a disease control rate of 56% and a median time to progression (TTP) of 3.3 months were described, all with a good toxicity profile at a dose of 360 mg per day mostly in Child-Pugh A patients (81%). The most frequent grade 3-4 adverse events (AEs) observed were haematological: neutropenia (38%), anemia (24%), and leucopenia (19%). Later a multicenter, randomized, placebo-controlled phase II trial included 107 patients with HCC who failed first-line systemic therapy[20]. Of these, thirty-eight patients received 360 mg of

tivantinib twice daily, and 33 patients received 240 mg, twice daily. Thirty-six patients were assigned to receive a placebo. Amedian TTP of 1.6 months was described in the tivantinib arm, which was modestly longer than 1.4 months in the placebo arm (HR, 0.64; p = 0.04). Of interest, the benefit in median TTP was greater in patients with high-MET tumors (n = 37), as determined by immunohistochemistry, when treated with tivantinib compared with placebo (2.7 months vs. 1.4 months; HR, 0.43; p = 0.03). These results favoring tivantinib must be considered carefully since they derive from a subgroup analysis. In addition, there were four reported deaths related to severe secondary neutropenia in the tivantinib arm, driving the investigators to decrease drug dose to 240 mg twice daily. Tivantinib is currently being evaluated in two phase III studies as second-line treatment: NCT01755767 in USA population with OS as primary endpoint, and NCT02029157 in Japanese population with progression free survival (PFS) as primary endpoint. The first preliminary results of both studies are expected in mid-2017.

Capmatinib (INC280)

This is a highly-selective, oral small molecule MET inhibitor with preclinical activity in human tumor models[21]. In a phase I trial[22] involving 33 patients with advanced solid tumors (45% HCC) that presented c-MET dysregulation, patients were treated in six dose cohorts of 100-600 mg twice daily. The most common drug related grade 3-4 adverse events were fatigue (9%) and decreased appetite (6%). Dose limiting toxicity occurred at 200 mg, 250 mg and 450 mg (1 patient each). Recommended phase II dose (RP2D) was established at 600 mg twice daily. Stable disease was reported in 24% heavily pretreated patients. There is an ongoing phase II trial (NCT01737827) to find out the safety and the beneficial effects of capmatinib in Asian patients with advanced HCC known to have dysregulation of c-MET pathway, with TTP as primary endpoint. The estimated primary completion date is May 2017.

Cabozantinib

Cabozantinib is an oral, small-molecule inhibitor of tyrosine kinases, including MET, VEGF receptors and AXL, and is currently approved for the treatment of patients with metastatic medullary thyroid and renal-cell cancer. A phase II trial of Cabozantinib[23] was carried out in 41 HCC patients with Child-Pugh A score and one or no prior systemic treatment. The overall disease control rate was 68% at week 12, slightly higher for the Asian population (73%). A median PFS of 4.2 months was reported, with an overall response rate of 5%. The most common toxicities were diarrhea (17%), palmar-plantar erythrodyesthesia (15%) and thrombocytopenia (10%). CELESTIAL (NCT01908426) is an ongoing phase III trial of Cabozantinib versus placebo in subjects with hepatocellular carcinoma who have received prior Sorafenib. The outcome from the first planned interim analysis of this study was announced on September 2016. Following this interim analysis, which was scheduled to take place when 50 percent of the events for the primary endpoint of OS had occurred, the trial's Independent Data Monitoring Committee determined that the study should continue without

modifications per the study protocol. The trial protocol calls for a second interim analysis to take place once 75 percent of events have been observed.

Foretinib (GSK1363089, XL880)

Multitargeted Tyrosine Kinase Inhibitor GSK1363089 is an orally bioavailable small molecule that acts as an ATP-competitive inhibitor of HGFR and VEGFR, mostly for MET and KDR. A phase I trial of this drug[24] was conducted in 40 patients who had metastatic or unresectable solid tumors for which no standard measures exist. The maximum tolerated dose was defined as 3.6 mg/kg, with a maximum administered dose of 4.5 mg/kg. Dose-limiting toxicities included grade 3 elevations in aspartate aminotransferase and lipase. Other grade III/IV adverse events include hypertension (24.3%) and fatigue (18.9%). Responses were observed in two patients with papillary renal cell cancer and one patient with medullary thyroid cancer. Stable disease was identified in 22 patients. Researchers did not mention the number of patients with HCC in their study. There is also a phase 1/2 study evaluating Foretinib[25] in 39 Child-Pugh A, Asian, advanced HCC-patients with no prior treatment. The ORR was 24% with an acceptable tolerability and safety profile, although two patients discontinued treatment due to toxicity. Most common adverse events were hypertension (36%), hyporexia (23%) and pyrexia (21%), and most common serious adverse events were encephalopathy (10%) and ascites (8%). There were no phase III trials with Foretinib in HCC patients at the time of writing this paper.

Antiangiogenic agents

Angiogenesis plays a central role in the development of HCC, as hypervascularity is a main HCC characteristic. Data from a meta-analysis indicate that increased levels of vascular endothelial growth factor VEGF are a marker of poor prognosis[26]. Micro- and macro- vascularization are crucial in HCC prognosis development, pathogenesis and in treatment and diagnosis; the abnormal vascularization is used to differentiate HCC when hepatic masses are observed through radiologic images. The best known angiogenic pathway is that promoted by VEGFR and its receptors (VEGFR) [27]. This pathway interacts with others to ultimately elicit the angiogenic effect. The angiopoietins (Ang) bind with the RTKs tyrosine kinase with immunoglobulin- like and EGF-like domains (Tie)1 and Tie2. Ang-2 is felt to increase the activity of VEGF. Multiple growth factors, including fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), and the transforming growth factors (TGF), have been found to increase VEGF-A expression. Tissue hypoxia has also been shown to promote VEGF-A, through the hypoxia-inducible factors (HIF) HIF-1α and HIF-2α. Although some antiangiogenic agents (brivanib[28], linifanib[29], sunitinib[30]) did not prove to be better than sorafenib in the first line of HCC treatment, the angiogenesis pathway is still an interesting target with great therapeutic potential, so there are several drugs in development.

Regorafenib

Regorafenib is an oral multikinase inhibitor structurally similar to sorafenib. This drug acts against VEGFR1–3, c KIT, tyrosine kinase with immunoglobulin-like and EGF-like domains-2, PDGFR- 2, FGFR-1, RET, RAF-1, BRAF, and p38 MAP kinase. Regorafenib is currently approved for the treatment of colorectal cancer after progression to chemotherapy and in third-line treatment in gastro-intestinal stromal tumors (GIST). In HCC patients, this agent was tested in a phase II study after failure to sorafenib[31]. Disease control was achieved in 26 (partial response n=1; stable disease n=25) out of 36 patients. Median TTP was 4.3 months and median OS was 13.8 months. Regorafenib had acceptable tolerability although 32 patients had treatment interruption due to drug-related adverse effects. These results were the basis for the development of the phase III trial RESORCE[32] of regorafenib in the second-line setting. This international, placebo-controlled study enrolled 573 patients with intermediate or advanced HCC that had progressed to sorafenib and its results were first presented at ESMO 2016. After a median of 3.6 months of treatment, patients on regorafenib showed a 38% reduction in the risk of death and a 54% reduction in the risk of progression or death compared to placebo. Median PFS was 3.1 months with regorafenib and 1.5 months with placebo, while median overall survival was 10.6 months for regorafenib and 7.8 months with placebo. Overall, 65.2% of patients on regorafenib showed complete, partial response or stable disease, compared to 36.1% in the placebo group. Regorafenib had a similar safety profile to sorafenib, with hypertension, hand-foot skin reaction, fatigue and diarrhea being the most common drug-related adverse effects. These results can lead to the approval of a new systemic drug in HCC, even in the second line setting, after 9 years.

Nintedanib

Nintedanib is an orally available small molecule multikinase inhibitor of VEGFR 1–3, PDGFR and FGFR. This drug is already approved for patients with metastatic non-small cell lung cancer (NSCLC) that has progressed to first-line chemotherapy. In HCC patients, nintedanib has been tested in two randomized phase I/II trials[33, 34] comparing its efficacy and safety versus sorafenib. One of these studies was carried out in European population and the other in Asian population. In both trials Nintedanib showed similar efficacy to sorafenib with respect to TTP and OS, with a manageable safety profile. These results warrant further studies of this agent in patients with advanced HCC.

Ramucirumab

Ramucirumab is a fully human G1 monoclonal antibody that binds to the vascular endothelial growth factor receptor-2 (VEGFR-2) and inhibits the downstream signaling induced by binding of VEGF ligands. REACH[35] was a randomised, double-blind, multicenter, phase 3 trial comparing Ramucirumab versus placebo as second-line treatment in patients with advanced HCC following first-line therapy with sorafenib. This trial enrolled 565 Child-Pugh A patients from 154 centers in 27 countries and it did not find significant

improvement in OS with ramucirumab (9.2 months vs. placebo, 7.6 months; p = 0.14). Despite these results, it should be noted that in patients with baseline serum alpha-fetoprotein (AFP) higher than P400 ng/mL, there was a survival benefit in the treatment arm (ramucirumab, 7.8 months vs. placebo, 4.2 months; p = 0.006). This data suggests that this subgroup of patients with high serum AFP values are more likely to respond to anti-VEGF2 therapy, although the pathophysiology that would explain this assumption is unknown. However, it is known from clinical studies that serum AFP level is a potent prognostic factor in advanced HCC[36], showing that AFP-positive HCC are biologically different from AFP-negative HCC. Nonetheless, AFP level was not a pre-stratified factor during randomization in the REACH study, so further studies are needed to support this finding. REACH-2 is an ongoing large-scale multicenter phase III study (NCT02435433) that repeats the design of the REACH study in a selected population enriched with high baseline AFP levels (P400 ng/mL or higher).

Lenvatinib (E7080)

This is an oral, multi-targeted tyrosine kinase inhibitor (TKI) of VEGF receptors 1, 2 and 3, fibroblast growth factor receptors 1, 2, 3 and 4, platelet-derived growth factor receptor alpha, RET and KIT signaling pathway. A phase I/II trial[37] tested this drug on HCC treatment-naïve patients. An interesting overall response rate of 33% was reported, with a median TTP of 12.8 months and an OS of 18.7 months. Nevertheless, toxicity was not negligible, with grade III-IV hypertension (50%), hyperbilirrubinemia (15%) and palmar-plantar erythrodysaesthesia syndrome (5%). There is an ongoing phase 3 trial (NCT01761266) to compare the efficacy and safety of lenvatinib versus sorafenib in first-line treatment of subjects with unresectable HCC. Its first results are expected in December 2017.

Axitinib

This small molecule is an indazole derivative that is a potent and highly selective inhibitor of VEGFR tyrosine kinase 1, 2, and 3. It is approved for second-line treatment of advanced renal-cell carcinoma. In HCC patients, axitinib was tested in a phase II trial[38] in the second-line setting. It met its primary endpoint with 42.3% tumor control rate at 16 weeks. In addition, a PFS of 3.6 months with a median OS of 7.1 months was reported. Most common grade 3/4 adverse events were hypertension, thrombocytopenia and diarrhea. Finally, four patients discontinued treatment due to AEs. There is no phase III trial ongoing at the moment.

mTOR pathway

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase which is a downstream target of the PI3K/Akt pathway. It plays an important role in the transcription and translation of proteins regulating cell proliferation, survival, and cell cycle, including key transcription factors cMyc, cyclin D1, and HIF-1a. mTOR can be divided in two different complexes: mTORC1, which activates

anabolism, and mTORC2, about which even less is known. In HCC, the mTOR pathway has been unregulated in up to 40%[39], thus inhibiting this pathway may be an optimal therapeutic approach.

Everolimus

Everolimus is an oral mTORC1 inhibitor. It has been approved for many tumors, such as renal cancer in first or in second line settings, and in breast cancer in combination with exemestan. In HCC, everolimus was tested in a phase I/II trial[40] that reported a median PFS and OS of 3.8 months and 8.4 months, respectively. The estimated PFS rate at 24 weeks was 28.6% and 10 mg/day was defined as the phase 2 dosage. Researchers also reported stability of the disease in 40% of the twenty-eight patients, with one patient achieving a partial response. Grade 3-4 adverse events included hypertransaminasemia (12%), lymphopenia (12%), and hyponatremia (8%). Based on these results, the placebo-controlled, phase III EVOLVE-1 trial[41] was conducted to evaluate the efficacy of everolimus in HCC tumors progressing to sorafenib. Unfortunately, no significant difference in OS was seen between treatment groups (7.6 months with everolimus vs. 7.3 months with placebo [HR=1.05; 95%, P=0.68]). Despite these results, a phase I trial[42] that explored the combination of everolimus with sorafenib was carried out. With twenty-five patients enrolled, it reported a disease stability above 40% with a good toxicity profile. Later, a phase II trial[43] randomized 106 patients to receive daily sorafenib 800 mg alone or with everolimus 5 mg, and no evidence was found that the drug combination improves the efficacy compared with sorafenib alone. Additionally, a higher-grade III-IV toxicity profile was found in the combination group (86% vs. 72%). For all this data, investigators concluded that further testing of this drug combination in molecularly unselected HCCs appears unwarranted.

Temsirolimus

Temsirolimus is a soluble ester of rapamycin which is administered intravenously. It binds to an intracellular protein (FKBP-12), and the protein-drug complex inhibits the activity of mTOR that controls cell division, resulting in a G1 growth arrest in treated tumor cells[44]. Temsirolimus was tried out in a phase I/II study[45] amongst thirty-five HCC patients, and there were 1 partial response and 20 stable diseases. The targeted median PFS endpoint was not reached (2.83 months, 95% C.I. 1.63-5.24). The median OS was 8.89 months (95% C.I. 5.89-13.30). Grade 3-4 adverse events included thrombocytopenia and hyponatremia, both with 11% of patients. A phase II trial[46] explored temsirolimus after sorafenib failure, reporting a median PFS of 17 weeks and a median OS of 30 weeks, with a disease control rate at 12 weeks of 43%. Grade 3-4 related adverse events occurring in over 10% of patients were thrombocytopenia, neutropenia, AST elevation and hyperglycemia. With these results, the authors speculate that intravenous administration may overcome compliance issues with the drug. Temsirolimus combination with sorafenib was tested in a phase I trial[47], describing a stable disease rate of 60%, and an 8% partial response. Grade 3 or 4 related adverse events at maximum tolerated

dose (MTD) included hypophosphatemia (33%), infection (22%), thrombocytopenia (17%), hand-foot syndrome (11%), and fatigue (11%). This combination is being tested in a phase II trial (NCT01687673) in the first-line setting, and primary results are expected in September 2017.

Other agents

An intense research activity regarding mTOR inhibitors is ongoing, with new agents that target both mTORC1 and mTORC2, preventing upregulation of AKT phosphorylation, and differentiating it from the rapamycin analogs. CC-223 is one of these new drugs, first presented in a phase I trial[48] that included different tumors. In HCC, a stable disease was reached in 10 out of 25 patients (40%). This finding must be confirmed in future studies in order to confirm the benefit of this new drug.

Immunotherapy

A high rate of spontaneous tumor regression has been observed in HCC, a phenomenon that has been explained from the perspective of tumor immunity[49]. In fact, the rationale of immunotherapy drugs is to block the inhibitory signals that cause tumor cells to evade the immune response unchained by host lymphocytes against tumor antigens. Local techniques such as radiofrequency and TACE in early HCC have proven to release tumor antigens, which are taken up by antigen-presenting cells and activate a tumor-specific immune response[50]. In addition, occasional objective tumor responses have been reported after adoptive immunotherapy using dendritic cells pulsed with tumor lysate[51] or lymphokine-activated killer cells plus recombinant interleukin 2[52]. From all these evidences, addressing HCC treatment from immunotherapy may be effective.

Tremelimumab

Tremelimumab (CP-675,206) is a fully human IgG2 monoclonal antibody that blocks the binding of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). This binding sends an inhibitory signal that serves as a natural brake for T cell activation, so CTLA-4 blockade releases this brake and enhances T cell activation and proliferation through various mechanisms still under intense study[53]. Tremelimumab was tested in twenty hepatitis C virus-related HCC patients in a phase II trial[54]. Of these patients, 43% had an altered liver function (Child-Pugh class B) and most of them were in the advanced stage. A disease control rate of 76.4% with a partial response of 17.6% was reported, with a good safety profile: no patient needed steroids because of severe immune-mediated adverse events. In addition, a significant drop in the viral load was reported. There are currently several ongoing studies testing this drug in combination with durvalumab (MEDI4736, another immunotherapy agent) as well as in combination with local ablative techniques (NCT02519348, NCT02821754, NCT01853618).

Nivolumab

Nivolumab is currently approved for the treatment of melanoma, renal cell carcinoma and NSCLC. It is a fully human IgG4 monoclonal antibody programmed death receptor-1 (PD-1) inhibitor that blocks interaction with its ligands, PD-L1 and PD-L2. This binding releases PD-1 pathway-mediated immune responses against tumor cells. A recent publication by Calderaro et al[55] indicates that PD-L1 expression by neoplastic cells in HCC is related to tumor aggressiveness (high AFP levels, satellite lesions, micro- and-macrovascular invasion and poor differentiation) and implies that the response to treatments targeting the PD-L1/PD-1 immune checkpoint could be restricted to particular HCC subtypes. A phase I/II trial (CheckMate-040) using this drug in the second line setting was presented at ASCO 2016[56] and it included HBV- and HCV-infected HCC patients. An objective response rate of 15% was reported and a median OS of 15.1 months. The responses were durable and they were observed regardless of PD-L1 status, and antiviral responses in HCV-infected patients were described. Most frequent grade 3/4 treatment-related adverse events consisted in AST increase (10%, of which one patient discontinued treatment) and lipase and ALT increase (6% each). There is currently an ongoing phase III trial (CheckMate-459, NCT02576509) of nivolumab versus sorafenib as first-line treatment in patients with advanced HCC, whereby the estimated primary completion date is May 2017. There are also other trials, all of them at an early stage of clinical development, of nivolumab in combination with several drugs: ipilimumab (NCT01658878), Yttrium Y90 glass microspheres (NCT02837029), galunisertib (LY2157299), an oral small molecule inhibitor of the TGF-β receptor I kinase (NCT02423343) and CC-122, an immunomodulatory drug that mimics interferon effect (NCT02859324).

Other immunotherapeutic antibodies

Pembrolizumab, the other anti-PD1 currently marketed, is undergoing intense investigation in several clinical trials in various stages of development. One of these is a phase III study (KEYNOTE-240, NTC02702401) of this drug vs best supportive care in advanced HCC patients after failure to sorafenib. Its primary endpoints are both PFS and OS and its estimated primary completion date is February 2019. Other ongoing studies with pembrolizumab worthwhile mentioning are two phase I trials in combination with antiangiogenic agents: one with lenvatinib (NCT03006926) and the other with nintedanib (NCT02856425). On the other hand, anti-PD-L1 antibodies are still in early stages of clinical development. Atezolizumab (MPDL3280A), which is already approved by FDA for second-line treatment of urothelial carcinoma, is being tested in HCC in two phase I studies. One of them in Chinese population with advanced solid tumors (NCT02825940) and the other in combination with bevacizumab and/or other treatments in USA and South-Korean patients with solid tumors (NCT02715531). Durvalumab, another anti-PD-L1 antibody, is being tested in two phase I studies: one in combination with ablative therapies in subjects with HCC or biliary tract carcinomas (NCT02821754) and the other with

ramucirumab in patients with advanced gastrointestinal or thoracic malignancies (NCT02572687).

Histone deacetylase inhibitors

It is known that epigenetic dysregulation has a central role in the genesis of HCC[57]. The balance of histone acetyltransferase and histone deacetylase (HDAC) maintains histone acetylation, which is associated with uncoiling of histone followed by gene expression. There is preclinical evidence both in human tumor xenografts and in cell lines suggesting that the inhibition of HDAC may induce apoptosis of HCC cells and expression of tumor suppressor gene[58,59]. Sensitivity to HDAC inhibitors has been associated with the homologue of Rad23 B (HR23B) protein in T-cell lymphoma. There are also studies showing that HR23B could determine, through interaction with HDAC6, the switch between apoptosis and autophagy of cancer cells[60,61]. Belinostat, an HDAC inhibitor, was tested in a phase I/II clinical trial with heavily pre-treated HCC patients[62]. It reported modest efficacy with a stable disease (SD) rate of 45.2%. To note is the fact that immunostaining of HR23B was associated with a higher rate of disease stabilization: 58% for the high-HR23B expression group vs. 14% for the low-HR23B expression group ($p = 0.036$). This suggests that HR23B expression could be used as a predictive biomarker for HDAC inhibitors in HCC treatment. These findings require further validation in clinical trials designed to test belinostat or other HDAC inhibitors in patients with HR23B-positive HCC.

Targeting cancer stem cells

Proliferation of stem cells has a central role in regenerating hepatic tissue. They are capable of unlimited self-renewal, tumor formation and differentiation into all tumor cell lineages. Some tumors originate from stem cells and may have different genetic alterations[63]. It is known that, although HCC originates from clonal cells, it may arise from hepatic stem cells[64]. As a consequence, the elimination of cancer stem cells could be a good approach in the treatment of HCC. The Wnt pathway is one of the most involved pathways in the ability to spawn tumors of these cancer stem cells[65]. A phase I clinical trial[66] of anticancer stem cell agent ipafricept (OMP-54F28, FZD8-Fc), decoy receptor for Wnt ligands, was conducted in patients with all kinds of advanced solid tumors. Although the researchers did not specify the number of patients with HCC, they reported 6 patients with prolonged stable disease out of a total of 25 patients. Ipafricept was well tolerated, as long as no grade 3-4 toxicities were reported. Another phase I trial with the same molecule in combination with sorafenib is being conducted in HCC patients. Its estimated primary completion date is April 2017. Amcasertib (BBI503) is an orally-administered investigational agent that targets STAT3 and beta-catenin, leading to inhibition of the critical pathways for maintaining cancer stem cells. This drug was tested in a phase I

trial[67] in patients with advanced solid tumors and reported a 55% of disease stabilizations with a median TTP of 16 weeks. At the moment there are two ongoing studies with amcasertib in HCC patients: a phase I/II trial in combination with sorafenib in patients who have not received systemic treatment (NCT02279719), and a phase II trial in cholangiocarcinoma and HCC patients who have exhausted all currently approved standard anti-cancer treatment options (NCT02232633).

Other approaches

Anti-glypican-3 (GC33)

Glypican 3 (GPC3) is a protein of the glypican family, which seems to play a vital role in developmental morphogenesis, and has been suggested as regulators for the Wnt and Hedgehog cell signaling pathways. It has additionally been suggested as regulator for fibroblast growth factor and bone morphogenic protein signaling[68]. In HCC, GPC3 is overexpressed and constitutes a poor prognostic factor after surgery[69]. GC33 is a fully humanized monoclonal antibody against GPC3 that was tested in a first-in-human phase I study[70] in patients with advanced HCC. This trial described a median TTP of 26.0 weeks in the GPC3 high expression group and 7.1 weeks in the low expression group (P = 0.033), with a good safety and tolerability profile. Despite these initial interesting results, the phase II trial of GC33[71] did not demonstrate benefit neither in terms of PFS nor in OS. The researchers attributed these results to the use of low doses. Further studies should be carried out to determine the actual potential of this drug.

Fibroblast growth factor receptor 4 (FGFR4)

This growth factor is mainly synthetized in hepatocytes to regulate bile acid secretion. There are several studies[72,73] showing that the FGFR4-FGF19 system plays an important role in HCC genesis and that FGF19 correlates with tumor progression and worse prognosis. Preclinical studies with HCC cell lines suggest that the copy number gain of FGF19, which occurs in a subset of HCC patients, could also be used as a predictive biomarker as long as it is associated with sensitivity to FGFR inhibition. Co-expression of FGFR4 and beta-Klotho (a type-I membrane protein that is related to β-glucuronidases) may also be associated with sensitivity to FGFR4 inhibitors[74,75]. At the moment, there are two phase I clinical trials evaluating two of this inhibitors, namely FGF401 (NCT02325739) and BLU554 (NCT02508467). Results of these two studies will help to determine the efficacy of FGFR4 inhibitors in patients with HCC. In addition, both clinical trials have incorporated biomarker analyses in both fresh biopsy and archival HCC tissue[76].

Refametinib (BAY 86-9766, RDEA119)

This is an orally bioavailable selective MEK inhibitor that specifically inhibits mitogen-activated protein kinase 1 (MAP2K1 or MAPK/ERK kinase 1), resulting

in inhibition of growth factor-mediated cell signaling and tumor cell proliferation. Preclinical studies have described the antitumor potential of this agent, alone and in combination with sorafenib[77,78]. Refametinib was tested in combination with sorafenib in a phase I clinical trial[79] with sixty-nine patients with several advanced solid tumors, including HCC. The drug was well-tolerated and showed some evidence of clinical benefit across a range of tumor types. In HCC patients, a phase II study[80] also in combination with sorafenib reported a disease control rate of 44%, with a PFS of 4.1 months and an OS of 9.7 months. KRAS mutation was predictive of better outcomes. Refametinib has been tested in Ras-mutated HCC in two single-arm phase-II trials: in first line combined with sorafenib (NCT01915602) and in second line versus placebo (NCT01915589). Both studies are completed and results are expected shortly.

CONCLUSIONS

Within the field of oncology, advanced HCC treatment remains a challenge. Since 2008, sorafenib remains as the first line treatment of this entity. However, after the regorafenib results presented last year at ESMO annual congress, it seems that sorafenib is going to have a new brother in arms in the fight against this disease, even in the second line setting. Despite negative data from some trials validating other VEGFR inhibitors, new TKIs are under intense investigation, and we are awaiting results. New drugs in advanced stage of clinical development inhibiting c-MET and mTOR pathways promise to challenge the solitary reign of sorafenib in front line HCC treatment. In addition, the arising of new comprehensive perspectives against carcinogenesis, such as immunotherapy -oncology's last great ace- and inhibiting cancer stem cells can also change the perspective in HCC treatment. Besides all this, recent advances in genomic medicine have opened the door for a deeper insight into the HCC molecular basis, raising the possibility of predicting responses to new agents and thus personalizing treatment in accordance with the molecular distinction of this tumor.

Drug	Type	Targets	Study
Tivantinib	Small molecule selective inhibitor	c-Met	Phase III (NCT01755767 (NCT02029157)
INC280	Small molecule selective inhibitor	c-Met	Phase II (NCT01737827)
Foretinib	Small molecule multikinase inhibitor	c-Met/VEGFR2	Phase I/II (NCT00920192)
Cabozantinib	Small molecule multikinase inhibitor	c-Met, RET, VEGFR1-3, c-KIT	Phase III (NCT01908426)
Regorafenib	Small molecule multikinase inhibitor	VEGFR1-3, c-KIT, TKI-like, EGF-like 2, PDGF2, FGF1, RET, RAF-1, BRAF, MAPK	Phase III (NCT01774344)
Nintedanib	Small molecule multikinase inhibitor	VEGFRs1-3, FGFR1, PDGFR	Phase I/II (NCT00987935 and NCT01004003)
Lenvatinib	Small molecule multikinase inhibitor	VEGFR 1-3, FGFR1, PDGFRa, RET, KIT	Phase III (NCT01761266)
Axitinib	Small molecule multikinase inhibitor	VEGFR 1-3	Phase II (NCT01210495)
Everolimus	Small molecule selective inhibitor	mTORC1	Phase III (NCT01035229)
Temsirolimus	Small molecule selective inhibitor	mTORC1	Phase II (NCT01567930)
CC-223	Small molecule selective inhibitor	mTORC1/mTORC2	Phase I/II (NCT01177397)
Tremelimumab	Fully human monoclonal antibody	CTLA-4	Phase II (NCT01008358)
Nivolumab	Fully human monoclonal antibody	PD-1	Phase III NCT02576509
Pembrolizumab	Fully human monoclonal antibody	PD-1	Phase III (NTC02702401)
Belinostat	Histone deacetylase inhibitor	HDAC enzymes	Phase I/II (NCT00321594)
OMP-54F28	Recombinant fusion antibody	Wnt	Phase I (NCT01608867 and NCT02069145)
BBI503	Small molecule selective inhibitor	B-Catenin/Stat3	Phase II (NCT02279719 and NCT02232633)
GC33	Fully human monoclonal antibody	Glypican-3	Phase II (NCT01507168)
Refametinib	Small molecule mitogen-activated protein kinase inhibitor	Mek 1-2	Phase II (NCT01915589 and NCT01915602)
FGF401	Small molecule selective inhibitor	FGFR4	Phase I (NCT02325739 and NCT02508467)

Table 1: Main agents investigated/under investigation in HCC with their respective most advanced trials.

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